Supplementation with a mixture of whole rice bran and crude glycerin on metabolic responses and performance of primiparous beef cows

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ABSTRACT - This study investigated the effect of a supplement containing whole rice bran and crude glycerin for 21 days before mating on metabolic, productive, and reproductive responses of 28 primiparous suckling beef cows. Cows were randomly assigned to a control group (CON, n = 14), grazing on grasslands, and a supplemented group (SUP, n = 14), grazing on grasslands and supplemented daily individually with 1 kg dry matter (DM) of whole rice bran + 550 mL crude glycerin (224 g kg^{-1} DM of methanol) per cow. After 33 days of natural mating, cows that had not expressed estrus were subjected to a fixed-time artificial insemination protocol. Ten days after the insemination program, bulls were reintroduced for 21 days. Supplementation increased milk yield (SUP: 5.7±0.2 vs. CON: 5.0±0.2 kg d^{-1}), milk protein content (SUP: 3.1±0.2 vs. CON: 2.8±0.2%), and body weight of cow (SUP: 379±2 vs. CON: 373±2 kg) and calf (SUP: 150±2 vs. CON: 142±2 kg). Supplementation improved the energy balance, increased plasma concentrations of cholesterol (SUP: 223.2±6.4 vs. CON: 202.1±6.4 mg dL^{-1}) and glucose (SUP: 72.0±1.2 vs. CON: 68.6±1.2 mg dL^{-1}), and reduced non-esterified fatty acids (SUP: 0.45±0.02 vs. CON: 0.56±0.02 mmol L^{-1}). The percentage of cows on superficial anestrous after supplementation was greater in SUP than in CON group (57 vs. 21%, respectively); however, no difference in final pregnancy rate was found (SUP: 79 vs. CON: 64%). There was no evidence that the ingestion of crude glycerin with high content of methanol induced clinical or hepatic disorders. Supplementation of whole rice bran and crude glycerin is not toxic, and can improve the energy balance, reflecting in increase in milk yield and calf growth, with a slight effect on the reproductive activity.

Key Words: methanol, milk protein, shallow anestrus

Introduction

In extensive pastoral systems for meat production, primiparous suckled cows have the lowest reproductive efficiency and wean the lightest calves, which reduces the productivity of the herd (Bellows et al., 1982). The main cause of reproductive failure is the prolonged post-partum anestrus, induced by undernutrition (Short et al., 1990; Hess et al., 2005) and suckling (Williams, 1990). The nutrient supply of grasslands during the winter is insufficient to meet the requirements of the growing fetus in the last third of the pregnancy period, causing a negative energy balance that continues during early postpartum due to the demand for milk production (Bell, 1995; Astessiano et al., 2013). The negative energy balance is evidenced by a decrease in body condition score (BCS) and endocrine changes, such as an increase in non-esterified fatty acids (NEFA) and a decrease in glucose and insulin, which have a negative impact on follicle growth and ovulation (Wiltbank, 1970; Mulliniks et al., 2011).

Postpartum supplementation can overcome, at least partially, pre-partum undernutrition (Perry et al., 1991; Ciccioli et al., 2003). Short-term supplementations before or during the mating period, with or without association with temporary weaning, are alternatives to increase pregnancy rates in cows with sub-optimal BCS (Pérez-Clariget et al., 2007; Soca et al., 2013). The supplement most frequently used in these studies has been whole rice bran, an energy nutrient with 130-180 g kg^{-1} of crude protein (CP) (Wang et al., 2012). On the other hand, the biodiesel industry has increased the availability of crude glycerin that can be used in ruminant nutrition (Donkin, 2008). The main component of crude glycerin is glycerol, a powerful gluconeogenic alcohol (Alexander et al., 2010). However, the main disadvantage of crude glycerin is that its methanol content can impair liver function (Schröder and Südekum, 1999).

The hypothesis of this study was that short-term supplementation before mating with whole rice bran and crude glycerin with high content of methanol improves the energy balance and the performance of primiparous

beef cows grazing grasslands without impairing liver function. The aim of this study was to evaluate the effect of supplementation for 21 day before mating with whole rice bran and crude glycerin with high level of methanol on body weight, body condition, milk production, hormonal and metabolic profiles, ovarian activity, pregnancy rate, and liver function in primiparous beef cows and the growth of their calves grazing grasslands.

Material and Methods

The experiment was conducted in an experimental station in eastern Uruguay (32º S, 54º W) according to the experimental procedures approved by the Animal Experimental Committee of Universidad de la República (UdelaR).

Twenty-eight pregnant Hereford, Aberdeen Angus, and crossbred heifers (n = 4, 10, 14; respectively) with 424±7 kg of body weight (BW) and 5.1±0.1 units of BCS (1-8 scale: 1 = very thin, 8 = very fat; Vizcarra et al., 1986) were monitored from 16±1 weeks pre-partum (early winter) until 47±1 days post-partum (DPP), when the supplementation period began.

At the start of supplementation (day 0), BW and BCS were 371±7 kg and 3.8±0.1, respectively. All the cows were suckling and in deep anestrus confirmed by the absence of a corpus luteum and the presence of follicles <9 mm in diameter in the ovaries in two ultrasound studies nine days apart (Wiltbank et al., 2002).

Cows were paired based on DPP, BCS, BW, genotype (crossbred vs. pure) and sex of the calf. One member of each pair was randomly assigned to one of the following treatments: Control group (CON, n = 14): grazing grasslands with no supplementation; and Supplemented group (SUP, n = 14): grazing grasslands and supplemented daily with 1 kg DM of whole rice bran + 550 mL crude glycerin per cow for 21 days before the mating period. Whole rice bran and crude glycerin were premixed before individual supplementation. The metabolizable energy (ME) available in the supplement was 5.50 Mcal d^{-1}, according to NRC (2001), and the CP content was 152 g d^{-1}.

The chemical composition of the supplement components was evaluated before use (Table 1). Whole rice bran and herbage (Table 1) were evaluated for ether extract (EE; AOAC, 1990; N. 954.02), ash and CP (AOAC, 2007; N.942.05, N.984.13; respectively), and neutral and acid detergent fiber (NDF and ADF; Van Soest et al. 1991). The chemical composition of crude glycerin was evaluated for ash (AOCS, 1973; Ea 2-38), glycerol (AOCS, 2012; Ea 6-51), water (AOCS, 2009; Ea 8-58), fat (AOAC, 1980; 14.019), and CP (AOAC, 2007; N.984.13)], and the methanol content was determined by gas chromatography.

Calves were separated from their mothers for the first 14 days of the supplementation period while the cows were supplemented (30 min) to avoid interference, but they remained within visual, auditory, and olfactory contact. During the last 7 days of the supplementation period, calves of the CON and SUP groups with 61±1 days of age were separated from their mothers and visual, auditory, and olfactory contact was prevented. During the temporary weaning, calves were kept separated in a small paddock and supplemented daily with 0.9 kg DM of alfalfa (*Medicago sativa*) hay bales per animal and 1.1 kg DM of early weaning ration (Bioración, Melo, Uruguay), containing 180 g kg^{-1} of CP, per animal. Free access to water and shade was provided.

The first mating period lasted 33 days and started when cows were at 68±1 DPP. The breeding soundness of the bulls was tested two months prior to the beginning of the breeding season. Estrus was detected 3 times per day (7.00 h, 13.00 h, and 19.00 h); a cow was considered in estrus when it accepted being mounted by the bull. Cows that did not show estrus during this period were subjected to a fixed-time artificial insemination program. The protocol started on 101±1 DPP in the morning; an inert silicone intravaginal device containing 1 g of progesterone (P$_4$; DIB®, Syntex Laboratory, Buenos Aires, Argentina) was placed and 2 mg of estradiol benzoate (Syntex Laboratory, Buenos Aires, Argentina) were injected. At the moment of DIB® withdrawal, in the morning of 108±1 DPP, 500 mcg of cloprostenol (Ciclase D®, Syntex Laboratory, Buenos Aires, Argentina) and 400 IU of equine chorionic gonadotrophin (Novormon®, Sintex Laboratory, Buenos Aires, Argentina) were injected. On the following day, 1 mg of estradiol benzoate was applied. All hormones were injected intramuscularly. The fixed-time artificial insemination was performed 52-56 h after removal of DIB®. This protocol of fixed-time artificial insemination

Table 1 - Chemical composition of whole rice bran, crude glycerin and herbage

Item	Whole rice bran	Crude glycerin	Herbage
Dry matter (g kg^{-1})	874	894	418
Ash (g kg^{-1} DM)	85	95	112
CP (g kg^{-1} DM)	149	7	84
NDF (g kg^{-1} DM)	185	-	691
ADF (g kg^{-1} DM)	61	-	320
EE (g kg^{-1} DM)	171	323	-
Glycerol (g kg^{-1} DM)	-	348	-
Methanol (g kg^{-1} DM)	-	224	-

DM - dry matter; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; EE - ether extract.

was the commercially recommended for suckled beef cows by the Laboratory Syntex S.A. (Buenos Aires, Argentina). Ten days after fixed-time artificial insemination, bulls were reintroduced with the cows for another 21 days.

All cows were managed as a single group during the entire experiment; they grazed together in the same pens of native grass, with forage availability greater than 2000 kg DM ha^{-1} (minimum: 2121±515, maximum: 6757±969 kg DM ha^{-1}). Every month, cows were weighed and forage availability was determined by the double sampling method (Haydock and Shaw, 1975) using a 50 cm × 50 cm square, with five points scale and two replicates, cutting the forage at ground level, and herbage allowance was estimated. Forage height was determined as described previously (Soca et al., 2007). The green/dry mass ratio was estimated by visual assessment in the sampling square. These determinations were performed before animals were placed in the pens. Forage height was always greater than 15 cm (minimum: 16±2, maximum: 26±4 cm). Green/dry mass ratio decreased towards the end of winter and increased in spring from 48/52 in August to 84/16 in November. The predominant species were *Axonopus* sp, *Paspalum dilatatum*, *Paspalum notatum*, *Paspalum quadrifarium*, *Stipa sp*, *Cynodon dactylon*, *Eryngium horridum*, and *Bothriochloa laguroides*. The average herbage allowance during the entire experiment was 24 kg DM (100 kg BW)$^{-1}$ [(minimum: 16, maximum: 37 kg DM (100 kg BW)$^{-1}$]. During supplementation, the cows remained in a paddock with a forage availability of 2121±515 kg DM ha^{-1}, 16±2 cm sward height, and 21 kg DM (100 kg BW)$^{-1}$ herbage allowance. Ten representative samples of herbage were taken and pooled for chemical composition analysis (Table 1).

Cow BCS was estimated by two trained technicians every 20 days from 16±1 weeks of pre-partum until calving and every 14 days from calving until the end of the first mating period. The correlation between technicians was 0.91, so the average of both values was used for the statistical analysis. Calf BW was recorded using an electronic scale (FX15, Iconix, Montevideo, Uruguay) at 47 (start of supplementation or day 0), 61 (beginning of temporary weaning with separation or day 14), 68 (end of temporary weaning with separation or day 21), and 82±1 (day 35) days of age and at definitive weaning (186±1 days of age or day 139).

Milk production was recorded on days 0, 14, 21, and 35 from the beginning of supplementation, using a portable milking machine according to the method described by Mondragon et al. (1983). In the morning, after cows received their meal, calves were separated and the udder

was emptied using 20 IU of oxytocin i/m (Neurofisin, Lab Fatro, Uruguay). Seven hours later, cows were milked again using the same methodology. The total milk was individually weighed on an electronic scale and 24 h production was estimated. On days 0 and 14, individual samples were taken and milk composition (fat and protein) was determined in the laboratory (COLAVECO; Colonia, Uruguay) using infrared radiation absorption.

Weekly, from day 0 to day 49, blood samples were collected by jugular venipuncture in Vacutainer® tubes with heparin (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Samples were centrifuged within the first hour of collection at 1530 g for 15 min and the plasma was collected and stored at –20 °C until processing.

Cows were monitored daily by a veterinary during the supplementation period and one week after. During this period, attention was especially paid to any observable change in behavior, eye alterations or impaired vision, changes in the respiratory rate and depth frequency of the chewing motion, or signs of hypoesthesia (Coppock and Christian, 2012). To evaluate possible hepatic damage due to ingestion of the methanol contained in the crude glycerin, another blood sample was collected at day 110 after the beginning of the supplementation using tubes without anticoagulant. Samples were immediately centrifuged, and the serum was frozen and transported to the laboratory. Liver function was studied through the concentrations of total protein, albumin, globulin, total bilirubin, aspartate amino transferase (ASAT), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT).

From day –9 to 49, the 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 size of the largest follicle was used to classify the type of anestrus. Cows with follicles >8 mm in diameter in two or more occasions without corpus luteum were considered in shallow anestrus and those with follicles ≤8 mm in diameter without corpus luteum were considered in deep anestrus. Resumption of ovarian activity was monitored by the concentration of progesterone (P_4), considering that cyclicity was reinitiated if a P_4 concentration ≥1 ng mL^{-1} was found in two successive samples with one week interval (Meikle et al., 2004) and a corpus luteum was identified in two ultrasound scans with a 7-day interval. Pregnancy was diagnosed by transrectal ultrasonography at 46 and 66 days after fixed-time artificial insemination.

Progesterone concentration was determined in all cows in samples collected on days 35, 42, and 49 from the beginning of supplementation. If a corpus luteum was observed by ultrasonography on any of those days, blood samples collected two weeks before and to two weeks after were also analyzed. The P_4 concentration was determined by solid phase radioimmunoassay using commercial kits (DPC, Diagnostic Products Co. Los Angeles, CA, USA). All samples were analyzed in one assay, with the standard curve and controls in duplicate and the samples in single. The assay sensitivity was 0.12 ng mL^{-1} and the intra-assay coefficients of variation for low (0.5 ng mL^{-1}), medium (2 ng mL^{-1}), and high (8 ng mL^{-1}) controls were 3.5, 2.6, and 2.2%, respectively.

Concentrations of insulin and metabolites were determined in samples from days 0, 7, 14, 21, and 28. Glucose, total protein, albumin, urea, cholesterol, and NEFA 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, respectively), with a sample volume and reagents adjusted to 96 cells and read in a Multiskan EX (Thermo Scientific, Waltham, Massachusetts, USA). The intra and inter-assay coefficients of variation for the high and low controls were less than 15%. Insulin concentration was determined by 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 1.1 uIU mL^{-1} and the intra-assay coefficients of variation for low (24.7 uUI mL^{-1}) and high (55.3 uIU mL^{-1}) controls were 4.9 and 5.1%, respectively.

The data were analyzed using SAS (Statistical Analysis System, version 9.2). The experiment was a completely randomized design and the individual cow was considered the experimental unit. Body condition score data were grouped in two different periods: monitoring phase (last third of gestation - beginning of supplementation) and experimental period (from the beginning of supplementation to the fixed-time artificial insemination). Data of milk production and composition, cow and calf weight, and concentrations of metabolites and insulin were analyzed using repeated measures analysis (MIXED procedure) with the date as the repeated factor. The following statistical model was applied:

$$Y_{ijk} = \mu + c_1 + T_i + D_j + TD_{ij} + E_{ijk},$$

in which μ = overall mean; c_1 = covariate with the initial value of the variable; T_i = effect of treatment; D_j = effect of date; TD_{ij} = effect of interaction between treatment and date; and E_{ijk} = residual error. Treatment, date, and the interaction between the two factors were included as fixed effect, and animal as the random effect. The first measures were used as covariates in the respective analysis. Data of BW of calves included the effects of sex, and birth weights were used as covariates. When the main effect was significant, the differences among means were analyzed using the Tukey-Kramer test.

Reproductive variables were analyzed using generalized model (GENMOD procedure) specifying the binomial distribution with logit transformation of the data (anestrus and pregnancy) or Poisson distribution (interval calving-conception). The model included treatment effects:

$$Y_{ij} = \mu + T_i + E_{ij},$$

in which: μ = overall mean; T_i = effect of treatment; and E_{ij} = residual error.

Correlation coefficients were estimated using the CORR procedure. Data were expressed as mean and standard error of the mean (Mean ± SEM) and considered statistically significant if P<0.05.

Results

During the monitoring phase, the BCS of the cows decreased (P<0.001) from 16±1 weeks pre-partum (early winter) to 47±1 DPP. Cows lost an average of 1.5±0.1 BCS units throughout this period, which corresponded to a loss of 1.2±0.1 units in the last gestation and 0.3±0.1 units in the postpartum period. The nadir of BCS was reached in the 4th week postpartum and remained low until the beginning of the supplementation period.

Supplementation did not influence the BCS (SUP: 3.9±0.1 vs. CON: 3.9±0.1; P = 0.257), and no interaction was found between supplementation and date (P = 0.540). Body weight was affected by supplementation (P = 0.044). Cows from the SUP group were heavier than cows from the CON group (SUP: 379±2 kg vs. CON: 373±2 kg; Table 2). Supplementation affected milk production (P = 0.017). Cows of the SUP group (5.7±0.2 kg d^{-1}) produced 14% more milk than the cows of the CON group (5.0±0.2 kg d^{-1}). The supplementation vs. date interaction was significant (P = 0.047, Table 2). Supplementation affected the BW of calves (P<0.001), and a treatment vs. date interaction was found (P<0.001). The calves from dams of the SUP group were heavier from days 14 to 35 than calves from CON group (Table 2). From days 0 to 14 of supplementation, while calves were suckling, they gained 0.26±0.07 kg d^{-1} more than the calves from CON dams (CON: 0.48±0.07 vs. SUP: 0.74±0.07 kg d^{-1}; P = 0.010). However, during

Table 2 - Body condition score (BCS), body weight (BW), and milk production of primiparous cows and BW of the calves whose mothers were not supplemented (CON) or supplemented for 21 days with whole rice bran and crude glycerin (SUP)

	BCS (1-8 scale)		Cow BW (kg)		Milk (kg d^{-1})		Calf BW (kg)	
	CON	SUP	CON	SUP	CON	SUP	CON	SUP
0	3.8±0.1	3.8±0.1	369±3	372±3	6.5±0.3	6.9±0.3	67±2	67±2
14	3.8±0.1	3.9±0.1	373±3b	382±3a	6.8±0.3b	8.1±0.3a	74±2b	78±2a
21	3.9±0.1	3.9±0.1	369±3	374±3	1.5±0.3	1.3±0.3	76±2b	80±2a
35	4.0±0.1	4.0±0.1	381±3	389±3	5.1±0.3b	6.3±0.3a	83±2b	88±2a
139[1]							142±2b	150±2a

Day 0 - start of supplementation at 47±1 days postpartum.
[1] Age at weaning: 186±1.4 days of age.
Means with different letters within rows differ (P<0.05) (overall mean ± standard error of the mean).

the temporary weaning with separation from their mothers, daily gain did not differ between groups (P = 0.380), and was lower (P<0.001) than in the previous period (0.20±0.05 kg d^{-1} for both groups). As expected, from days 0 to day 35, a positive correlation was found between BW gain of the calves and milk production of their dams (r = 0.34; P<0.001). At definitive weaning, calves from supplemented dams were on average 8 kg heavier (P = 0.029) than calves from CON dams (Table 2).

The supplement did not affect the milk fat content, expressed as a percentage (P = 0.225) or as the total content (P = 0.794). No effect of date (P = 0.127) or treatment vs. date interaction (P = 0.178) was found. The average fat percentage and total content was 3.0±0.1% and 216±10 g d^{-1}, respectively. On the contrary, supplementation increased (P<0.001) milk protein content (CON: 2.9±0.1 vs. SUP: 3.1±0.1%), and a treatment vs. date interaction was found (P<0.001). In cows from the SUP group, the milk protein content increased from days 0 to 14 (2.9±0.1 to 3.3±0.1%; P<0.001), while in cows of the CON group, it remained unchanged (2.9±0.1 to 2.9±0.1%; P = 0.820).

Supplementation did not affect plasma concentrations of total protein (SUP: 75.6±1.1 vs. CON: 76.3±1.0 g L^{-1}; P = 0.627), albumin (SUP: 33.0±0.6 vs. CON: 32.6±0.6 g L^{-1}; P = 0.619), or urea (SUP: 14.4±0.9 vs. CON: 15.3±0.9 mg dL^{-1}; P = 0.486). There was also no effect of the supplementation vs. date interaction on albumin (P = 0.687, Figure 1a) or total protein concentration (P = 0.578, Figure 1c); however, the urea concentration was affected by this interaction (P = 0.036). One week after the beginning of supplementation (day 7), cows in the SUP group had lower urea values (P = 0.012) than cows in the CON group, but these differences disappeared thereafter (Figure 1b).

Cows in the SUP group had higher plasma glucose concentrations than cows in the CON group (CON: 68.6±1.2 vs. SUP: 72.0±1.2 mg dL^{-1}; P = 0.028). Glucose concentration increased in SUP cows and differed (P = 0.001) from CON cows on day 28 (Figure 1e). The plasma cholesterol concentration

was higher (P = 0.029) in SUP (223.2±6.4 mg dL^{-1}) than in CON cows (202.1±6.4 mg dL^{-1}), and on day 14, SUP cows had the highest cholesterol concentration, differing from CON cows (P = 0.005; Figure 1g). Supplementation decreased (P<0.001) the concentration of NEFA (SUP: 0.45±0.02 vs. CON: 0.56±0.02 mmol L^{-1}). The concentration of NEFA in the SUP cows decreased (P = 0.017) and remained low from days 7 to 14 (Figure 1f).

Insulin concentration in SUP cows was higher (P = 0.011) than in CON cows (8.3±0.4 vs. 7.0±0.3 uIU mL^{-1}). Insulin increased and was higher (P = 0.004; Figure 1d) than the CON cows in the first seven days of supplementation.

Independently of the BCS at calving, all cows were in deep anestrus when supplementation began. Twenty-one days after the introduction of bulls (day 42 after the start of supplementation), 36% more cows of the SUP group were in shallow anestrus than cows of CON group (Table 3).

Table 3 - Percentage of primiparous cows not supplemented (CON) and supplemented for 21 days before mating with whole rice bran and crude glycerin (SUP), in shallow anestrus from the start of supplementation until 21 days of mating, pregnancy rates at 33 (early pregnancy), 43 (fixed-time artificial insemination pregnancy), and 74 (total pregnancy) days of mating, and calving - conception interval (days)

	Nutritional treatment		
	CON	SUP	P-value
Shallow anestrus	21 (3/14)	57 (8/14)	0.049
Early pregnancy	7 (1/14)	7 (1/14)	0.998
Fixed-time artificial insemination pregnancy	23 (3/13)	46 (6/13)	0.221
Total pregnancy	64 (9/14)	79 (11/14)	0.401
Calving-conception interval	108±5	110±5	0.770

Shallow anestrus was defined as the presence of follicles >8 mm in the absence of corpus luteum in two or more ultrasound studies at 7-day intervals. Cows that were in anestrus in the first 33 days of mating entered the fixed-time artificial insemination program. Ten days after fixed-time artificial insemination, cows were naturally rebred for 21 days. The entire mating period lasted 74 days. Numbers in brackets represent number of cows.
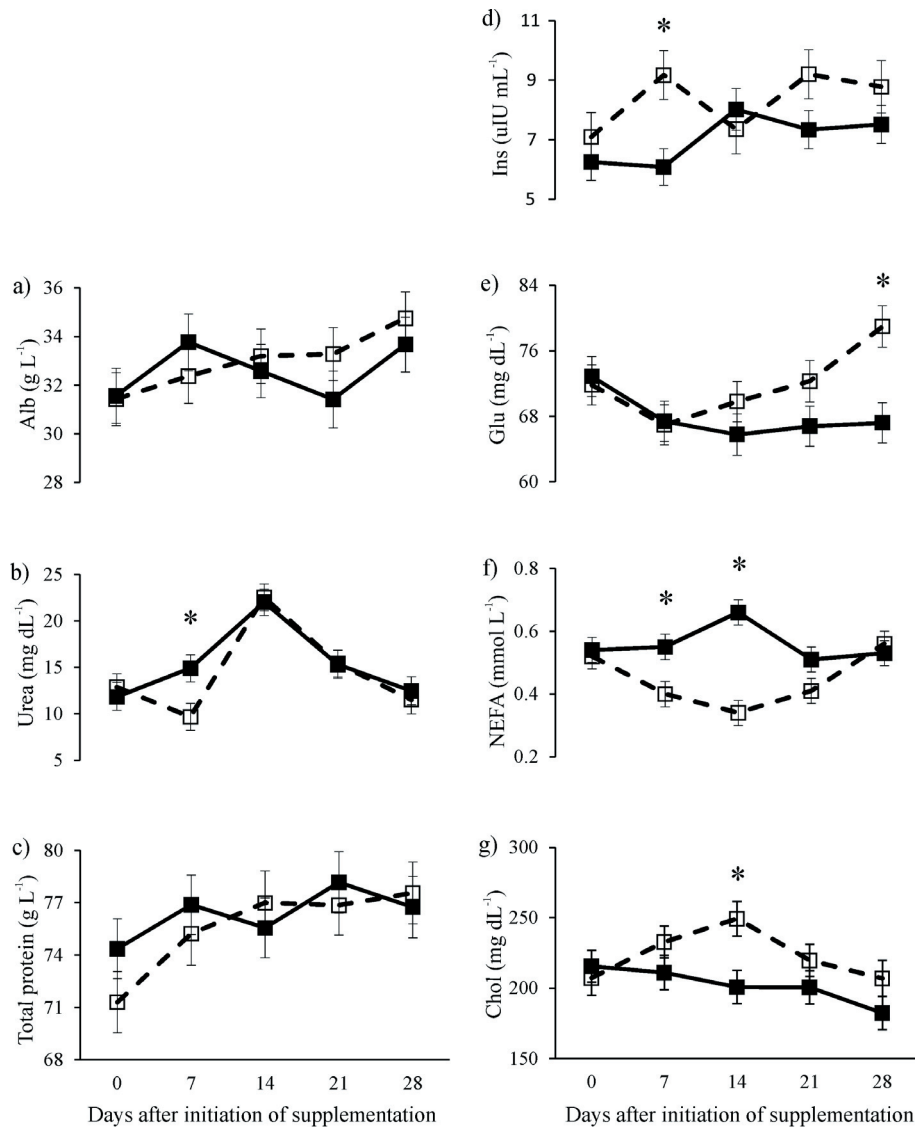
Day 0 - start of supplementation, at 47±1.4 days postpartum.
Differences between treatments are indicated by * when P<0.05.

Figure 1 - Concentrations of insulin and metabolites in primiparous cows non-supplemented (■) and supplemented for 21 days with whole rice bran and crude glycerin (□).

In the first 33 days of the mating period, only two cows — one of each group — were detected in estrus, and both became pregnant. The pregnancy rate after fixed-time artificial insemination in SUP cows was twice that of CON cows; however, this difference was not statistically significant (Table 3). After the second period with the bulls, more cows became pregnant (8/17; 47%), and no differences between treatments were found (P = 0.410). The final pregnancy rate was not different between groups. The type of anestrus on day 21 of the mating period influenced overall pregnancy rate (P = 0.026). Indeed, more cows showing shallow anestrus became pregnant (91%; 10/11) compared with cows showing deep anestrus (53%; 9/17).

No clinical signs of methanol intoxication were observed during the supplementation period or one week after. Hepatic functionality, evaluated by the concentrations of total protein, albumin, globulin, total bilirubin, ASAT, ALP, and GGT performed on all the cows 110 days after the start of supplementation, showed no sign of hepatic damage.

Discussion

In extensive grassland cow-calf systems, the last third of pregnancy and early post-partum occurs during winter months, when forage availability and quality are lower

than in fall or spring (Carámbula, 1991). Cows, in these conditions, show a negative energy balance and lose BCS (Soca et al., 2014a). As reported in other countries (Houghton et al., 1990; Perry et al., 1991; Stalker et al., 2006), and in our conditions (Quintans et al., 2010; Scarsi, 2012), in the present work, the loss of BCS was greater during pre-partum than early postpartum. As a consequence, BCS at calving was lower than those recommended (4.5 units; scale of 1-8) to obtain a pregnancy probability similar to or greater than 70% (Orcasberro et al., 1994).

The nadir of BCS was observed in the 4th week of the postpartum and remained low until supplementation began. The postpartum supplementation had no effect on BCS and stimulated only a transient increase in BW. The effect of the pre-mating supplementation on BCS is not consistent and seems to depend, at least partially, on the supplement used. Astessiano et al. (2013) and Soca et al. (2013) used a supplement based on whole rice bran and reported no effect on BCS. However, cows grazing on pasture improved with *Lotus subbiflorus* cv. Rincón, during the same period, increased BCS (Astessiano et al., 2012).

Supplementation increased milk production as has been reported previously in dairy (Reis and Combs, 2000; Bargo et al., 2002), dual purpose (Aguilar-Pérez et al., 2009), and beef cows (Perry et al., 1991; Lalman et al., 2000). The observed increase in milk protein content was also reported in dairy cows, and is attributed to a higher energy intake by cows supplemented with concentrates (Dillon et al., 1997; Reis and Combs, 2000; Bargo et al., 2002) and with crude glycerin (Bodarski et al., 2005). At the beginning of the supplementation, cows were in a physiological period (47±1.4 DPP) in which the mammary gland is prioritized in the partitioning of nutrients (Bauman and Currie, 1980), so the supplement increased milk production and calf growth.

Neville (1962) suggested that during the first 60 DPP, milk production and weight gain of calves are linked, and as calves begin to consume grass, this ratio decreases. The increase in the availability of milk with greater protein content for calves from the SUP cows determined an increase in their daily weight gain. Calf daily weight gain decreased during the temporary weaning with separation from their mothers and increased again after calves returned with them. These findings give major support to the concept that the development of calves is milk-dependent up to 90 days of age (Grings et al., 2008; Quintans et al., 2010). The daily gains observed after temporary weaning with separation are in agreement with those reported by other authors (Beal et al., 1990). Calf daily gains during the evaluated period (0.54 and 0.60 kg d^{-1}, CON and SUP groups, respectively) were similar to those reported by Quintans et al. (2010)

(0.65 kg d^{-1}), and Soca et al. (2014b) (0.50 kg d^{-1}). These authors worked with multiparous and primiparous grazing cows, respectively, with similar BCS to those of the cows used in the present work. The greater BW shown by calves from SUP cows during the supplementation period remained until the final weaning, which is in agreement with the results reported by Astessiano (2010). These results show that short-term supplementation before mating increases the productivity of primiparous cows.

Beef heifers in anestrus show the highest NEFA plasma concentrations, which reflect their negative energy balance (Bossis et al., 2000). The frequency of LH pulses is negatively correlated with plasma concentration of NEFA in primiparous suckling beef cows (Grimard et al., 1995). In addition, an increase in NEFA plasma concentration could have a negative effect on the ovarian function (Bossis et al., 1999). In the present work, the plasmatic concentration of NEFA was different between treatments, and reflected a different adipose tissue rate of lipolysis (Lucy, 2003). These results suggest that SUP cows had a better energy balance than CON cows. Moreover, plasma cholesterol concentration was greater in SUP than in CON cows. The plasma cholesterol concentration increases in supplemented dairy cows as a consequence of an increase in energy intake (Cavestany et al., 2005). In summary, in the present work, the plasma concentrations of NEFA, cholesterol, and glucose suggest an improvement in the energy balance of SUP cows (Bossis et al., 1999; Lucy, 2003).

A high energy intake increases the size of the follicles and the number of large follicles (diameter >10 mm) in beef cows (Perry et al., 1991; Aguilar-Pérez et al., 2009) and dairy cows (Lucy et al., 1991). It is possible that nutrition has a direct effect on the ovary, rather than an indirect effect via the hypothalamic-pituitary axis (Khireddine et al., 1998). At the beginning of supplementation, all cows were in deep anestrus, but 21 days after the mating period had begun, more SUP cows were in shallow anestrus than CON cows. Taking into account that one of the most important criteria to classify anestrus is the size of the follicle (Wiltbank et al., 2002), it is conceivable that the extra energy consumed by SUP cows had a stimulatory effect on folliculogenesis. However, either because the temporary weaning with separation failed to stimulate LH pulsatility, or because the supplement did not reach the levels required for this event to occur, the cows remained in anestrus. There is a positive correlation between the concentration of insulin and the reproductive response (Sinclair, 2008) and between insulin concentration and size of follicles (Khireddine et al., 1998). Although the number of SUP cows that became pregnant doubled the number of cows in the CON group, this

difference was not significant, possibly because of the low number of animals and the binomial nature of this variable. However, the better results obtained with cows in shallow than deep anestrus after fixed-time artificial insemination reinforces the positive effect of the supplement on the reproductive function (Khireddine et al., 1998). Considering that the cows were primiparous, it is possible to think that the energy partitioning followed the priorities described by Short et al. (1990), so after they achieved the maintenance requirements, milk production had the highest priority, followed by their own growth and reproduction activity had the last.

Cows of SUP group consumed 110 g of methanol d^{-1} during 21 days. During this period, no clinical sings that could be associated with methanol intolerance were observed. Moreover, the concentrations of total protein and albumin during the entire monitored period did not differ between groups, reflecting that liver synthesis of protein seemed not to be affected. The study of liver function at 110 days after the beginning of supplementation did not show impaired liver function. These findings are in agreement with those reported by Winsco et al. (2011), who infused 0 to 210 g of methanol day^{-1} directly into the rumen of steers and did not observe adverse effects on intake, digestion, or ruminal fermentation. These authors suggested that cattle could tolerate methanol consumption that largely exceeds the current recommendation of the United States Pharmacopeia (150 ppm) or the European Pharmacopeia (2000 ppm). In agreement, Dasari (2007) and Elam et al. (2008) suggested that maximum recommended levels of methanol should be revised; an issue that requires further research.

Conclusions

In primiparous beef cows grazing native grass, pre-mating supplementation with whole rice bran and crude glycerin with high content of methanol improves energy balance and increases milk yield and calf growth without showing signs of toxicity, with a slight effect on the reproductive activity.

Acknowledgments

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