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Metabolic and endocrine profiles of primiparous beef cows grazing native grassland. 2. Effects of body condition score at calving, type of suckling restriction and flushing on plasmatic and productive parameters

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Abstract. The objective of the present experiment was to analyse the effect of body condition score (BCS) at calving, type of suckling restriction and flushing on metabolic and endocrine profiles, and productive and reproductive responses of primiparous beef cows grazing native grassland. Primiparous beef (n = 56) cows in anestrus classified by BCS at calving (low $\leq$3.5 and moderate $\geq$4; 1–8 visual scale) were assigned randomly to four treatments in a two by two factorial arrangement of type of suckling restriction and flushing. Type of suckling restriction started at 55 $\pm$ 10 days postpartum (DPP $\pm$ s.e.m.; Day 0 = initiation of the treatment) and consisted of applying nose plates to calves for 12 days (i.e. TS treatment) or 5 days of isolation of the cow–calf pair, followed by applying nose plates to calves for 7 days as calves were reunited with their mothers (i.e. IS treatment). Immediately after the suckling restriction treatments were finished, the breeding season started, and each cow received (flushing group) or not (control group) 2 kg/day (fresh basis) of whole-rice middling for 22 days. The BCS was superior in moderate-BCS cows through the experiment. The type of suckling restriction did not affect any plasma parameter, but insulin-like growth factor I (IGF-I) concentrations increased in all cows during suckling restriction. Cholesterol concentration was affected by flushing $\times$ day interaction ($P < 0.05$), while insulin and IGF-I concentrations were affected by the interaction among BCS at calving, flushing and days ($P < 0.03$). Flushing increased cholesterol concentration in both BCS groups at calving, while insulin and IGF-I concentrations increased during flushing only in moderate-BCS cows. Suckling restriction, flushing, and BCS at calving did not affect calf weight or milk production. Moderate BCS cows had a shorter postpartum anestrous interval (PPI) (98 vs 123 DPP; $P < 0.01$). Isolated type of suckling reduced postpartum anestrous interval when compared with TS treatment (97 vs 115 DPP, $P < 0.05$). Early pregnancy rate was greater in flushed than in control cows (0.8 vs 0.55, $P < 0.01$) and in moderate-BCS cows than in low-BCS cows (0.84 vs 0.46, $P < 0.01$). Total pregnancy rate was also greater in flushed and moderate cows and tended to be affected by the interaction between flushing and BCS at calving ($P = 0.06$; flushed cows: moderate = 1 vs low = 0.5, $P < 0.08$; and control cows: moderate = 0.8 vs low = 0.4, $P < 0.09$). These results confirmed the great value of suckling restriction and flushing during post-calving and relevance of BCS at calving as a link between energetic nutrition and metabolic and reproductive processes in primiparous beef cows grazing native grassland.

Additional keywords: body condition at calving, flushing, metabolic response, suckling.

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Introduction

In grazing cow–calf systems, weaning rate and calf weight at weaning determine the economics of the enterprise and can be partly explained by the postpartum anestrous interval (PPI; Hess *et al*. 2005). Body condition score (BCS) at calving, as an indicator of maternal energy status, and suckling were the most important factors that controlled PPI, probability of pregnancy and weaning percentage of primiparous beef cows grazing native pastures (Quintans *et al*. 2010; Soca *et al*. 2013). The interaction between variability in forage production within and among years, and the high and constant stocking rate, would explain the inability to achieve an optimum BCS at calving in primiparous beef cows (5 in 1–8 visual scale, Vizcarra *et al*. 1986; Soca *et al*. 2013). This suggests that use of tactical interventions based on suckling restriction and/or increased cow energy intake postpartum could redistribute nutrients and energy so as to stimulate initiation of postpartum oestrous cycles and thereby improve pregnancy rates (Wettemann *et al*. 2003; Hess *et al*. 2005; Waterman and Butler 2010).

Suckling restriction with calf nose plates for 12 days reduced daily milk production, increased concentrations of glucose and insulin and follicle size and reduced PPI (Alvarez-Rodríguez *et al*. 2010; Quintans *et al*. 2010). Indeed, insulin and other hormones such as insulin-like growth factor I (IGF-I) are known to stimulate follicular growth (Spicer *et al*. 2002). Moreover, the isolation of the cow–calf pair has been postulated as a powerful neuroendocrine signal to improve the frequency and amplitude of luteinising hormone pulses and PPI (Stevenson *et al*. 1997). However, it is not known whether the type of suckling restriction (i.e. isolated calf–cow pair vs restricted suckling) affects the metabolic hormone concentrations that could explain differential PPI responses (Soca *et al*. 2013).

Supplementation with fatty acids (Lake *et al*. 2006) or gluconeogenic specific precursors (Mulliniks *et al*. 2008) for long periods improved reproductive efficiency and calf weight per cow exposed to breeding. Nevertheless, in grazing beef systems of South America, such medium- to long-term supplementation of beef cows is generally not economically viable (Soca *et al*. 2007). In beef cows, the effect of short-time energy supplementation (flushing) has been evaluated in a few studies and increases in follicular size, lifespan of corpus luteum, progesterone concentrations and early pregnancy rates have been reported (Khireddine *et al*. 1998; Soca *et al*. 2013).

Body condition score at calving has been proposed as an indicator of metabolic memory (Blanc *et al*. 2006), and has been associated with the length of PPI, conception and maintenance of pregnancy (Hess *et al*. 2005; Soca *et al*. 2013). Moreover, the effect of suckling control on IGF-I concentrations and PPI in primiparous beef cows was dependent on BCS at calving (Stagg *et al*. 1998). However, we could find no records on metabolite and metabolic hormone profiles associated with interventions based simultaneously on suckling restriction and flushing in primiparous beef cows grazing native pastures. We hypothesise that effects of the type of suckling restriction (i.e. isolated calf–cow pair vs restricted suckling) and flushing on PPI and pregnancy rates in primiparous cows are modulated by BCS at calving, and that the differential biological responses involve changes in metabolites and metabolic hormones.

The objective of the present experiment was to evaluate the type of suckling restriction and flushing with rice middling supplementation during 22 days at 55 ± 10 (days postpartum, DPP), on metabolite and endocrine profiles, and their relationships with PPI, probability of pregnancy rate, milk production and calf weight in primiparous cows of different BCS at calving, grazing native pastures.

Materials and methods

Location and pasture description

The experiment was carried out at the Experimental Station of the Faculty of Agronomy, Salto, Uruguay (31°23′S, 57°55′W), during spring and summer in 2005–2006. Cows and calves grazed native pasture on basaltic soils (Berretta *et al*. 2000). The most frequent pasture species found were *Paspalum notatum* (19%), *Axonopus affinis* (11%), *Botrhiochloa laguroides* (6%), *Paspalum dilatatum* (6%), *Coelorachis selloana* (6%), *Stipa setigera* (6%), *Piptochaetium montevidensis* (5%) and *Desmodium incanum* (4%). Forage quantity was determined by the double-sampling method (Haydock and Shaw 1975) at calving, and during the breeding period. Forage height was determined as described previously (Soca *et al*. 2007). In addition, representative pasture forage samples were collected and dried in a forced-air oven at 60°C, and ground to pass a 1-mm mesh Wiley mill. Forage samples were analysed in the Laboratory of Animal Nutrition (School of Agronomy, Montevideo, Uruguay) for DM, crude protein (CP), neutral detergent fibre (NDF) and ash (AOAC 2000). Forage quantity during late gestation, calving and breeding was 800 ± 130, 300 ± 200 and 700 ± 400 kgDM/ha and 3 ± 1, 2.0 ± 0.8 and 4.0 ± 0.7 cm, respectively. Chemical composition of forage was similar to that reported by Berretta *et al*. (2000).

Experimental design

All experimental procedures were approved by the Animal Experimentation Committee (CHEA) of the University of Uruguay (Montevideo). To evaluate effects of type of suckling restriction and flushing, primiparous Hereford cows (n = 56) with normal calving were used. Cows had 3.7 ± 0.4 units of BCS (visual scale 1–8, Vizcarra *et al*. 1986) and 365 ± 15 kg of bodyweight (BW) at calving. Cows were classified in moderate-BCS (≥4; n = 27) and low-BCS (≤3.5; n = 29) groups, according to their BCS at calving. The BCS at calving was not due to pre- or postpartum dietary treatments, because all cows were managed together in the same pasture throughout the experiment. Cows and their calves were randomly assigned, according to calving date, BCS at calving, and calf sex to treatments (i.e. type of suckling restriction and flushing; Soca *et al*. 2013) in a two by two factorial design. At the beginning of the treatment (Day 0 of the experiment = initiation of the suckling restriction at 55 ± 10 DPP), all cows were in anestrus as confirmed by blood progesterone concentrations.

The type of suckling restriction consisted of (1) temporary suckling control (TS) by applying nose plates to calves for 12 days without separation of the cow–calf pair, or (2) isolated temporary suckling control (IS) which consisted of complete isolation (>5000-m distance) of the cow–calf pair for 5 days, followed by applying nose plates to calves for 7 days when the cow–calf pair was reunited. During the 5 days of complete separation in the IS treatment, calves did not have visual or auditory contact with their mothers and remained in corrals with hay, supplement based in corn and barley grain (89% DM, 18% CP and 12.54 MJ/kg DM of metabolisable energy) and water *ad libitum*. The TS treatments concluded at 67 ± 10 DPP, and cows were then assigned to nutritional treatments consisting of (1) *flushing group* in which cows were offered 2 kg/day (fresh basis) of whole rice bran (86.5% DM, 13.5% CP, 44% NDF and 13.5% ether extract) for 22 days (from Day 12 to Day 34 of the experiment), or (2) *control group* which received no supplementation. During flushing, both groups grazed different paddocks of the same pastures and the supplement was offered in feeders with individual separations of 100 cm. Individual intake was checked by observing each cow for the entire session of supplementation (15 min). On the same day, the nutritional treatments were initiated; two bulls per group with proven fertility (McGowan *et al*. 1995) were placed and rotated with the cows during 83 days.

Animal measurements

Cow BCS (visual scale 1–8, Vizcarra *et al*. 1986) was determined every 20 days from Day 0 to Day 150 DPP by one trained observer. Changes in BCS between calving and the day of BCS nadir and between calving and the start of the breeding (ΔBCS) were calculated. Six cows of each treatment were selected for recording milk production on Days 0, 12, 22 and 50 by the weigh–suckle–weigh method, with a total separation of the cow–calf pair to 12 h (Williams *et al*. 1979). Pregnancy diagnoses were performed at 42 days (early pregnancy rate) after the introduction of the bulls and were confirmed at 30 days (total pregnancy rate) after bull withdrawal, using a linear array real time ultrasound scanner with 5 MHz rectal transducers (Aloka SSD-500, Hitachi Aloka Medical, Tokyo, Japan). Identification of a sharply demarcated black area with an ecogenic picture of an embryo within the uterus and/or observation of the heart beat were used as criteria for pregnancy (sensitivity of 94.8%, specificity of 95.3%, positive predictive value 97.7% and negative predictive value 89.8%; Taverne *et al*. 1985).

Calves were weighed at birth, at 73 ± 10 days of age (Day 18 of experiment), and at definitive weaning at 188 ± 10 days of age (Day 123 of experiment) without fasting, and average daily gain (ADG) was calculated.

Blood sampling and metabolite and hormone determinations

Blood samples were collected weekly with vacutainer tubes with lithium heparin from 40 to 100 DPP, refrigerated at 5°C until centrifuged (2000*g*, 15 min) within 2 h post-sampling and plasma aliquots were stored at −20°C until analyses. All assays were performed in the Laboratory of Nuclear Techniques (Veterinary Faculty, Montevideo, Uruguay).

Progesterone (P4) concentrations were determined by a direct solid-phase radioimmunoassay (RIA) by using a commercial kit (Diagnostic Product Co., Los Angeles, CA, USA). The RIA had a sensitivity of 0.1 ng/mL. Intra-assay CVs for low (0.8 ng/mL), medium (2.2 ng/mL) and high (8 ng/mL) controls were 6.6%, 6.4% and 6.3%, respectively. The inter-assay CVs for the same controls were 9.8%, 6.4% and 6.1%, respectively. The PPI was defined as the interval from calving to the day of the first of two consecutive weekly blood samples with progesterone ≥1 ng/mL.

Cortisol concentrations were determined by a direct solid-phase RIA using a commercial kit (Diagnostic Product Co.). The RIA had a sensitivity of 0.51 μg/dL. The intra-assay CVs for low (1.5 μg/dL), medium (5.5 μg/dL) and high (13.0 μg/dL) controls were 13.7%, 9.1% and 8.2%, respectively.

Cholesterol, insulin and IGF-I were determined as described in the companion paper (Soca *et al*. 2014).

Statistical analyses

All data were analysed as a completely randomised design by using the SAS Systems program (SAS Institute, Cary, NC, USA). Metabolic, endocrine profiles, BCS evolution, milk production and calf weight were analysed by repeated measures using the MIXED procedure, with days as the repeated effect and first-order autoregressive as the covariance structure and the degrees of freedom were adjusted by the Kenward–Rogers method. The model included BCS at calving, type of suckling restriction, flushing, days, and all first-, second- and third-order interactions as fixed effects. For calf-weight analysis, sex was also included as a fixed effect and age as a covariable. Initial analyses included all possible interactions, but only significant ($P < 0.05$) components were retained in the model. Least square means were compared using the Tukey–Kramer test. Probability of early and total pregnancy rate (number of cows pregnant/total cows) was fitted using the GLIMMIX procedure with the binomial distribution and the logit as the link function specified. The effects of BCS at calving, type of suckling restriction and flushing as fixed effects on PPI were analysed by lineal model (GLM procedure, SAS Institute). Variables that better explained the PPI variations were studied using the stepwise REG procedure.

Results

Body condition score

Evolution of BCS was affected by BCS at calving ($P < 0.01$), days ($P < 0.01$) and the interaction between BCS and days ($P < 0.06$), but not by the type of suckling restriction, flushing or their interaction. The BCS of moderate-BCS cows was greater ($P < 0.01$) than that of low-BCS cows throughout the experimental period, with a maximum difference of BCS between groups observed at Day 150 (end of the experimental period; Fig. 1).

Plasma cholesterol and hormone determination

The effects of BCS at calving, type of suckling restriction, flushing and their interaction on the metabolic and endocrine responses are shown in Table 1. All variables were affected by days and/or the interaction between BCS at calving

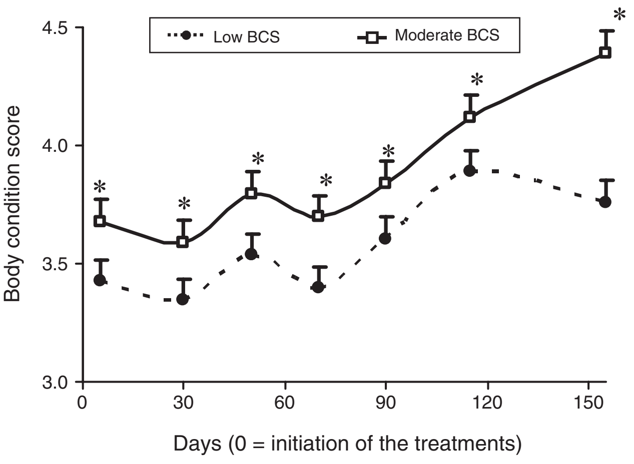

Fig. 1. Evolution of body condition score (BCS) from calving before, during and after suckling-restriction and flushing treatment in cows calving with moderate (≥ 4; $n = 27$) or low (≤ 3.5 $n = 29$) BCS (least square means ± s.e. of estimation).

and days. The type of suckling restriction or its interaction with days did not affect any variable, while the interaction between flushing and days affected cholesterol concentrations and the triple interaction including BCS at calving, flushing and days affected insulin and IGF-I concentrations.

Table 1. *F*-tests of fixed effects included in the model for metabolites and hormones in primiparous beef cows under grazing conditions
Fixed effects were body condition score (BCS) at calving, suckling restriction (S), flushing (F), days and interactions. IGF-I, insulin-like growth factor I

Parameter	F	S	Days	BCS	BCS × days	F × days	BCS × F × days
Cortisol	0.5	0.36	<0.01	0.76	0.48	0.38	0.25
Cholesterol	0.27	0.92	<0.01	0.07	0.94	<0.01	0.93
Insulin	0.28	0.71	<0.01	<0.01	0.03	0.12	0.04
IGF-I	0.07	0.96	<0.01	<0.01	<0.01	0.62	<0.01

In both BCS groups, cholesterol concentrations increased during flushing (Day 30, 85 DPP) and decreased immediately after (P < 0.01) (Fig. 2*a*, *b*). No difference in cholesterol concentrations between control and flushed groups could be observed 2 weeks after the flushing treatment finished (Day 48).

Insulin concentrations were greater in moderate- than in low-BCS cows (10.6 ± 0.8 vs 7.5 ± 0.8 µIU/mL, respectively). During flushing, insulin concentrations increased in moderate- but not than in low-BCS cows (Fig. 2*c*, *d*). Insulin concentrations decreased immediately after the flushing finished.

Concentrations of IGF-I were greater in moderate- than in low-BCS cows (70 ± 4 vs 46 ± 3 ng/mL, respectively). Plasma IGF-I increased during the suckling restriction, decreasing again after

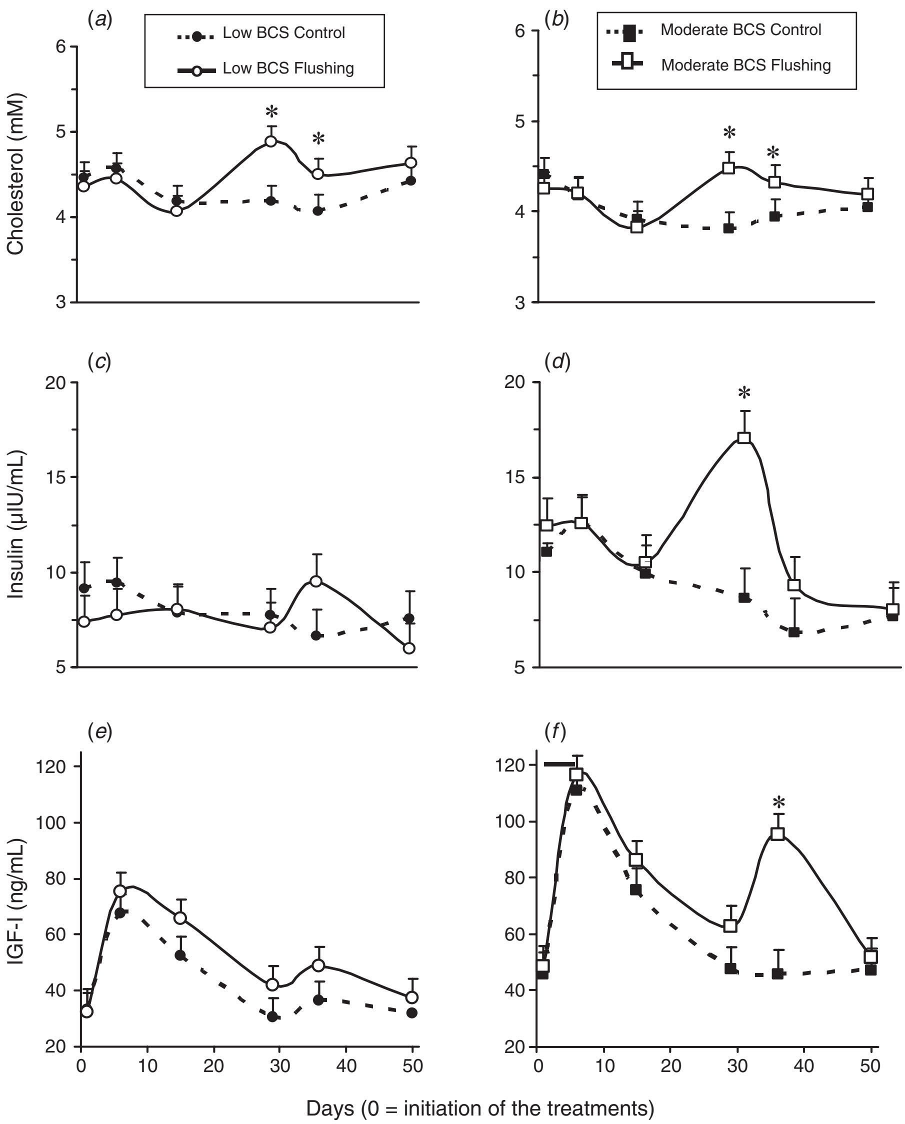

Fig. 2. (*a*, *b*) Plasma cholesterol, (*c*, *d*) insulin and (*e*, *f*) insulin-like growth factor I concentrations before, during and after suckling-restriction and flushing treatment in cows with moderate (≥4; n = 27, *a*, *c*, *e*) or low (≤3.5, n = 29, *b*, *d*, *f*) body condition score at calving (least square means ± s.e. of estimation).

suckling restriction treatments ended (Fig. 2*e*, *f*). A 2–3-fold increase in IGF-I concentrations was observed immediately after the flushing treatment ended in moderate-, but not in low-BCS flushed cows (Fig. 2*e*, *f*). This IGF-I increase was observed later than the insulin increase in moderate-BCS flushed cows.

Cortisol concentrations decreased from Day 0 to Day 15 (from 2.2 $\pm$ 0.13 to 1.5 $\pm$ 0.12 μg/dL) and remained low thereafter until Day 50 (data not shown). No differences among groups were detected.

Calf weight and milk production

Cow BCS at calving, type of suckling restriction, flushing and their interactions did not affect calf weaning weight, average daily gain or cow milk production (Table 2). Milk production was affected by days ($P < 0.01$) as decreased by 33% during suckling restriction (from Day 0 to Day 12, 4.9 $\pm$ 0.2 to 3.3 $\pm$ 0.2 L/cow.day) and increased again at Days 22 and 50 (4.0 $\pm$ 0.1 and 4.9 $\pm$ 0.3 L/cow.day, respectively).

Reproductive responses

At the end of the suckling-restriction treatments (Day 12, 67 DPP) and 2 weeks after the flushing (Day 50, 105 DPP), 13% and 43% of total cows were cycling, respectively. Cow BCS at calving, ΔBCS, the interaction among type of suckling restriction and BCS at calving and IGF-I concentration at the initiation of the experiment (Day 0) explained 75% of the variation in PPI. Moderate-BCS cows had reduced PPI when compared with low-BCS cows (Table 2). For every unit of improvement in BCS at calving and ΔBCS, PPI was reduced ($P < 0.01$) by 46 $\pm$ 9 and 23 $\pm$ 5 days, respectively. The IS reduced PPI when compared with TS (Table 2). Concentrations of IGF-I at Day 0 were greater in cycling than non-cycling cows at Day 50 (45 vs 33 $\pm$ 2.5 ng/mL, respectively, $P < 0.01$).

The BCS at calving and flushing affected early and total pregnancy rates but the type of suckling restriction and its interaction with flushing did not affect them (Table 2). Early and total pregnancy rates were greater in moderate- than low-BCS cows. Early pregnancy rate tended to be greater while total pregnancy rate was greater in flushed than in control cows.

Discussion

The present study has shown that the interventions used (suckling restriction and flushing) were novel, short-duration and low-cost signals to improve the metabolic status of primiparous anestrous beef cows with suboptimal BCS (<5, scale 1–8, Soca *et al*. 2013). Suckling restriction, independently of its type (TS or IS) or BCS at calving, reduced milk production and increased IGF-I concentrations. However, BCS at calving modulated plasma insulin and IGF-I increases during or after flushing. Observed changes in the endocrine milieu were consistent with known effects of insulin and IGF-I on ovarian function (Spicer *et al*. 2002), thereby contributing to the reduction in PPI. Neither suckling restriction nor flushing modified BCS, since it was maintained from Day 0 to Day 90 of the experiment, which could reflect a steady-state in cow energy balance. This could be explained by the difficulty of primiparous beef cows grazing swards with low forage height to increase forage intake and fulfil their requirements. The increase in cow BCS from Day 90 to Day 150 could reflect the improvement in pasture quantity as discussed previously (Soca *et al*. 2013). The greater BCS in moderate BCS cows was consistent with their greater insulin and IGF-I concentrations throughout the experiment, reflecting their better metabolic status. The upregulation of hormone levels could have been in response to increased body reserves and/or increased DM intake (Blanc *et al*. 2006; Waterman and Butler 2010; Scarlato *et al*. 2011). Moreover, moderate-BCS cows of the present experiment presented greater insulin and IGF-I and lower non-esterified fatty acid concentrations before calving (Soca *et al*. 2014).

Both types of suckling restriction limited calf suckling and contributed to explain the reduction in milk production, as reported previously (Alvarez-Rodríguez *et al*. 2010; Quintans *et al*. 2010). The reduction in milk production was associated with an immediate increase (at least two-fold) in IGF-I concentrations. Indeed, lactation is often accompanied with low IGF-I concentrations (consequence of growth hormone–IGF-I axis uncoupling, Rhoads *et al*. 2004), which has been reported as a strategy for nutrient prioritisation for the mammary gland. Thus, IGF-I increase during suckling restriction is

Table 2. Effect of suckling restriction (S), flushing (F) and body condition score (BCS) at calving on productive and reproductive responses in primiparous beef cows (estimated values)

S, suckling restriction; IS, isolated calf–cow pair for 5 days and suckling control with nose plates for 7 days; TS, temporary suckling control with calf nose plates during 12 days while calves remained with their mothers; F, flushing; NF, control; L, low body condition at calving (≤3.5); M, moderate (≥4); EP, probability of early pregnancy; TP, probability of total pregnancy; PPI, postpartum anestrous interval. Interactions between main fixed effects (ST = suckling treatment; F = flushing treatment) were not significant ($P > 0.34$)

Parameter	Effects									*P*-value		
	S			F			BCS			S	F	BCS
	IS	TS	s.e	F	NF	s.e	L	M	s.e			
Productive response												
Milk production (kg/day)	4.00	3.80	0.40	3.80	3.70	0.30	3.70	4.00	0.30	0.40	0.30	0.20
Daily weight gain (kg/day)	0.50	0.60	0.08	0.45	0.50	0.08	0.50	0.60	0.07	0.30	0.20	0.10
Calf weaning weight (kg)	156	158	4.00	153	161	5.00	151	158	8.00	0.40	0.50	0.10
Reproductive response												
PPI (days)	97	115	5.0	106	106	4.0	94	119	3.00	0.01	0.87	0.01
EP	0.7	0.6	0.08	0.8	0.6	0.08	0.50	0.80	0.06	0.77	0.08	0.01
TP	0.9	0.9	0.05	1.00	0.6	0.05	0.50	1.00	0.06	0.73	0.02	0.01

consistent with the reduction in energy requirements and similar findings were reported within 5 days of suckling restriction, with or without calf isolation (Stagg *et al*. 1998). However, suckling restriction did not affect insulin concentrations in contrast to previous experiments in multiparous cows (Quintans *et al*. 2010). This could be due to the important restriction in forage quantity (energy intake) in the present study and/or limited ability of the primiparous cow to increase intake (Blanc *et al*. 2006). This increment in IGF-I without changes in insulin is inconsistent with previous reports in dairy cows (Rhoads *et al*. 2004), implicating the importance of other mechanisms controlling IGF-I secretion as discussed previously (Waterman and Butler 2010).

The flushing with rice middling increased cholesterol concentrations in both BCS groups, probably due to fat percentage and/or fatty acid composition of this supplement, as reported previously (Williams and Stanko 2000). However, the flushing effect on insulin and IGF-I profiles was dependent on BCS at calving, since no increases were observed in low-BCS cows. The increases found in moderate-BCS flushed cows were similar to the rapid increase in insulin concentrations observed after glucose infusion in beef cows with better BCS (Vieira *et al*. 2012). Moreover, greater glucose and leptin concentrations and a differential hepatic gene expression of enzymes that regulate energy metabolism in cows with greater BCS have also been postulated to explain, at least partially, the better metabolic status (Vizcarra *et al*. 1998; León *et al*. 2004; Astessiano *et al*. 2012). The differences in the patterns of metabolic response to the flushing treatment in moderate- vs low-BCS cows may reflect the influence of the metabolic memory. For example, it has been shown in sheep that insulin and IGF-I responses to the same nutritional treatment depended on body reserves (Fernández-Foren *et al*. 2011). This suggests that the pancreas, liver and adipose tissue control harmonically the metabolic flux, as well as regulating the endocrine activity of each other (Yoshida *et al*. 2007). In addition, greater cow body reserves could have improved feed (forage and supplement) utilisation efficiency and/or reduced grazing costs (by modifying cow grazing strategy) as suggested previously (Dhuyvetter and Caton 1996), thus, explaining also plasma insulin and IGF-I increases found only in moderate cows.

Milk production and calf weight were not affected by flushing, in contrast with a previous report from our group when improved pastures were used as the flushing feed and no suckling restriction was applied (Astessiano *et al*. 2012). Although no effect of tactical interventions (suckling restriction and flushing) and BCS at calving was observed on productive parameters, the reproductive indicators were affected by them.

The IS reduced the PPI in 20 days when compared with TS control, as previously reported (Soca *et al*. 2013). Indeed, Stagg *et al*. (1998) reported that isolated suckling-restricted cows increased follicle size and luteinising hormone pulses when compared with non-isolated suckling-restriction treatment, which, in interaction with greater IGF-I concentrations, promotes ovulation. Even if IGF-I concentrations increased during the suckling-restriction period, neither IGF-I nor cortisol were affected by the type of suckling restriction; thus, other neuroendocrine mechanisms may be involved in the effect of type of suckling on PPI as proposed (Waterman and Butler 2010). The reduction in PPI and greater early and total pregnancy rates in moderate- than in low-BCS cows found is consistent with the concept that the brain integrates both information of body reserves (metabolic memory) and signals from the current energy intake to withstand future environmental challenges to the reproductive cycle (Blanc *et al*. 2006). However, the BCS of the moderate-BCS cows was also lower than the optimum (Soca *et al*. 2013), which would further emphasise the importance of suckling restriction and flushing to improve the metabolic status and reproductive performance.

Although the number of animals to evaluate pregnancy responses is limited, flushing improved early and total probability of pregnancy rates, as previously reported in a larger number of animals (Soca *et al*. 2013). We did not find any report of short-term flushing on pregnancy rates, but long-term supplementation with gluconeogenic precursors and fat improved pregnancy rates (Hess *et al*. 2005). Nutritional strategies that increase P4 concentrations in cattle before or after breeding have been positively associated with pregnancy rates, given that P4 is required for proper establishment and maintenance of pregnancy (Khireddine *et al*. 1998; Cooke *et al*. 2012). This is consistent with the greater concentrations of the P4 precursor – cholesterol – found in flushed cows, regardless of their BCS at calving. However, 100% vs 50% of moderate- and low-BCS cows, respectively, were pregnant at the end of the breeding season, which cannot be explained by cholesterol concentrations. The insulin and IGF-I increases found only in moderate-BCS cows could explain this better fertility in flushed cows (Soca *et al*. 2013), as has been previously reported (Sinclair *et al*. 2002). Indeed, insulin and IGF-I are involved in embryo growth and implantation (Cooke *et al*. 2012).

Conclusions

Our data suggest that in primiparous beef heifers grazing native pastures, body energy reserves significantly modulate reproductive responses to the effects of suckling restriction and flushing on nutrient availability and metabolic and endocrine status.

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