BRUNA LIMA CHECHIN CATUSSI

Prepartum and/or postpartum supplementation with low-moisture molasses blocks to optimize fertility and calf performance in primiparous beef cows

São Paulo

2021

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Dissertation submitted to the Postgraduate Program in Animal Reproduction of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Master's degree in Sciences.

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Advisor:

Prof. Dr. Pietro Sampaio Baruselli

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CERTIFICADO

Certificamos que a proposta intitulada "Suplementação no pré-parto e / ou pós-parto com blocos para otimizar a fertilidade e o desempenho dos bezerros de primíparas de corte.", protocolada sob o CEUA nº 8169050819 (ID 006865), sob a responsabilidade de **Pietro Sampaio Baruselli** *e equipe; Bruna Lima Chechin Catussi* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 14/08/2019.

We certify that the proposal "Prepartum and/or Postpartum supplementation with low-moisture molasses blocks to optimize fertility and calf performance in primiparous beef cows.", utilizing 840 Bovines (males and females), protocol number CEUA 8169050819 (ID 006865), under the responsibility of **Pietro Sampaio Baruselli** *and team; Bruna Lima Chechin Catussi* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 08/14/2019.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 11/2019 a 05/2020 Área: Reprodução Animal

Origem:	Animais de proprietários						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	28 a 32 meses	N:	420
Linhagem:	Nelore			Peso:	300 a 450 kg		
Origem:	Animais de proprietários						
Espécie:	Bovinos	sexo:	Machos e Fêmeas	idade:	0 a 8 meses	N:	420
Linhagem:	Nelore			Peso:	30 a 250 kg		

Local do experimento: O experimento será realizado nos piquetes com pastagem natural. E os manejos reprodutivos, pesagem dos animais, coleta sanguínea e ultrassonografia de carcaça serão realizados no tronco de contenção individual, em um curral de manejo da própria fazenda.

Comentário da CEUA: Sem óbice ético para realização do exeprimento.

São Paulo, 12 de março de 2021

Prof. Dr. Marcelo Bahia Labruna
Coordenador da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Camilla Mota Mendes
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

EVALUATION FORM

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Date: _____/_____/_____

Committee Members

Prof.____

Institution: ______________________________Decision: _________________

Prof.____

Institution: ______________________________Decision: ________________

Prof.____

Institution: ______________________________Decision: ________________

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RESUMO

CATUSSI, B. L. C. **Suplementação com blocos no pré e/ou pós-parto para otimizar a fertilidade e o desempenho dos bezerros de primíparas de corte**. 2021. 67 f. Dissertação (Mestrado em ciências), Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2021.

O manejo nutricional do rebanho bovino é fator determinante para o sistema de produção na pecuária de corte, com impactos diretos no metabolismo e nas funções reprodutivas. Com o objetivo de estudar estratégias de suplementação no pré e/ou pós-parto foram utilizadas 417 novilhas Nelores gestantes. Os efeitos da suplementação semanal com blocos multinutricionais (à base de melaço e monensina) durante o pré (90 dias antes do parto; D-90) e/ou pós-parto (120 dias após o parto) foram avaliados sobre o desempenho reprodutivo da primípara e de sua progênie. As novilhas foram alocadas aleatoriamente em quatro grupos experimentais: 1) Grupo CC: as novilhas receberam suplemento mineral convencional em pó (0,06% do PV) oferecido diariamente (suplementação controle; C) antes e após o parto (n = 108); 2) Grupo CB: Novilhas receberam C antes do parto e suplemento mineral proteico em blocos (0,07% do PV) oferecido semanalmente (suplementação em bloco; B) após o parto (n = 117); 3) Grupo BC: recebeu B antes e C após o parto (n = 103) e 4) Grupo BB: recebeu B antes e B após o parto (n = 89). Durante os períodos pré e pós-parto parâmetros produtivos [peso corporal (PC); escore de condição corporal (ECC); espessura de gordura subcutânea na costela (EGSU) e na garupa (EGP); e peso corporal dos bezerros (PCB)] foram avaliados. Amostras de sangue foram coletadas em um subgrupo de animais (n= 120) no D-90, D40 e D80 para análise hormonal e metabólica (D0=parto). Todos os animais foram sincronizados para IATF usando protocolo a base de estradiol/progesterona no D40 e D80 (não gestantes à 1ª IATF). O diagnóstico de gestação foi realizado 30 dias após IATF e 30 dias após o final da estação de monta. Todos os dados foram analisados por contrastes ortogonais [C1: Controle *vs.* suplementação com blocos (CC *vs.* BB + BC + CB); C2: B em ambos os períodos (pré e pós-parto) *vs.* B em um dos períodos (pré ou pós-parto; BB *vs.* BC + CB); C3: B apenas durante o pré-parto *vs.* B apenas durante o pós-parto (BC *vs.* CB). A suplementação com blocos (C1) aumentou a taxa de prenhez à primeira IATF ($P = 0,04$) e a taxa prenhez final ($P = 0,05$). Houve interação tempo*PC ($P<0,0001$) e tempo*ECC ($P<0,0001$). Vacas suplementadas apresentaram maior PC somente no D40 ($P=0,03$) e ECC somente no parto (D0; $P= 0,04$) e no D40 ($P=0,02$). Além disso, vacas suplementadas tiveram maior EGSU ($P = 0,03$) e EGP ($P = 0,03$) no D40. As concentrações de insulina (P=0,008) foram maiores para vacas suplementadas. Houve interação tempo*glicose (P = 0,0002). As concentrações de glicose foram maiores somente no D40 nas vacas suplementadas. Foi verificado interação tempo*PCB (P<0,0001). O PCB foi superior para bezerros nascidos de vacas suplementadas aos 80 (P=0,03) e aos 120 (P<0,001) dias de idade. Entretanto, não foram verificadas diferenças aos 170 dias (P=0,55) e ao desmame (P=0,38). Embora não houve diferenças no desempenho reprodutivo no C2 (P> 0,15), o PCB foi maior para bezerros nascidos de vacas suplementadas antes e após o parto aos 80 (P<0,001), 120 (P<0,001), 170 (P=0,002) e 210 (P=0,02) dias de vida. Apesar de nenhuma diferença reprodutiva observada no C3 (P>0,8), o ECC ao parto foi maior para vacas suplementadas apenas no pré-parto (P<0,001) e no D170 foi maior para vacas suplementadas apenas durante o pós-parto (P<0,001). Os bezerros de vacas suplementadas apenas durante o pós-parto foram mais pesados somente aos 120 dias de idade (P=0,002). Em resumo, independentemente do período de tratamento, a suplementação com blocos aumentou a taxa de prenhez à primeira IATF e a taxa de prenhez final. Adicionalmente, a suplementação com blocos durante os períodos pré e pós-parto melhorou o ganho de peso da progênie até o desmame. A suplementação com blocos pode ser uma ferramenta para otimizar a fertilidade e o desempenho dos bezerros de primíparas Nelore.

Palavras-chave: Nutrição; Bovinos de corte; reprodução; inseminação artificial

ABSTRACT

CATUSSI, B. L. C. **Prepartum and/or postpartum supplementation with low-moisture molasses blocks to optimize fertility and calf performance in primiparous beef cows.** 2021. 67 f. Dissertação (Mestrado em ciências), Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2021.

Nutritional management of the herd is a determining factor for the production system in beef cattle, as the nutritional and metabolic state of the animal directly affects their reproductive functions. In order to study supplementation strategies, 417 pregnant Nelore heifers were used to evaluate the effects of block supplementation (offered weekly) during pre (90 days before calving: D-90) and/or postpartum (120 days after calving) on reproductive and progeny performance. Heifers were randomly allocated in four experimental groups: 1) Group CC: heifers received mineral supplement in loose meal form (0.06% of BW) offered daily (control supplementation; C) before and after parturition (n=108); 2) Group CB: received C before parturition and mineral protein supplement in block form (0.07% of BW) offered weekly (block supplementation; B) after parturition (n=117); 3) Group BC: received B before and C after parturition (n=103) and 4) Group BB: received B before and B after parturition (n=89). During pre and postpartum periods, the performance [body weight (BW); body condition score (BCS); subcutaneous backfat thickness (BFAT); rump fat thickness (RFAT), and calf body weight (CW)] were evaluated. Blood samples were collected on a subset of animals (n=120) at D-90, D40 and D80 for metabolites and hormonal analysis (D0=parturition). All animals were synchronized for FTAI using estradiol/progesterone-based protocol at D40 and D80 (non-pregnant at 1st FTAI). Pregnancy diagnosis was performed 30 days after FTAI and 30 days after the end of the breeding season (BS). All data was analyzed by orthogonal contrasts [C1: Control *vs.* block supplementation (CC *vs.* BB+BC+CB); C2: B in both periods (pre and postpartum) *vs.* B in one of the periods (pre or postpartum; BB *vs.* BC+CB); C3: B only during prepartum *vs.* B only during postpartum (BC *vs.* CB). Block supplementation (C1) increased pregnancy at first FTAI (P=0.04) and overall pregnancy rate (P=0.05). There was interaction time*treatment for BW (P<0.0001) and BCS (P>0.0001). Supplemented cows had greater BCS only at parturition (D0; P=0.04)) and at D40 (P=0.02) and greater BW only at D40. Also, supplemented cows had greater BFAT (P=0.03) and RFAT (P=0.03) at D40. There was interaction time*treatment for glucose concentrations (P=0.0002), which were higher for supplemented cows only at D40 (P=0.01).

Insulin concentrations were higher for supplemented cows ($P=0.008$). Furthermore, time*treatment interaction for CW ($P<0.0001$) was observed. CW was superior for calves born by supplemented cows at 80 and 120 days old ($P\leq0.03$), but not at D170 ($P=0.55$) and at weaning ($P=0.38$). Although no differences in reproductive performance in C2 ($P>0.15$), the CW was higher for calves born by cows supplemented before and after parturition at 80 ($P<0.001$), 120 ($P<0.001$), 170 ($P=0.002$) and 210 ($P=0.02$) days old. No reproductive differences were observed in C3 ($P>0.8$). Nevertheless, BCS at parturition was greater for cows supplemented only during prepartum ($P<0.001$). Furthermore, BCS at D170 was greater for cows supplemented only during postpartum ($P=0.001$). Calves born by supplemented cows only during postpartum were heavier only at 120 days old ($P=0.002$). In summary, regardless of period of treatment, block supplementation increased pregnancy at first FTAI and overall pregnancy rate. Additionality, block supplementation during both pre and postpartum periods improved progeny weight gain until weaning. Block supplementation can be a tool to optimize fertility and calf performance in Nelore primiparous cows.

Keywords: Nutrition; beef cattle; reproduction; artificial insemination

FIGURE LIST

TABLE LIST

SUMMARY

1. GENERAL INTRODUCTION

The demand for food is increasing due to the accelerated growth of the global population. Thus, there is a need for the expansion of sustainable meet production (FAO, 2017). In this scenario, Brazil has a distinguished position in the global beef industry as it possesses vast extensive territory as well as an expansive capacity for large-scale food production.

According to ABIEC (2020), the number of bovines slaughters in Brazil reached 43.3 million, with an estimated production of 10.49 million tons of carcass equivalent, representing 14.8% of world meat production in 2019. Despite this positive scenario, Brazilian beef cattle production still has low production efficiency. Although Brazil has the largest commercial cattle herd in the world (213.68 million heads), ranks second classification of meat production, led by the United States, which produces 17.3% of the world meat production with 94,5 million heads (ABIEC, 2020).

To be successful in beef cattle production, attention must be paid to the females and their reproductive efficiency. In Brazil, low reproductive efficiency is noted in the beef herd: heifers presenting 42 months first calving and cows presenting 17 months calving interval and 65% of calves produced per cow per year (Baruselli and Vieira, 2015). Although reproductive failure may occur for several reasons, nutrition management is often an important contributing factor (D'Occhio et al., 2019a; Keisler and Lucy, 1996; Short and Adams, 1988; Wiltbank et al., 1962). Most Brazilian commercial farms have tropical grazing systems, consisting of seasonal variations in forage production (Abeygunawardena and Dematawewa, 2004; Latawiec et al., 2014; Santos et al., 2014). Consequently, beef cows may spend most of their pregnancy period during the dry season, which is characterized by high fiber content and reduced mass of forage available (Ayres et al., 2014; Santos et al., 2014; Gouvêa et al., 2018). Since pasture often does not provide enough nutrients in quantity and or quality, females do not fulfill their role of resumption of fertile ovarian cycles after calving, resulting in a failure in re-conceive and calve annually (Hess et al., 2005).

After calving, the secretion of LH is initially low and pulsatile discharges present low frequency (Yavas and Walton, 2000). As postpartum proceeds, about thirty days are required for completion of uterine involution in beef cows. Also, there is an increase in the LH pulses until 4 to 5 pulses per 10 hours period, necessary to support the final stages of follicular development, ensuing to the first ovulation postpartum (Crowe et al., 2014).

Nutrition intake, before and after calving, has been demonstrated to influence the duration of the postpartum anestrous interval and pregnancy rates in beef cattle (Ciccioli et al., 2003; D’Occhio et al., 2019a; Diskin and Kenny, 2016). If nutrient intake is insufficient, cows' body reserves become depleted and BCS declines (Crowe et al., 2014). According to several researchers, BCS at calving has proven to be key indicators in estimating the reproductive performance and subsequent pregnancy rates of beef cows (Ayres et al., 2014; DeRouen et al., 1994; Hess et al., 2005; Marques et al., 2016). Ayres and coauthors (2014) reported that cows with moderate to good BCS at calving can undergo a decline in BCS during postpartum; however, they still have a higher re-conception rate than cows with poor BCS at calving. After calving, when nutritional requirements are increased, cattle can often enter a negative energy balance and lose BCS. Consequently, cattle can enter the breeding season (BS) with poor BCS, affecting reproductive function and performance (Meteer et al., 2015).

Metabolites and hormones could mediate the effects of nutrient intake on reproductive function (Keisler and Lucy, 1996). Concentrations of glucose in plasma are affected by BCS, and inadequate availability of utilizable glucose reduces the hypothalamic release of GnRH (Mulliniks et al., 2012; Vizcarra et al., 1998). Insulin is responsive to nutrition and has a crucial role in glucose utilization by maternal somatic tissues including reproductive tissues and facilitating the production of insulin-like growth factor 1 (IGF1) by the liver (Laskowski et al., 2016). Nutrition also influences IGF1 concentrations that are positively associated with circulating of insulin, glucose, and cow body condition (Samadi et al., 2013). Steroidogenesis of follicular cells is supported by IGF1 (Zulu et al., 2002). IGF-1 increases the sensitivity of follicular cells to FSH and LH, which would promote steroidogenesis resulting in follicular growth and maturation (Meteer et al., 2015; Stewart et al., 1996). A better understanding of the interactions between nutrition, metabolic hormones and postpartum reproductive performance in beef cattle grazing subtropical pastures could lead to management strategies that produced an earlier resumption of ovulation and pregnancy after calving for cows on subtropical pastures.

In undernutrition conditions, extra attention should be taken in primiparous cows (Freetly et al., 2006). The energy required by cows is increased with lactation, and additional energy is necessary for growth in first-calf beef cows. Therefore, in this category is required a greater BCS at parturition than a mature cow to achieve satisfactory pregnancy rates (Ciccioli et al., 2003; Vizcarra et al., 1998). DeRouen et al. (1994) reported that primiparous calved BCS between 6 and 7 (scale 1-9) had greater pregnancy rates (87.0 and 90.7%) than those with a BCS of 4 and 5 (64.9 and 71.4%). Additionally, there is evidence that thin primiparous at calving respond to increased postpartum nutrient intake, improving reproductive performance (Ciccioli et al., 2003; Diskin and Kenny, 2016; Spitzer et al., 1995). In this sense, the development of nutritional programs based on supplementation to enhance the reproductive efficiency of beef cows could be a strategy to improve herd productivity.

In addition to correcting nutrient deficiencies, nutritional supplementation allows for greater use of the forage (Kunkle et al., 2000). Mainly, when low-quality forages are not limited by quantity, protein supplements are considered the main limiting nutrient (DelCurto et al., 2000). In fact, protein supplementation for beef cows fed tropical forages has a positive effect on forage dry-matter intake, digestibility, nutrient concentration in the rumen, crude protein (CP) intake, BW, BCS, blood metabolites and reproductive performance (da Silva et al., 2017; Quintans et al., 2016; Wilson et al., 2016a).

The most appropriate period for a cow's supplementation (before and/or after calving) is not yet clear in the literature. Many studies have been evaluated the supplementation during the last trimester of gestation, with positive effects BCS at calving (Bohnert et al., 2013; Stalker et al., 2006), on postpartum anestrous (da Silva et al., 2017; Lents et al., 2008) and on pregnancy rates (Hess et al., 2005). However, other experiments found no effect of prepartum supplementation on reproductive performance and metabolic parameters (Shoup et al., 2015; Summers et al., 2015). Furthermore, the supplementation during the postpartum period also influences reproductive functions (Randel, 1990), but is most pronounced in cows calving in thin to moderate BCS (Ciccioli et al., 2003; Lalman et al., 1997). Enhancing postpartum nutritional status promotes fat deposition, which may be a prerequisite to re-establish secretion of LH and follicular growth (Diskin and Kenny, 2016; Grimard et al., 1995). Additionally, improving maternal nutrition during postpartum increases dam milk yield and promotes calf weight gain at weaning (Callaghan et al., 2020).

Improving reproductive efficiency is a major goal of cow-calf production systems. Thus, it is essential to develop technologies to collaborate increasing productivity on farms, optimizing the breeding systems and the profitability of the herds.

2. HYPOTHESES

All hypotheses are described below and illustrated in Figure 1.

2.1. HYPOTHESIS OF CONTRAST 1 (Block supplementation effect, regardless of period: pre and postpartum)

Regardless of treatment period (pre and postpartum), block supplementation improves BCS, BW, subcutaneous fat thickness (SFAT), metabolites/hormones concentrations, and thereby enhances pregnancy per AI (P/AI) in Nelore primiparous cows compared to control group. Also, calves born by supplemented cows have greater weight gain until weaning.

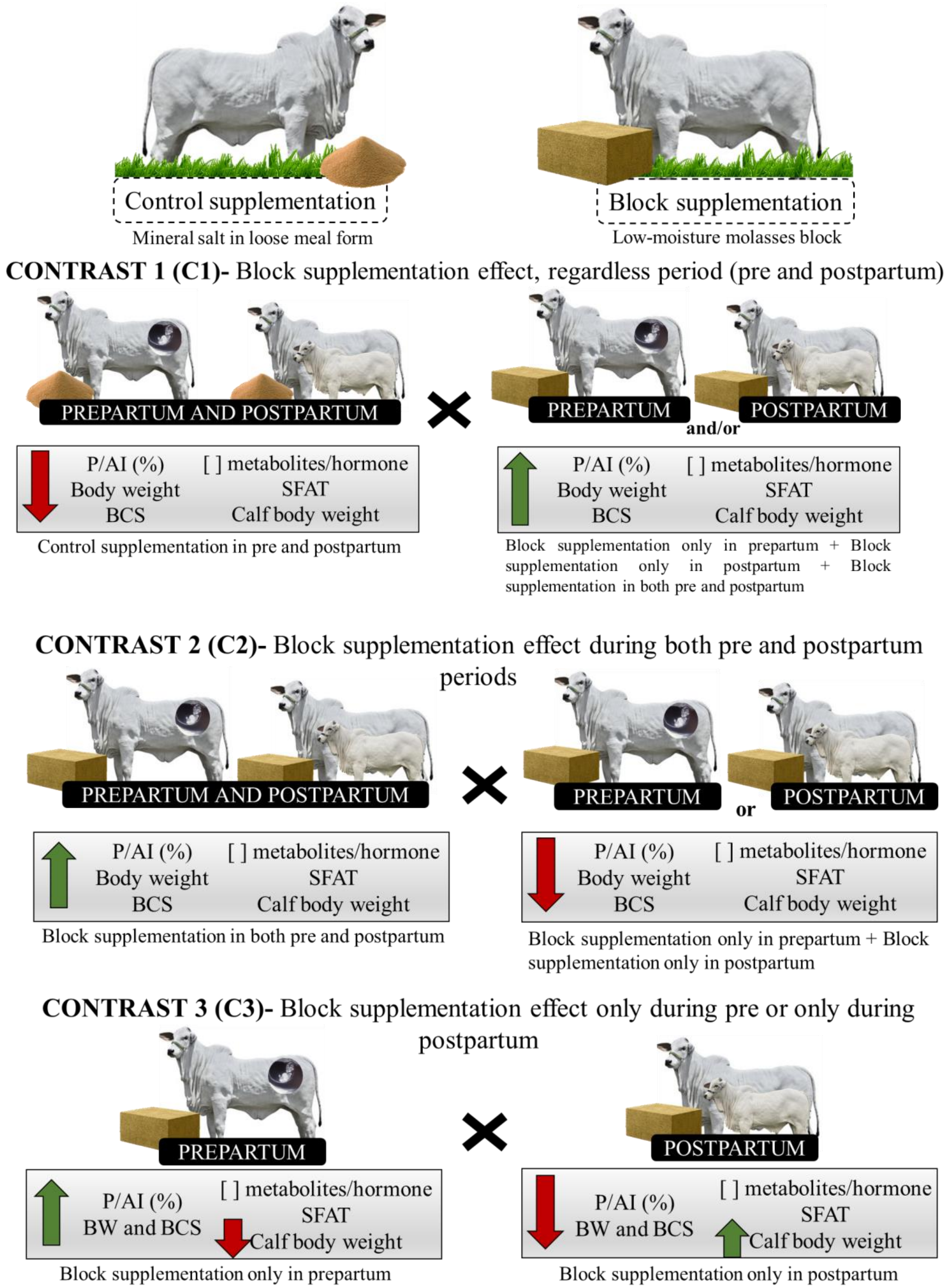
2.2. HYPOTHESIS OF CONTRAST 2 (Block supplementation effect during both pre and postpartum periods)

Block supplementation during both pre and postpartum periods promotes greater BCS, BW, SFAT, metabolites/hormones concentrations, and thereby enhances pregnancy rates in Nelore primiparous cows compared to cows supplemented only in prepartum or only in postpartum period. Also, calves born by supplemented cows during both periods have greater weight gain until weaning.

2.3. HYPOTHESIS OF CONTRAST 3 (Block supplementation effect only during pre or postpartum)

Block supplementation during only prepartum period increases BCS, BW, SFAT, metabolites/hormones concentrations, and thereby enhances pregnancy rates in Nelore primiparous cows compared to cows supplemented only in the postpartum period. However, calves born by supplemented cows only during postpartum have greater weight gain until weaning.

Figure 1- Hypothetical model design



3. OBJECTIVES

Evaluate the effects of block supplementation during prepartum (90 days before parturition) and/or postpartum (120 days after parturition) in Nelore primiparous cows on:

- Body condition score during pre and postpartum periods,
- Body weight during pre and postpartum periods,
- Subcutaneous fat thickness in postpartum,
- Insulin and IGF1concentrations during pre and postpartum periods,
- Glucose and urea concentrations during postpartum period,
- Cyclicity rate at the onset of synchronization protocol for FTAI,
- Pregnancy rate at 1st and 2nd FTAI,
- Overall pregnancy rate at the end of the breeding season,
- Pregnancy losses,
- Calves weight gain until weaning.

4. PREPARTUM AND/OR POSTPARTUM SUPPLEMENTATION WITH LOW-MOISTURE MOLASSES BLOCKS TO OPTIMIZE FERTILITY AND CALF PERFORMANCE IN PRIMIPAROUS BEEF COWS.

ABSTRACT

Pregnant Nelore heifers (n=417) were used to evaluate effects of block supplementation during pre (90 days before calving; D-90) and/or postpartum (120 days after calving) on reproductive and progeny performance. Heifers were randomly allocated in four experimental groups: 1) Group CC: heifers received mineral supplement in loose meal form (0.06% of BW) offered daily (control supplementation; C) before and after parturition (n=108); 2) Group CB: received C before parturition and mineral protein supplement in block form (0.07% of BW) offered weekly (block supplementation; B) after parturition (n=117); 3) Group BC: received B before and C after parturition (n=103) and 4) Group BB: received B before and after parturition (n=89). During pre and postpartum periods the performance [body weight (BW); body condition score (BCS); subcutaneous backfat thickness (BFAT); rump fat thickness (RFAT) and calf body weight (CW)] were evaluated. Blood samples were collected on a subset of animals (n=120) for metabolites and hormonal analysis. All animals were synchronized for FTAI using estradiol/progesterone-based protocol at D40 and D80 (non-pregnant at 1st FTAI). Pregnancy diagnosis was performed 30 days after FTAI and at the end of the breeding season (BS). All data was analyzed by orthogonal contrasts [C1: Control *vs.* block supplementation (CC *vs.* BB+BC+CB); C2: B in both periods (pre and postpartum) *vs.* B in one of the periods (pre or postpartum; BB *vs.* BC+CB); C3: B only during prepartum *vs.* B only during postpartum (BC *vs.* CB). Block supplementation (C1) increased pregnancy at first FTAI ($P=0.04$) and overall pregnancy rate ($P=0.05$). There was an interaction time*treatment for body weight (BW; $P<0.0001$) and body condition score (BCS; $P>0.0001$). Supplemented cows had greater BCS only at parturition (D0; $P=0.04$) and at D40 ($P=0.02$) and greater BW ($P=0.03$) only at D40. Block supplementation increased subcutaneous backfat ($P=0.03$) and rump fat thickness ($P=0.03$) and insulin concentrations ($P=0.008$). There was an interaction time*treatment for glucose ($P=0.0002$), which were higher for supplemented cows only at D40 ($P=0.01$). Although no differences in reproductive performance in C2 ($P>0.15$), the CW was higher for calves born by cows supplemented before and after parturition at 80

($P<0.001$), 120 ($P<0.001$), 170 ($P=0.002$) and 210 ($P=0.02$) days old. In summary, regardless of period of treatment, block supplementation increased pregnancy at first FTAI and overall pregnancy rate. Additionality, block supplementation during both pre and postpartum periods improved progeny weight until weaning. Block supplementation can be a tool to optimize fertility and calf performance in Nelore primiparous cows.

Keywords: Nutrition; Beef cattle; Reproduction; FTAI, Pregnancy, Calves.

4.1. Introduction

Nutritional management is considered one of the most important factors that affect the reproduction of beef cattle (Armstrong et al., 1992; D'Occhio et al., 2019b; Wiltbank et al., 1962). Brazilian commercial farms have the highest concentration of births in the dry season and during the transition to the rainy season. Consequently, beef cows spend most of their peripartum period with a low-quality forage available (Santos et al., 2014), which may lead to an inadequate intake of nutrients (Gouvêa et al., 2018; Stobbs, 1975).

The higher gestational energetic/protein demand in the last third of gestation plus inadequate nutrient intake results in low BCS at parturition and negative energy balance in early postpartum (Sotelo et al., 2018; Mulliniks et al., 2012). As already noted, low energy reserves compromise the postpartum anestrous interval (Spitzer et al., 1995; Hess et al., 2005) and pregnancy rates in beef cows submitted to fixed timed artificial insemination (FTAI; Ayres et al., 2014). This relationship is especially critical for primiparous, due to the additional demands needed to continue their own growth combined with the stress of first lactation (Ciccioli et al., 2003). Thus, inadequate nutrient intake before and/or after calving has greater detrimental effects on reproductive performance in primiparous than mature cows (Moura et al., 2020; Sotelo et al., 2018; Summers et al., 2015).

Supplementation programs for beef cows during prepartum and/or postpartum can be an alternative for improving the nutritional efficiency in grazing systems, especially when protein supplements are used (DelCurto et al., 2000). Protein supplements can improve the activity of the rumen microbiota and fiber degradation, allowing for a better utilization of the forages, particularly during the dry season (Kunkle et al., 2000; Meteer et al., 2015). Studies have shown that supplemental protein for beef cattle grazing low-quality forage has a positive effect on forage intake, BW, BCS, blood metabolites and reproductive efficiency (D'Occhio et al., 2019b; DelCurto et al., 2000; Wilson et al., 2016). However, nutritional supplementation programs considerably increase production costs in beef cattle systems, such as the vehicle maintenance, fuel and labor costs that are required for daily supplemental feeding (Moura et al., 2020). Low-moisture block supplements consist of molasses, with ingredients that supply nutrients such as protein, minerals and vitamins (Cassini and Hermitte, 1992). These blocks have unique characteristics that limit the intake and can therefore be delivered less frequently (once a week or every 10 days) while avoiding overconsumption. Furthermore, molasses blocks improve forage intake, digestion, and the grazing of underutilized pastures, proving to be a potential strategy to decrease production costs and better the nutritional status of beef cattle (Bailey and Welling, 2007; Löest et al., 2001; Moriel et al., 2019; Stephenson et al., 2016).

Thus, we hypothesized that block supplementation offer weekly improves the reproductive efficiency of primiparous beef cows and their progeny performance. The current study aimed to evaluate the effects of block supplementation during pre (90 days before calving) and/or postpartum (120 days after calving) periods on pregnancy rate and metabolic/hormonal characteristics of grazing primiparous Nelore cows as well as their progeny growth.

4.2. Material and Methods

The experiment was conducted in a commercial farm located in Santa Rita do Pardo, Mato Grosso do Sul, Brazil, from June 2018 to May 2019. All animal-related procedures used in this study were approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo, Brazil) under protocol number 8169050819.

4.2.1. *Animals, experimental design and treatments*

A total of 417 Nelore *(Bos indicus)* heifers in the final trimester of their pregnancy were assigned to this experiment. Heifers were 31.6 ± 2.3 months of age (mean ± SE), weighed 438.8 ± 3.5 kg and BCS (1 to 5 scale) of 2.94 ± 0.03 at the beginning of the supplementation period (D-90). Treatments consisted of control supplementation (C: mineral supplement in loose meal form; 0.06% of BW; offered daily) used routinely on the farm, and molasses-monensin block supplementation (B: mineral protein supplement in block form; 0.07% of BW; offered weekly) recommended by Minerthal Nutritional Products Ltd (São Paulo, Brazil), offered in the proportion of 1 block (25kg): 10 cows (Table 1). A quantity of supplements was chosen to meet the protein requirements of primiparous cows, according to recommendations of Nutrient Requirements of Zebu and Crossbred Cattle (Valadares Filho et al., 2016).

Table 1. Average chemical composition of supplements provided from day -90 to 120.

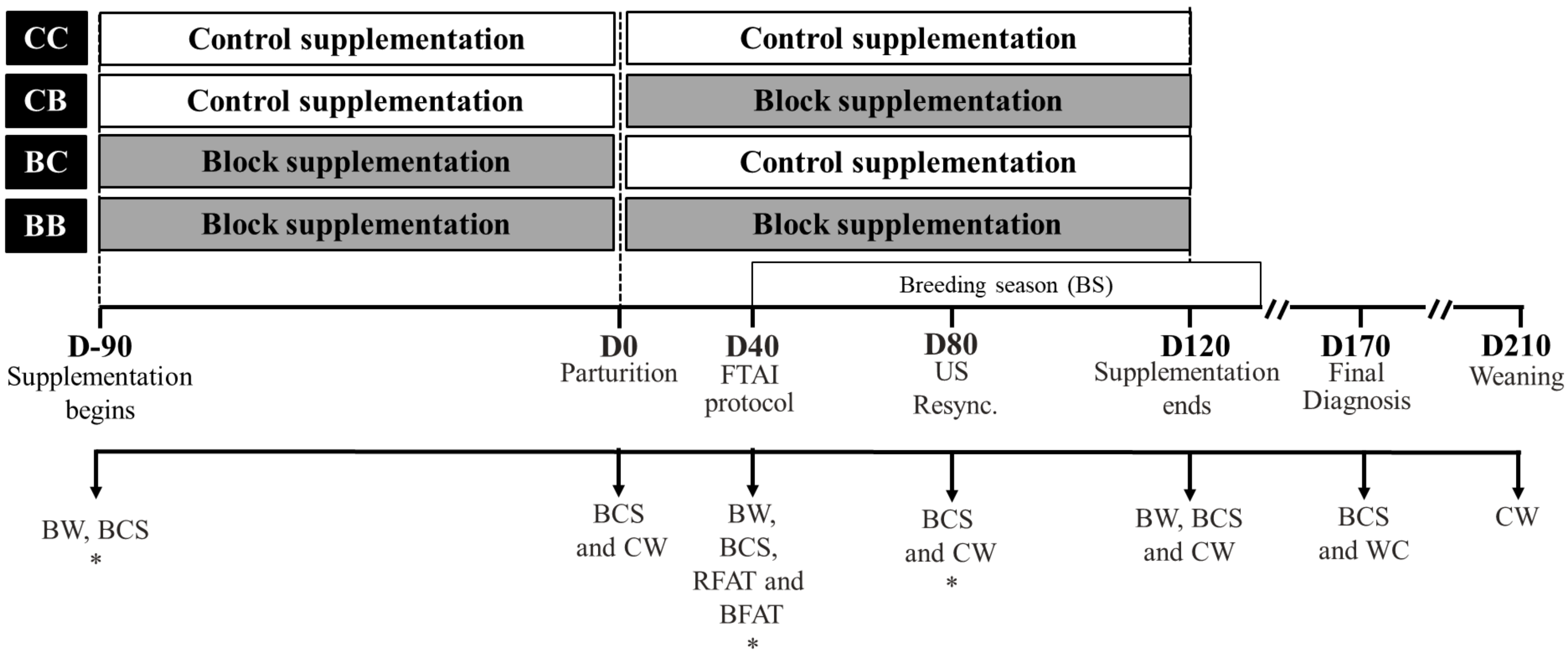
Item[2]	Supplement[1]	
	C	B
DM, %	92.5	90.0
TDN, %	35.0	40.0
CP, %	40.0	35.0
NPN, %	5.1	3.5
Ca, g/kg	80	65
P, g/kg	30	20
Na, g/kg	50	40
S, g/kg	5	4
Zn, mg/kg	1400	920
Cu, mg/kg	400	230
I, mg/kg	20	13.5
Co, mg/kg	22	17.5
Se, mg/kg	7.2	9
Mn, mg/kg	430	380
Sodium monensin, mg/kg	-	300

[1] C= Control supplementation offered daily at 0.06% of body weight/animal/day; B= Block supplementation offered weekly at 0.07% of body weight/animal/day.
[2]DM=dry matter; TDN = total digestible nutrients; CP = crude protein; NPN = non protein nitrogen; Ca = calcium; P = phosphorous; Na = sodium; S= sulfur; Zn = zinc; Cu = cooper; I = iodine; Co = cobalt; Se = selenium; Mn = manganese.

At the start of the supplementation period (90 days before the expected date of parturition) heifers were randomly distributed (according to predict calving date, BW and BCS), as seen in Figure 2, in 4 treatments: 1) Group CC: heifers received C 90 days before and 120 days after calving (n=108); 2) Group CB: heifers received C 90 days before and B 120 days after calving (n=117); 3) Group BC: heifers received B 90 days before and C 120 days after calving (n=103) and 4) Group BB: heifers received B 90 days before and 120 after calving (n=89). The cows were distributed in 4 paddocks, 2 groups of cows received B and 2 groups received C. Total supplementation period was 210 days: 90 days before parturition until the second pregnancy check (D120). After the supplementation period, all cows received C until the end of the BS.

During the trial period, the animals were kept in rotational grazing systems containing 8 paddocks of 39 hectares each. The groups of cows were rotated through the grazing systems every 7 days to avoid the effect of variation among pastures. The pasture was composed of grasses of the genus Urochloa brizantha. All the paddocks had a food court containing feeders which provided at least 20 cm of linear feeder space per animal, avoiding competition between animals.

Figure 2. Experimental timeline. Ninety days before parturition (D-90) initiated the supplementation period, heifers were evaluated BW, BCS and blood sample (*) was collected (subset). At parturition calves were weighed and BCS of cows were evaluated. Forty days after calving (D40), cows were evaluated BW, BCS, RFAT and BFAT. At the same time, cows were synchronized to FTAI and blood samples were collected (subset). Eighty days after calving (D80), blood sample was collected (subset), BCS and pregnancy diagnosis was performed. Non-pregnant cows were resynchronized to a second FTAI and CW was evaluated. A second pregnancy diagnosis was performed (D120) and BCS, BW and CW were evaluated. A final diagnosis for overall pregnancy rate (1st + 2nd + natural mating) was performed on D170, and BCS and CW were evaluated. At the weaning (D210), all the calves were weighed.



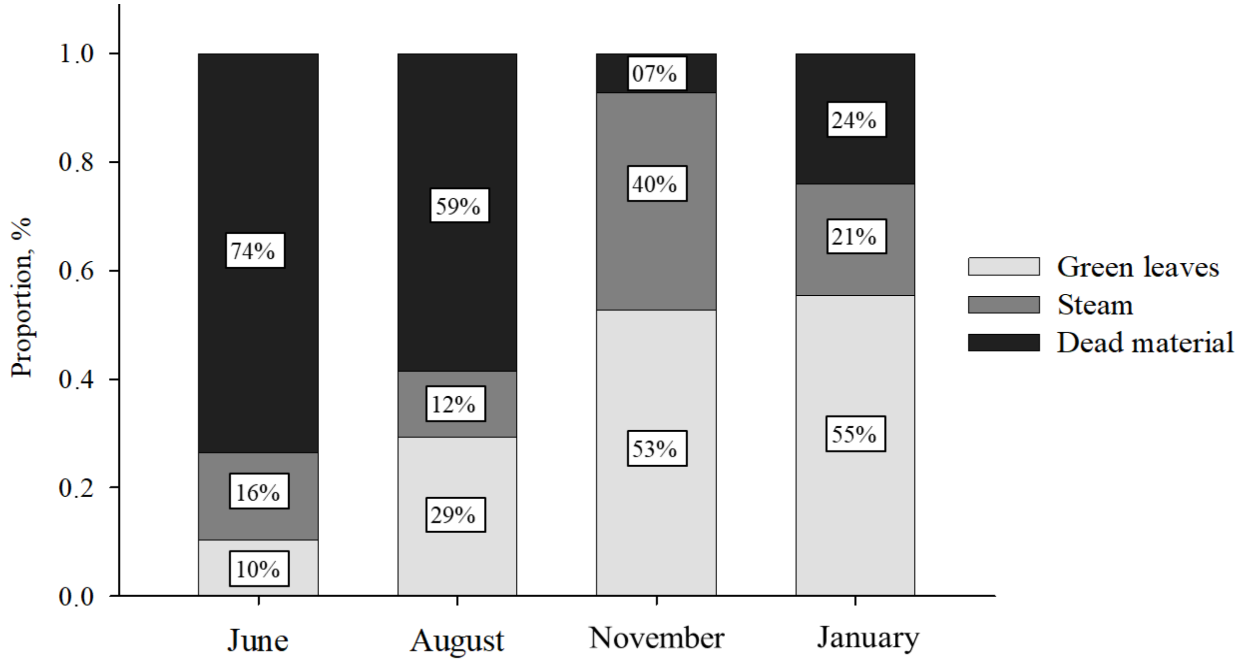
Prior to grazing, forage samples were collected by hand-plucked sampling four times: June (Beginning of supplementation), August, November, and January (end of supplementation). Samples were cut at the ground level from five delimited areas (0.5 x 0.5 m), selected randomly in each paddock to quantify herbage mass (Table 2). The green leaves were separated from the other structural components of the pasture (stem and dead material), weighed, pre-dried in a forced air oven at 55°C for 72 hours, and ground in a Willey mill with a 1-mm sieve. The mean chemical analysis of the green leaves is presented in Table 2, and the botanic composition of pastures in Figure 3.

Table 2. Herbage mass (HM) of pastures and average chemical composition of the green leaves sample collected during the experimental period.

	Month[1]			
Item[2]	June	August	November	January
HM, kg DM/ha	2.237	1.493	1.702	1.366
Green leaves, %	10	29	53	55
CP, %	14.8	14.3	8.8	9.6
Ca, %	3.1	2.9	3.0	2.3
P, %	2.1	1.5	2.1	1.6
Cu, mg/kg	6.21	6.49	4.34	3.48
Mn, mg/kg	82.66	77.63	52.26	32.53
Zn, mg/kg	17.84	16.53	15.23	7.04

[1] Forage samples were collected by hand-plucked over the time before the grazing by females.
[2] HM= herbage mass; Green leaves= percentage of green leaves on pasture; CP= crude protein; Ca= calcium; P=phosphorus; Cu= cooper; Mn= manganese; Zn= zinc.

Figure 3. Botanic composition of pastures (8 paddocks/ 39 ha each) over the time.



4.2.2. *Ovulation synchronization protocol for FTAI*

Cows were assigned to FTAI protocol at 40.7 ± 7.8 days postpartum. On a random day of the estrous cycle (Day 0 of protocol), all cows received an intravaginal device with 0.6 g of progesterone (P4; Fertilcare 600, MDS, Brazil) and 2.0 mg intramuscular injection (IM) of estradiol benzoate (Fertilcare sincronização, MSD). At the same time, the cows were classified as cyclic if they had a *corpus luteum* (CL) detected by an ultrasonography exam (DP-2200 VET; Mindray, China). Eight days later (Day 8 of protocol), the P4 device was removed, and 0.530mg of sodic cloprostenol (Ciosin, MSD), 1mg of estradiol cypionate (Fertilcare ovulação, MSD), and 300 IU of equine chorionic gonadotropin (Folligon, MSD, Brazil) was given IM. The cows were inseminated 48 hours after P4 device removal (Day 10 of protocol), by the same technician, using two semen batches from a previously tested bull. The semen batches were homogeneously distributed between the experimental groups.

Pregnancy diagnosis was perfomed by transrectal ultrasonography 30 days after the first AI (D80). Non-pregnant cows were resynchronized to a second FTAI using the same hormonal protocol described above. Fifteen days after the second FTAI, all the cows were exposed to natural mating (NM) with clean-up bulls at a proportion of 1 bull: 20 cows until the end of the BS

(D140). Thirty days after the second FTAI (D120), the resynchronized cows had their pregnancy diagnostic. All animals were examined by transrectal ultrasonography 30 after the end of the BS (D170) to determine pregnancy status and pregnancy loss.

4.2.3. *Cow and Calf performance*

All animals had their BCS evaluated at five different times during the experimental period: D-90 (before parturition); D0 (parturition); D40; D80 and D120. The BCS attributed to each animal was performed using the visual technique (Ayres et al., 2009) by the same trained technician. Animals were classified using a 1 (very thin) to 5 (very fat) point scale, with a difference of 0.25 points from one class to the next. Furthermore, animals were weighed at three different moments: D-90; D40 and D120. BW was obtained using a digital balance which all the animals were weighed individually at the same time of day. The calves had their body weight (CW) evaluated on D0 (at birth), D80, D120 (end of the supplementation), D170, and D210 (at weaning) to estimate growth and weight gain.

4.2.4. *Subcutaneous fat thickness evaluation*

Forty days after parturition, at the onset of the synchronization of the FTAI protocol (D40), subcutaneous backfat thickness (BFAT) and subcutaneous rump fat thickness (RFAT) were measured in all the animals. Ultrasound measurements were taken with an Aloka 500 SV (Hitachi Aloka Medical America, Inc., Wallingford, CT) instrument equipped with a 3.5- MHz 172-mm linear transducer. Measurements of BFAT were taken in a transverse orientation between the 12th and 13th ribs approximately 10 cm distal from the midline. To RFAT the transducer was linearly positioned between hooks and pins at the sacral examination site and moved slightly until the correct image was formed, allowing for the visualization of the superior limit of the biceps femuris muscles. Ultrasound images were processed using Lince software (M & S Consultoria Agropecuária Ltda., Pirassununga, Brazil).

4.2.5. *Blood sampling, Metabolites and Hormone Determinations*

Blood samples were taken over time on a subset of cows (n=120), using a tube through the coccygeal vein/artery. Blood samples were collected to measure glucose and urea concentrations during postpartum (D40 and D80), using a Vacutainer tube containing EDTA and sodium fluoride (BD Vacutainer® Fluoreto/EDTA, São Paulo, Brazil). A second blood sample was collected in a tube containing gel for serum separation and clot activation (BD Vacuntainer® SST II Plus, São Paulo, Brazil) to analyze insulin-like growth factor-1 (IGF1) and insulin concentration during prepartum (D-90), and postpartum (D40 and D80). Centrifugation of both tubes (2000 × g for 20 min) was performed to separate plasma and serum. Plasma/serum was removed and stored at −20°C for further analysis.

Serum concentrations of insulin were measured via a commercial RIA kit (Sigma, St. Louis, EUA), as previously described (Lacau-Mengido et al., 2000). Intra- and inter assay coefficients of variation (CV) were 9.9% and 14.9%. Serum concentrations of IGF1 were analyzed in duplicate samples using in house competitive enzyme-linked immunosorbent assays (cELISA; Ansh labs, EUA) for bovine with the amplification biotin-streptavidin peroxidase system (Maioli and Nogueira, 2017). Intra- and inter assay CV were 7.4% and 11.0%. Commercial enzymatic-colorimetric kits were used to determine plasma concentrations of glucose (K0827; Bioclin, Brazil) and urea (K047; Bioclin, Brazil).

4.2.6. *Statistical analysis*

The experiment followed a randomized complete block design, with random effect of group (group of cows/calf in a pasture in which treatment was applied) nested within treatment identifies the group as the experimental unit.

Distributions of the residuals of continuous data, such as cow and calf performance and metabolites and hormone profile, were evaluated for normality using graphical diagnostics, and data transformation was performed when appropriate. Variables that did not follow these assumptions were transformed accordingly and outliers were removed when necessary. Data were analyzed by the GLIMMIX procedure of SAS (SAS/STAT ver. 9.4) using the following model adapted to St-Pierre, 2007:

$$Yijk = \mu + Di + Tj + Di*Tj + ak + \beta X + eijk,$$

where *Yijk* = dependent variable; μ = overall mean; *Di* = fixed effect of treatment; *Tj* = random effect of time; *Di X Tj* = interaction between treatment and time; *ak* = random effect of animal within group of animals; βX is the covariate adjustment for each animal; and *eijk* = residual error.

Unstructured method was used to calculate the covariance structure. The Kenward-Roger method was used to calculate the denominator degrees of freedom. Unstructured UN(1) was the best covariance structure based on the smallest Akaike's information criterion values. Other covariance structures were tested including compound symmetry, heterogeneous compound symmetry, first-order autoregressive and heterogeneous autoregressive. In addition, the data from the first sampling date of BW, BCS, CW, insulin and IGF-1 were added as a covariate in the statistical model.

Contrasts were constructed to evaluate the treatments: block supplementation effects [Contrast 1 (C1): Control x Block supplementation (CC x BB+BC+CB)]; Block supplementation effects in both pre and postpartum periods [Contrast 2: pre and postpartum x pre or postpartum (BB x BC+CB)] and Block supplementation effects only during prepartum or only during postpartum. [Contrast 3 (C3): prepartum x postpartum (BC x CB)]. Data are presented as means ± standard error of the mean, obtained using PROC MEANS of SAS. Statistical significance was defined as $P \leq 0.05$.

Variables with a binomial distribution, such as cyclicity rate, pregnancy per AI (**P/AI**), pregnancy per NM and pregnancy loss, were analyzed by logistic regression using GLIMMIX procedure (SAS/STAT ver. 9.4). Initial models contained the following categorical explanatory variables as fixed effects: treatment, cyclic status (cyclic or noncyclic), AI technician, sire, straw(sire), BCS change (gained, maintained, or lost) and their first order interaction. Animal within group of animals were included as random effects in the model. Selection of the fixed effects model that best fit the data for each variable of interest was performed by finding the model with the lowest value for the Akaike information criterion using a backward elimination procedure that sequentially removed all variables with $P \geq 0.10$ from the model. Final models included the fixed effects of treatment and the random effects of animal within group of animals. The same contrasts were constructed to evaluate the treatments. Statistical significance was defined as $P \leq 0.05$.

Logistic regression curves were obtained using the coefficients generated by the "interactive data analyses" of the SAS statistical program and the formula y = exp (α × X + b) / [1 + exp (α ×

$x + b)]$, where: y = probability of pregnancy success; exp = exponential, α = slope of the logistic equation; b = intercept of the logistic equation; X = significant variable.

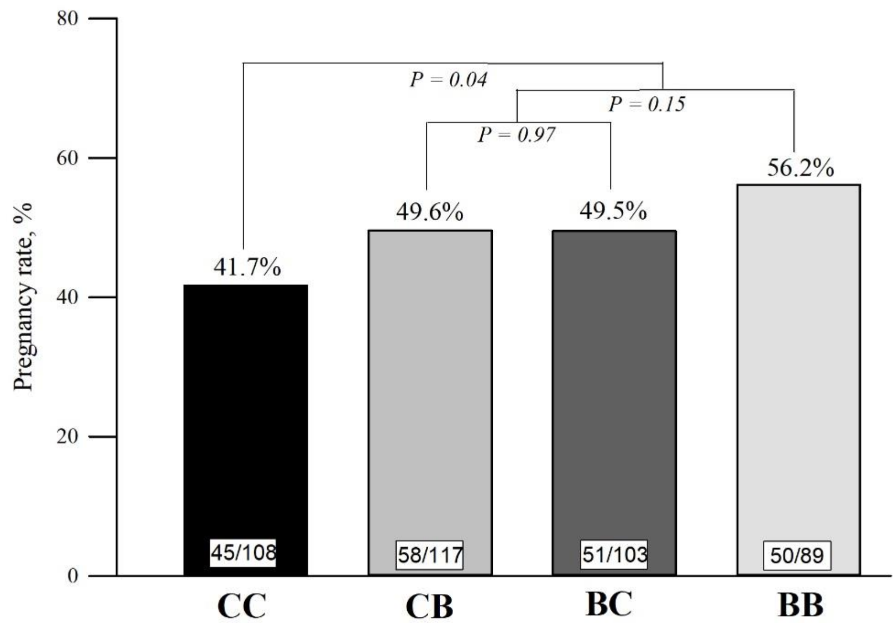
4.3. Results

4.3.1. Reproductive performance

The reproductive performance according to groups is presented in Table 3. The pregnancy rate at first FTAI was 9.8 percentage points greater for cows treated with blocks [Block supplementation: 51.5% (159/309) *vs.* Control: 41.7% (45/108); P_{C1}=0.04], compared to control cows (figure 4). Moreover, there was a block supplementation effect on the overall pregnancy rate at end of the BS, after two consecutive FTAI's and NM [Block supplementation: 84.1% (260/309) *vs.* Control: 76.9% (83/108); P_{C1}=0.05]. Despite the evident positive effects of block supplementation on the pregnancy rate at first FTAI and at the end of the BS, no differences for cyclicity (P_{C1}=0.39), pregnancy at second FTAI (P_{C1}=0.70), or pregnancy at NM (P_{C1}=0.28) were observed.

The block supplementation during pre and postpartum periods (C2) did not affect the cyclicity rate (P_{C2}=0.51), pregnancy at first (P_{C2}=0.15) and second FTAI (P_{C2}=0.93), pregnancy at NM (P_{C2}=0.72), or overall pregnancy rate (P_{C2}=0.54), when compared with cows supplemented only during pre or postpartum. Likewise, no reproductive differences were observed for C3. The cyclicity (P_{C3}=0.97), pregnancy at first (P_{C3}=0.97) and second FTAI (P_{C3}=0.95), pregnancy at NM (P_{C3}=0.51), and overall pregnancy rate at end of the BS (P_{C3}=0.81) were similar between cows supplemented only in prepartum compared with cows supplemented only in postpartum. The pregnancy loss after the first and second FTAI did not differ between any contrast ($P \geq 0.3$).

Figure 4. Pregnancy rate (%) at the first FTAI according to groups in primiparous Nelore cows.



Orthogonal contrasts: C1 (Block supplementation effect): Control (CC) *vs.* block supplementation (BB+BC+CB); C2 (Block supplementation effect on pre and postpartum): Pre and postpartum (BB) *vs.* Pre or postpartum (BC+CB) and C3 (Pre or postpartum effect): prepartum (BC) *vs.* postpartum (CB).

Table 3.Effects of block supplementation during pre and/or postpartum on reproductive performance in primiparous Nelore cows.

Variable[1]	Groups CC	CB	BC	BB	P value[2] C1	C2	C3
Postpartum cyclicity rate at D40, % (n/n)	15.7 (17/108)	18.8 (22/117)	18.4 (19/103)	22.5 (20/89)	0.39	0.51	0.97
Pregnancy rate at first FTAI, % (n/n)	41.7 (45/108)	49.6 (58/117)	49.5 (51/103)	56.2 (50/89)	0.04	0.15	0.97
Pregnancy loss of first FTAI, % (n/n)	8.9 (4/45)	8.6 (5/58)	5.9 (3/51)	10.0 (5/50)	0.98	0.30	0.60
Pregnancy rate at second FTAI, % (n/n)	46.0 (29/63)	47.5 (28/59)	48.1 (25/52)	48.7 (19/39)	0.70	0.93	0.95
Pregnancy loss of second FTAI, % (n/n)	3.4 (1/29)	7.1 (2/28)	4.0 (1/25)	5.3 (1/19)	0.98	0.97	0.63
Pregnancy rate at NM, % (n/n)	35.9 (14/39)	49.5 (19/38)	41.9 (13/31)	53.8 (14/26)	0.28	0.72	0.51
Overall pregnancy rate at end of BS, % (n/n)	76.9 (83/108)	83.8 (98/117)	82.5 (85/103)	86.5 (77/89)	0.05	0.54	0.81

[1] Cyclicity rate= presence of corpus luteum at the onset of the FTAI protocol (D40); FTAI = fixed-time artificial insemination; NM= natural mating; Overall pregnancy rate= 1st FTAI+ 2nd FTAI+ NM; BS = breeding season.

[2] Orthogonal contrasts: C1 (Block supplementation effect): Control (CC) *vs.* block supplementation (BB+BC+CB); C2 (Block supplementation effect in both pre and postpartum periods): Pre and postpartum (BB) *vs.* Pre or postpartum (BC+CB) and C3 (Pre or postpartum effect): prepartum (BC) *vs.* postpartum (CB).

4.3.2. Cow and calf performance

There was interaction between time and treatment for BW ($P<0.0001$; Table 4), BCS ($P<0.0001$; Table 4) and CW ($P<0.0001$; Table 5). Supplemented cows (C1) had greater BW only at D40 (Block supplementation: 408.7±1.99 *vs.* Control: 400.8±3.25 kg; $P_{C1}=0.03$), but not at D-90 ($P_{C1}= 0.83$) or D120 ($P_{C1}= 0.16$). A greater BCS at parturition (Block supplementation: 3.06±0.01 *vs.* Control: 3.00±0.03; $P_{C1}=0.02$) and at D40 (Block supplementation: 2.91±0.02 *vs.* Control: 2.83±0.03; $P_{C1}= 0.01$) was found for supplemented cows compared to control cows; However, no effects for BCS at D-90 ($P_{C1}= 0.15$), D120 ($P_{C1}= 0.56$) or D170 ($P_{C1}= 0.34$) were found among groups.

Calves body weight was higher for calves born by cows treated with block supplementation compared to those born by cows from the control group at birth (Block supplementation: 35.5±0.24 *vs.* Control: 34.6±0.42 kg; $P_{C1}=0.01$), at D80 (Block supplementation: 101.3±0.91 *vs.* Control: 97.8±1.37 kg; $P_{C1}=0.03$) and at D120 (Block supplementation: 132.6±1.05 *vs.* Control: 123.9±1.59 kg; $P_{C1}<0.01$). Nevertheless, there was no difference for CW at D170 ($P_{C1}=0.55$) and at weaning ($P_{C1}=0.38$; Table 5).

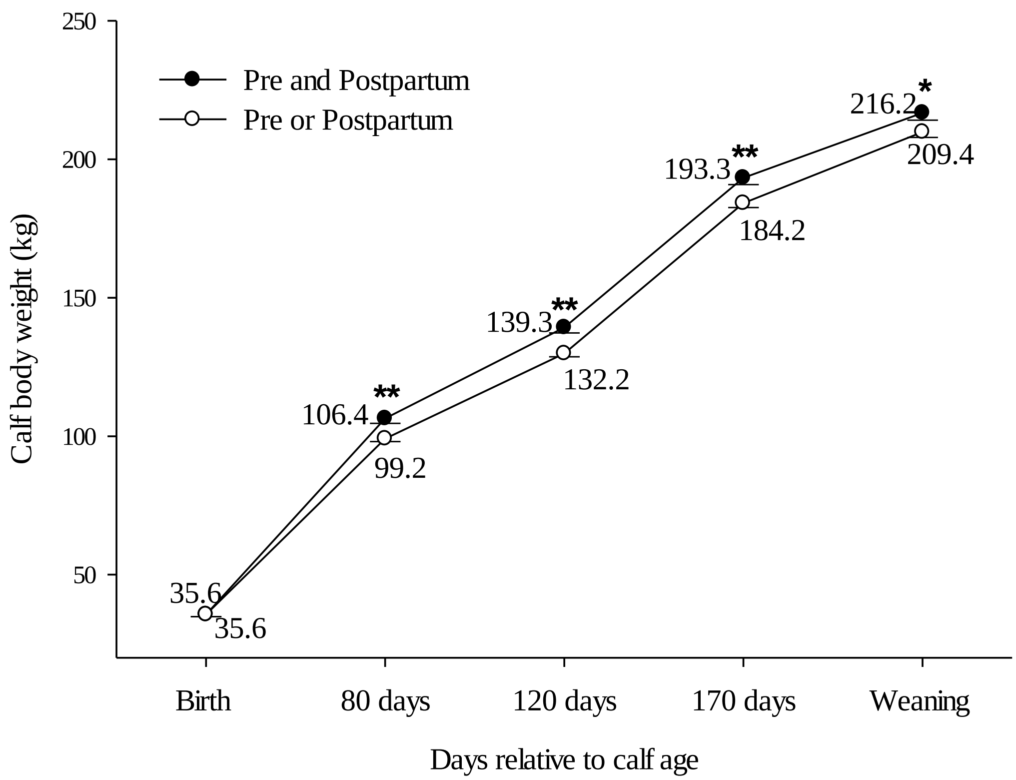
Comparing block supplementation during pre and postpartum *vs.* either pre or postpartum (C2), effect for BCS and CW was observed. However, no effect was found for BW. Cows treated during both pre and postpartum showed higher BCS only at D80 compared to cows treated during either pre or postpartum (Pre and postpartum: 2.98±0.04 *vs.* Pre or postpartum: 2.88±0.02; $P_{C2}=0.02$; table 4). No effect was observed at D-90 ($P_{C2}=0.45$), D0 ($P_{C2}=0.25$), D40 ($P_{C2}=0.21$), D120 ($P_{C2}=0.65$) or D170 ($P_{C2}=0.33$) for BCS. The calves' performance for C2 is presented in figure 5. Calves born by cows supplemented with blocks during pre and postpartum showed the same birth weight as calves born by cows supplemented only in pre or postpartum ($P_{C2}=0.89$). However, CW was greater at 80 (Pre and postpartum: 106.4±1.74 *vs.* Pre or postpartum: 99.2±1.04; $P_{C2}<0.001$), 120 (Pre and postpartum: 139.3±1.97 *vs.* Pre or postpartum: 129.9±1.20; $P_{C2}<0.001$), 170 (Pre and postpartum: 193.3±2.44 *vs.* Pre or postpartum: 184.2±1.57; $P_{C2}=0.002$) and 210 (Pre and postpartum: 216.2± 2.03 *vs.* Pre or postpartum: 209.4±1.48; $P_{C2}=0.02$) days old for calves born by cows treated with blocks during both pre and postpartum.

Block supplementation only during prepartum *vs.* only during postpartum (C3) showed effects for BW, BCS and CW. Cows supplemented during postpartum period had greater BW at

D40 (Prepartum: 400.9±3.10 *vs.* postpartum: 412.2±3.46; P_{C3}<0.001), but not at D-90 (P_{C3}=0.18) or D120 (P_{C3}=0.11). The BCS at parturition was higher in cows treated during prepartum than cows treated during postpartum (Prepartum: 3.11±0.01 *vs.* postpartum: 3.00±0.01; P_{C3}<0.01). However, at the end of the BS (D170), cows treated in postpartum had greater BCS (Prepartum: 2.74±0.02 *vs.* postpartum: 2.87±0.01; P_{C3}=0.001; Table 4).

No effect of block supplementation was observed among the groups for CW at birth (P_{C3}=0.16), 80 (P_{C3}=0.75), 170 (P_{C3}=0.93), or 210 days old (P_{C3}=0.74). However, at the end of the supplementation period (D120), calves born by cows supplemented only during postpartum had greater CW when compared to calves born by cows treated only during prepartum (Prepartum: 126.5 ± 1.65 *vs.* postpartum: 132.9 ± 1.69 kg; P_{C3}=0.002; Table 5).

Figure 5. Block supplementation effect during pre and postpartum period for calf body weight evaluated over the time. C2 (Block supplementation effect in both pre and postpartum periods): Pre and postpartum (BB) *vs.* Pre or postpartum (BC+CB).



** Indicate a difference (P<0.01).
* Indicate a difference (P<0.05).

Table 4. Effect of prepartum and/or postpartum supplementation with blocks on body weight (kg) and body condition score (1–5 point scale) of primiparous Nelore cows evaluated at different times.

Itens[1]	Treatment				SEM	P value[2]				
	CC	CB	BC	BB		T	T x Treat	C1	C2	C3
BW, Kg						<0.0001	<0.0001	0.83	0.98	0.76
90 days prepartum[a]	435.8	441.0	473.1	440.1	1.78	-	-	0.25	0.68	0.18
40 days postpartum[b]	400.8	412.2	400.9	413.3	1.70	-	-	0.03	0.16	0.001
120 days postpartum[d]	432.9	431.3	432.9	429.0	1.79	-	-	0.16	0.22	0.11
BCS, 1-5						<0.0001	0.002	0.05	0.03	0.60
90 days prepartum[a]	2.91	2.95	2.93	2.94	0.01	-	-	0.15	0.45	0.64
Parturition	3.01	3.00	3.11	3.10	0.01	-	-	0.04	0.25	<0.001
40 days postpartum[b]	2.83	2.90	2.90	2.95	0.02	-	-	0.02	0.21	0.99
80 days postpartum[c]	2.84	2.91	2.85	2.98	0.02	-	-	0.12	0.02	0.12
120 days postpartum[d]	3.00	3.07	2.99	3.04	0.02	-	-	0.56	0.65	0.20
170 days postpartum[e]	2.78	2.87	2.74	2.84	0.01	-	-	0.34	0.33	0.001

SEM= Standard error of the mean.

[1] BW= body weight (kg); BCS= body condition score (1-5 point scale).

[2] Orthogonal contrasts: C1 (Block supplementation effect): control (CC) *vs.* block supplementation (BB+BC+CB); C2 (Block supplementation effect in both pre and postpartum periods): Pre and postpartum (BB) *vs.* Pre or postpartum (BC+CB) and C3 (Pre or postpartum effect): prepartum (BC) *vs.* postpartum (CB); T= Time, days relative to calving; T x treat= interaction between sampling time and treatment.

[a] 90 days prepartum= at the beginning of supplementation (D-90)

[b] 40 days postpartum= at the onset of the synchronization protocol (D40)

[c] 80 days postpartum= at pregnancy diagnosis and resynchronization (D80)

[d] 120 days postpartum= at the end of supplementation and second pregnancy diagnosis (D120)

[e] 170 days postpartum= at final pregnancy diagnosis after two FTAI’s and natural mating (D170)

Table 5. Effect of prepartum and/or postpartum supplementation for calf performance evaluated at 5 different times.

Items[1]	Treatment				SEM	P value[2]				
	CC	CB	BC	BB		T	T x Treat	C1	C2	C3
CW, Kg						<0.0001	<0.0001	0.11	0.20	0.13
Birth	34.6	35.4	35.7	35.6	0.65	-	-	0.01	0.89	0.16
80 days[a]	97.8	98.7	99.7	106.4	0.78	-	-	0.03	<0.001	0.75
120 days[b]	123.9	132.9	126.5	139.3	0.91	-	-	<0.001	<0.001	0.002
170 days[c]	182.9	183.7	184.8	193.3	1.18	-	-	0.55	0.002	0.93
Weaning[d]	207.7	208.8	210.0	216.2	1.45	-	-	0.38	0.02	0.74

SEM= Standard error of the mean

[1] CW= Calf body weight.

[2] Orthogonal contrasts: C1 (Block supplementation effect): Control (CC) *vs.* block supplementation (BB+BC+CB); C2 (Block supplementation effect in both pre and postpartum periods): Pre and postpartum (BB) *vs.* Pre or postpartum (BC+CB) and C3 (Pre or postpartum effect): prepartum (BC) *vs.* postpartum (CB). T= Time, days relative to calving; T x treat= interaction between sampling time and treatment.

[a] 80 days= Calf body weight at 80 days old (D80)

[b] 120 days= Calf body weight at 120 days old and at the end of the supplementation period (D120)

[c] 170 days = Calf body weight at 170 days old (D170)

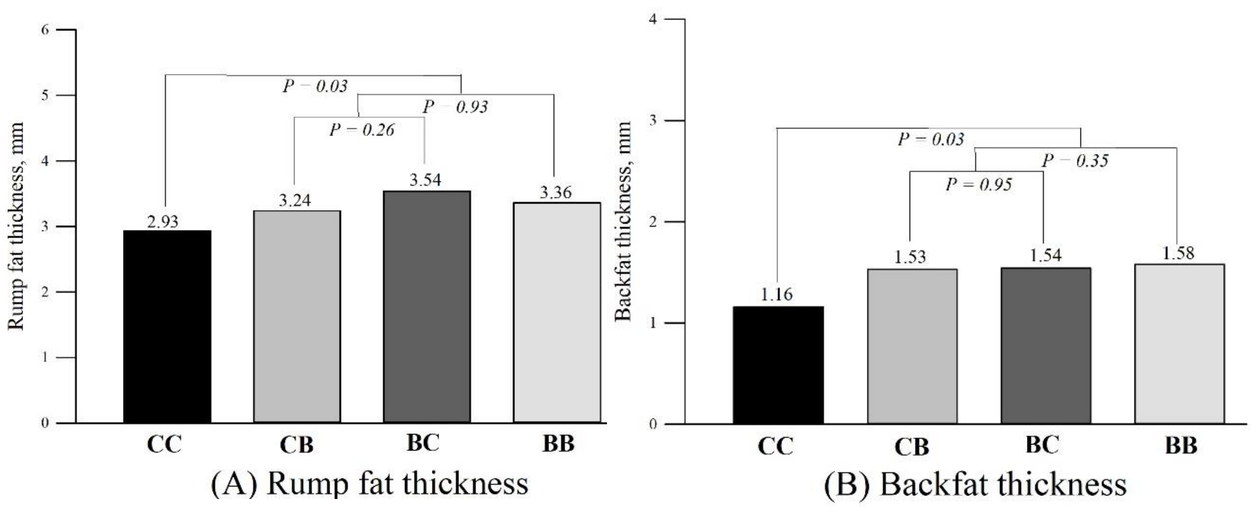
[d] Weaning= Calf body weight at 210 days old (D210)

4.3.3. Subcutaneous fat thickness

The subcutaneous fat thickness according to each group is presented in figure 6. The rump fat thickness was greater for cows supplemented with blocks than control cows (3.36±0.10 *vs.* 2.93±0.17 mm; P_{C1}=0.03). Likewise, there was a block supplementation effect for backfat thickness (Block supplementation: 1.58±0.07 *vs.* Control: 1.16±0.16 mm; P_{C1}=0.03). The block supplementation during pre and postpartum (C2) did not affect RFAT (P_{C2}=0.93) and BFAT (P_{C2}=0.35). Additionally, there were no differences for RFAT (P_{C3}=0.26) and BFAT (P_{C3}=0.95) in cows supplemented only during pre or postpartum (C3).

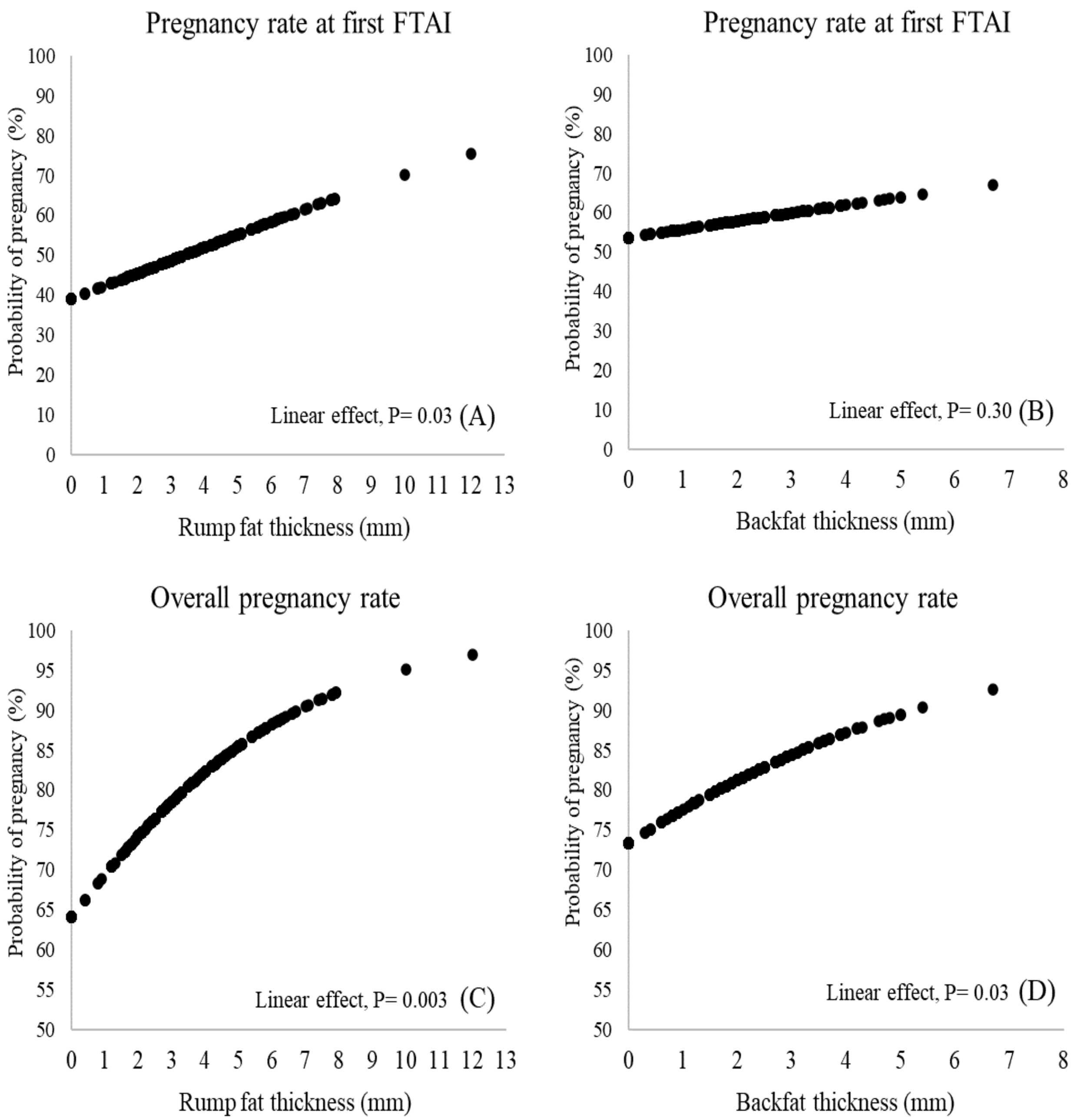
The probability of primiparous to becoming pregnant at first FTAI (P = 0.03) and at the end of the BS (P = 0.003) increased linearly as RFAT increased on D40. BFAT also showed an effect on pregnancy probability at the end of the BS (P=0.03). However, pregnancy probability at first FTAI was not influenced (P = 0.30) by BFAT on D40. Data are presented in Figure 7.

Figure 6. Subcutaneous fat thickness (mm) 40 days after parturition according to groups in primiparous Nelore cows. Subcutaneous rump fat thickness (RFAT; figure 6A) and subcutaneous backfat thickness (BFAT; figure 6B).



Orthogonal contrasts: C1 (Block supplementation effect): Control (CC) *vs.* block supplementation (BB+BC+CB); C2 (Block supplementation effect in both pre and postpartum periods): Pre and postpartum (BB) *vs.* Pre or postpartum (BC+CB) and C3 (Pre or postpartum effect): prepartum (BC) *vs.* postpartum (CB).

Figure 7. Probability of pregnancy in primiparous Nelore cows (n = 417) according to subcutaneous fat thickness at D40 (onset of FTAI protocol). Probability of pregnancy at first FTAI according to RFAT (Figure 7A) and BFAT (Figure 7B) and at the end of the breeding season (overall pregnancy rate) according to RFAT (Figure 7C) and BFAT (Figure 7D).



4.3.4. Metabolites and hormone profile

The metabolite and hormone concentrations over time are presented in Table 6 and figure 8, according to each group. There was an interaction between time and treatment for glucose concentration (P=0.002), and urea concentration (P=0.07), but not for insulin (P=0.91) and IGF-1 (P= 0.79) concentration. Block supplementation effect (C1) was observed for glucose at D40 (Block supplementation= 101.5±1.9 *vs.* Control= 89.9±4.9 mg/dL; P_{C1}=0.01), but not at D80 (P_{C1}=0.15). Likewise, the urea concentration was higher for supplemented cows when compared with control cows at D40 (10.31±0.7 *vs.* 14.03±0.8 mg/mL; P_{C1}=0.03), but not at D80 (P_{C1}=0.41). Insulin serum concentration was higher for cows supplemented with blocks (P_{C1}=0.008). However, no effect for IGF-1 concentration was observed among groups (P_{C1}=0.24).

Cows supplemented with blocks during both pre and postpartum did not differ for glucose (P_{C2}=0.76), insulin (P_{C2}=0.13), IGF-1 (P_{C2}=0.35) or urea concentrations (P_{C2}=0.08) compared to cows supplemented during either pre or postpartum (C2). Furthermore, there was no effect of block supplementation in only pre or postpartum (C3) for insulin (P_{C3}=0.26) or IGF-I (P_{C3}=0.99) concentrations. Nevertheless, plasma concentration of glucose at D80 was greater in cows supplemented only during prepartum (Prepartum= 108.3±5.6 mg/dL *vs.* Postpartum= 89.3±6.7; P_{C3}= 0.03), but no effect was observed at D40 (P_{C3}=0.65). The urea concentration at D40 was higher in cows that were only supplemented during postpartum (Prepartum= 10.81±0.9 *vs.* Postpartum= 16.24±1.6 mg/dL; P_{C3}=0.004), but not at D80 (P_{C3}=0.91).

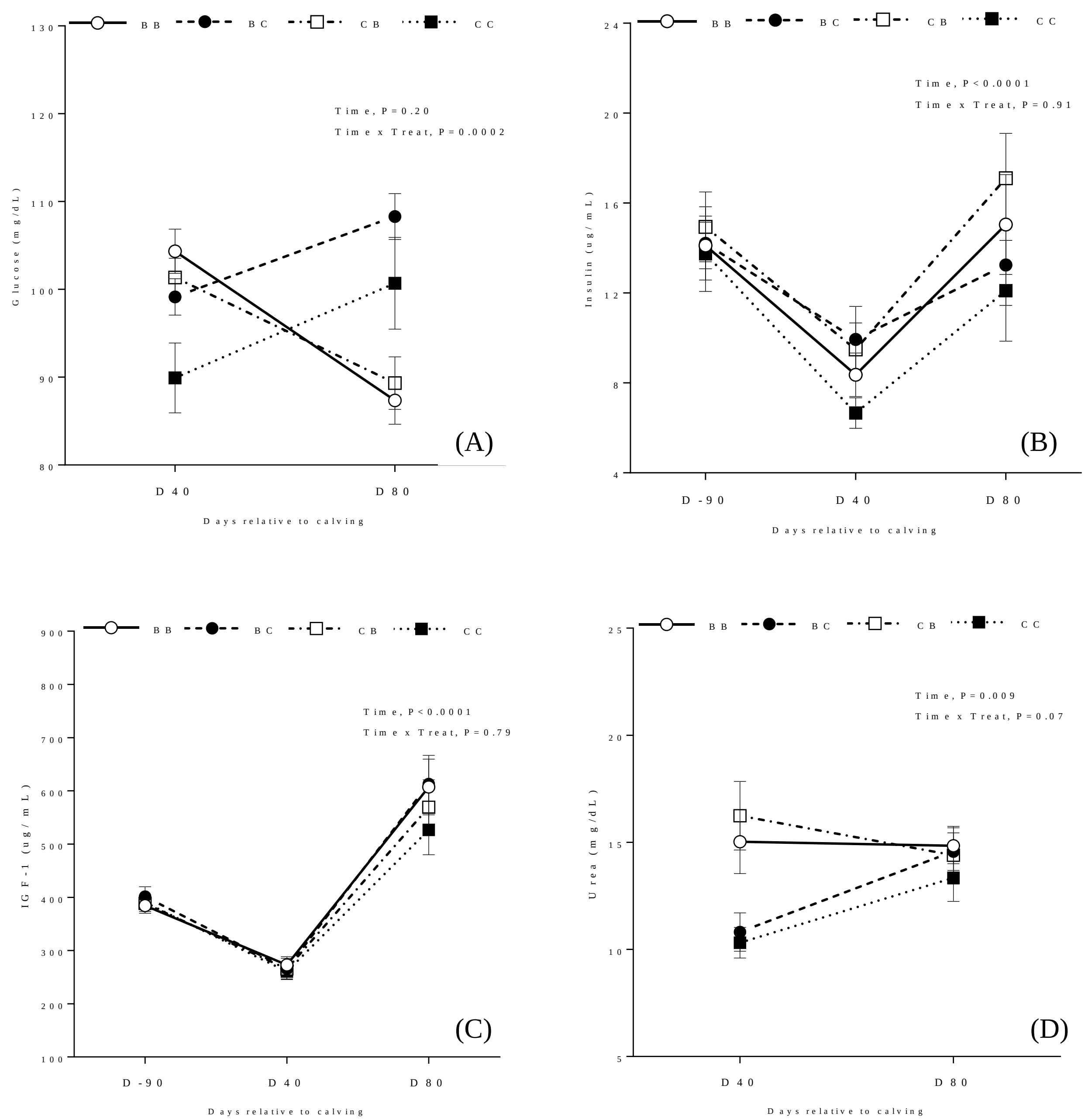
Table 6. Effect of prepartum and/or postpartum supplementation with blocks on metabolic and hormone concentration of primiparous Nelore cows evaluated at different times.

Itens[1]	Treatment				SEM	P value[2]				
	CC	CB	BC	BB		T	Treat x T	C1	C2	C3
Insulin, µg/ mL	**(10.80)**	**(13.88)**	**(12.39)**	**(12.55)**	0.49	<0.0001	0.91	0.008	0.13	0.26
90 days prepartum[a]	13.75	14.94	14.21	14.11	0.78	-	-	-	-	-
40 days postpartum[b]	6.66	9.49	9.93	8.63	0.58	-	-	-	-	-
80 days postpartum[c]	12.10	17.11	13.25	15.04	1.05	-	-	-	-	-
IGF-1, ng/ mL	**(388.5)**	**(405.8)**	**(423.8)**	**(418.7)**	11.16	<0.0001	0.79	0.24	0.35	0.99
90 days prepartum[a]	390.6	388.7	401.3	384.6	7.57	-	-	-	-	-
40 days postpartum[b]	260.7	265.4	265.7	273.1	7.59	-	-	-	-	-
80 days postpartum[c]	526.9	569.4	613.0	607.4	20.70	-	-	-	-	-
Glucose, mg/dL						0.20	0.0002	0.83	0.76	0.20
40 days postpartum[b]	89.9	101.6	99.1	104.3	1.83	-	-	0.01	0.34	0.65
80 days postpartum[c]	100.7	89.3	108.3	87.4	3.16	-	-	0.15	0.11	0.03
Urea, mg/dL						0.009	0.07	0.04	0.19	0.22
40 days postpartum[b]	10.31	16.24	10.81	15.03	0.70	-	-	0.03	0.33	0.004
80 days postpartum[c]	13.34	14.40	14.57	14.44	0.51	-	-	0.41	0.97	0.91

( ) The mean of the treatments is identified in parentheses.
SEM= Standard error of the mean.
[2]Orthogonal contrasts: C1 (Block supplementation effect): Control (CC) *vs.* block supplementation (BB+BC+CB); C2 (Block supplementation effect in both pre and postpartum periods): Pre and postpartum (BB) *vs.* Pre or postpartum (BC+CB) and C3 (Pre or postpartum effect): prepartum (BC) *vs.* postpartum (CB).
[a] 90 days prepartum= at the beginning of supplementation (D-90).
[b] 40 days postpartum= at the onset of the synchronization protocol (D40).

[c] 80 days postpartum= at pregnancy diagnosis and resynchronization (D80)

Figure 8. Effect of prepartum and/or postpartum supplementation with blocks on glucose plasma concentration (A), insulin serum concentration (B), IGF-1 serum concentration (C) and urea plasma concentration (D) of primiparous Nelore cows evaluated at different times.



4.4. Discussion

In the present study, supplementation with low-moisture molasses blocks increased BCS at parturition, improved the pregnancy at first FTAI, as well as the overall pregnancy rate at the end of the BS. Furthermore, cows supplemented with blocks had great BW, BCS and a high index of subcutaneous backfat and rump fat thickness at the onset of the synchronization protocol, confirming our initial hypothesis. Several studies have shown a positive relationship between high BCS at parturition and fertility (Ayres et al., 2014; DeRouen et al., 1994; Hess et al., 2005; Shoup et al., 2015; Vizcarra et al., 1998). Additionally, other authors have demonstrated a strong correlation between subcutaneous fat index and the amount of fat in the carcass (Ayres et al., 2009; Williams, 2002). Body energy reserves are an essential source of readily available energy for reproduction, and beef cows had a greater likelihood of conceiving postpartum if they had greater BCS and rump fat thickness at parturition and during postpartum (Ayres et al., 2014; D'Occhio et al., 2019a; Hess et al., 2005; Wiltbank et al., 1962).

Nutrition impacts reproduction through various changes in metabolic hormones (D'Occhio et al., 2019b). Blood glucose can be used as measures of the energy status, and it is the primary fuel source used by the central nervous system which plays a major role in the release of GnRH (Hess et al., 2005; Meteer et al., 2015; Short and Adams, 1988; Vizcarra et al., 1998). In the present study, the glucose concentration was higher for cows supplemented with blocks in postpartum. The composition of the block used in this study contains sodium monensin. Ionophores as monensin have been used in supplementation programs to grazing ruminants, especially with low-quality forages (Bohnert et al., 2016). Monensin increases the production of propionate and decreases the acetate/propionate ratio, improving DM and protein digestibility, and increasing gluconeogenesis and glucose turnover (Schelling, 1984). Researchers reported that monensin supplementation decreases the interval between parturition and first estrus in beef cows (Hardin and Randel, 1983), and increases dominant follicle diameter in beef heifers (Reed and Whisnant, 2001), as well as in postpartum Nelore cows (Matos et al., 2004). It is important to mention that the experimental design does not allow us to understand the exact effect of monensin, but it makes sense to speculate that a greater glucose concentration found 40 days on postpartum may be associated with the monensin treatment.

Insulin and IGF-I synthesis are directly influenced by energy intake and circulating glucose concentrations (Laskowski et al., 2016; Vizcarra et al., 1998). The insulin concentration was higher for cows supplemented with blocks, although IGF-I was only numerically greater. All these changes in the endocrine and metabolic profile in cows supplemented with blocks may explain the higher pregnancy rate in the first TAI at the early postpartum (Figure 4). For pasture-based systems, high pregnancy rates at the beginning of the BS are critical for herd profitability (Sá Filho et al., 2013). According to Baruselli et al. (2018), cows exposed to FTAI at the beginning of the BS calved earlier, weaning heavier calves, and had improved probability of re-conception in the subsequent BS.

A greater overall pregnancy rate at the end of BS was verified in this study, which means more calves would be born in the next season. Besides the increase on reproductive performance, cows supplemented with blocks presented good BCS at parturition and during the postpartum. Moreover, calves born by cows supplemented with blocks were heavier from 80 to 170 days old (end of supplementation period=120 days). Previous studies observed that cows with good BCS at calving tend to wean heavier and healthier calves and this has important implications for young heifers destined to become breeders (D'Occhio et al., 2019a; Freitas et al., 2021).

Although supplementation with blocks during pre and postpartum *vs.* during either pre or postpartum did not promote sufficient changes in reproductive performance (C2), primiparous cows supplemented in both periods (pre and postpartum) had greater BCS at 80 days postpartum than cows supplemented in only one period (pre or postpartum). Improving maternal nutrition during postpartum improves milk yield and the higher calf nutrient intake acts upon the somatotropic axis, increasing calf growth and weight at weaning (Callaghan et al., 2020). As a result, CW was higher until weaning for calves born by cows treated before and after parturition (figure 5). This data corroborates with other authors that have shown a positive effect on calf growth at weaning when cows were supplemented during both pre and postpartum periods (Spitzer et al., 1995; Stalker et al., 2006). The main focus for beef cow supplementation is usually to improve reproductive functions, but the enhancement of the nutritional status of beef cow diets may also influence the development of the future calf (Bohnert et al., 2013; Wu et al., 2004).

When comparing block supplementation in only pre and only postpartum periods (C3), a positive effect for block supplementation in prepartum was observed for BCS at parturition. However, 170 days after parturition cows supplemented in postpartum showed greater BCS.

Primiparous cows appear to be more sensitive to nutrient intake and consequently, BCS changes more drastically comparing to mature cows (Moura et al., 2020; Spitzer et al., 1995).

At the onset of the synchronization protocol, plasma concentrations of urea were higher for cows supplemented with blocks during postpartum. Blood urea nitrogen is traditionally a biological marker for CP or rumen degradable protein (Hill et al., 2018; Sotelo et al., 2018). Studies have documented that the relationship between blood urea nitrogen and fertility in dairy cattle is negatively correlated (>19mg/DL results in low fertility; Butler et al., 1996; Rhoads et al., 2006). Conversely, in beef cattle, urea concentration is not negatively associated with pregnancy risk (Gunn et al., 2016), whereas optimal urea concentration in beef cows ranges from 10 to 25 mg/ dL (Gunn et al., 2016; Hill et al., 2018). Our data showed that regardless of treatment, cows had urea concentration ranging from 10 to 16 mg/ dL, which suggest that all the cows in the present study consumed adequate amounts of CP. The blocks supplements are highly palatable, which gives them the ability to mask undesirable flavors, such as urea and monensin (Kunkle et al., 2000; Moriel et al., 2019; Trater et al., 2003). Hence, this particularity of block supplements may be associated with higher urea concentrations 40 days postpartum for cows supplemented only in the postpartum period.

Additionally, cows supplemented only in postpartum had greater glucose concentration at D80, which means better metabolic/nutritional status. Hence, the calves born by cows treated in postpartum were heavier at 120 days old. Notwithstanding, no CW differences at weaning were observed, probably because the supplementation ended at 120 days old and not at weaning (210 days old).

In the present study, it was observed a high probability of primiparous to become pregnant at first FTAI and at the end of the BS according to RFAT. However, lower relationships were observed for BFAT and pregnancy probability. Studies have found that the RFAT measure is an indicator of total carcass fat, however, BFAT is directly related to carcass yield grade (Williams, 2002). These characteristics of fat deposition in the carcass can explain the differences in the accuracy of the pregnancy probability analysis between RFAT and BFAT found in the current research.

In conclusion, regardless of period of treatment, block supplementation increased BCS at parturition, pregnancy rate at first FTAI and overall pregnancy rate. Likewise, forty days postpartum, BW, BCS, RFAT, BFAT and glucose concentration were greater for supplemented cows. Also, supplemented cows had greater insulin concentrations. Block supplementation during both pre and postpartum periods improved progeny growth until weaning. Under the conditions of the current experiment, block supplementation only during pre *vs.* only during postpartum did not affect reproductive performance in primiparous cows. Block supplementation can be a tool to optimize fertility and calf performance in Nelore primiparous cows, facilitating nutritional management on farms.

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5. GENERAL CONCLUSION

5.1. CONCLUSIONS OF CONTRAST 1 (Block supplementation effect, regardless period: pre and postpartum)

Primiparous cows supplemented with blocks had greater BW (D40), BCS (D0, D40, D80), subcutaneous fat thickness (D40), glucose (D40) and insulin concentrations. Furthermore, pregnancy rate at first FTAI and overall pregnancy rate (1st FTAI + 2nd FTAI + NM) were greater for supplemented than control cows. However, IGF1 and urea plasma concertation did not differ among groups. Although calves born by supplemented cows were heavier 80 and 120 days old, no difference was observed at weaning. ***The initial hypothesis was partially confirmed.***

5.2. CONCLUSIONS OF CONTRAST 2 (Block supplementation effect during both pre and postpartum periods)

Block supplementation during both pre and postpartum periods improved cow BCS (D80) and progeny growth until weaning. However, there were no differences for BW, subcutaneous fat thickness, metabolites, and hormones, nor pregnancy rates. ***The initial hypothesis was partially confirmed.***

5.3. CONCLUSIONS OF CONTRAST 3 (Block supplementation effect only during pre or postpartum)

Primiparous cows supplemented only during prepartum had greater BCS at parturition (D0). However, the BW and urea concentration was lower at D40 for cows supplemented during prepartum than postpartum. Cows supplemented during prepartum had higher glucose concentrations at D80. No differences for SFAT, insulin and IGF-1 concentrations and pregnancy rates were observed among groups. The supplementation during postpartum increased calf body weight at 120 days old, but not at weaning. ***The initial hypothesis was not confirmed.***

6. PRACTICAL IMPLICATIONS

In Brazil, beef cattle are maintained in pasture conditions, which present high variability in quantity and quality during the year. Considering climatic conditions in the southern hemisphere, the nutritive value of forages is often limited in winter (dry season), which coincides in general with the last trimester of gestation in beef cows. In this context, nutritional supplementation programs become necessary to attend to the high gestational energetic/protein demand and improve reproductive efficiency as well as cattle productivity. However, feeding supplements can be expensive and increase production costs, including expenses associated with the purchase of the feed as well as the labor required for daily supplementation. Therefore, technology that facilitates farm management, reduces costs and increases production must be investigated further.

Low-moisture molasses blocks are a popular supplementation strategy in some countries, such as the United States, Australia, and New Zealand, due to their convenience. The block technology offers an advantage over loose meal supplements because it limits intake. Therefore, the blocks can be delivered to the herd less frequently (once a week), reducing farm labor requirements. Also, block self-limiting intake characteristics allow grazing animals continuous access to the supplement and intake can occur more than once a day. Moreover, molasses blocks are highly palatable, which allow block formulations to contain high levels of unpalatable ingredients, such as monensin and urea, which can increase forage intake and digestion.

Despite all the advantages of block supplementation mentioned above, its cost is generally higher than a conventional supplement. Thus, an analysis was carried out to assess the economic impact of block supplementation in a commercial Brazilian farm (Table 7). The costs were calculated based on the actual purchase price (December 2020), including delivery to the farm, to assign a value to the block supplement (R$2.77/kg) and control supplement (R$1.84/kg; $1.00 = R$5.20). The labor and fuel costs considered the frequency and distance to deliver the supplements in the paddocks. The sale value of calves at weaning (R$1,856/calf and R$10/kg) was the 2-yr average price by CEPEA (2019 to 2020). Furthermore, it can be assumed that calves born in the first FTAI could have an additional weight gain of 10kg by the next weaning due to the optimal birth season. In addition, this fact leads to a reduced number of resynchronization protocols needed among the cows (R$60 per protocol). The total costs for block supplementation during pre and postpartum (BB) were higher among groups. On the other hand, the reproductive and productive gains exceeded the costs, generating an economic return of around R$ 22,049.32 per 100 supplemented cows. From these data is possible to calculate the return on investment of this supplementation program. Each R$ 1.00 invested on block supplementation, there is a return of R$ 2.60 for the beef producer. The block supplementation in only one of the periods (pre or postpartum) also brings gains to the producer in relation to the control supplementation. However, block supplementation in both periods is 5 times more profitable.

The results of the present study provide an opportunity for farmers to consider the use of block supplementation as a practical tool for enhancing reproductive performance and weight gain of calves, contributing to the economic gains of livestock.

Table 7. The economic impact of different supplementation strategies. For analyses was considered 100 animals for each group.

Costs

	CC	CB	BC	BB
Supplement cost	R$ 10.411,27	R$ 12.352,62	R$ 11.028,07	R$ 12.969,42
labor and fuel cost	R$ 1.219,09	R$ 754,68	R$ 870,78	R$ 406,36
Total expenses	**R$ 11.630,36**	**R$ 13.107,30**	**R$ 11.898,85**	**R$ 13.375,78**

Revenue

	CC		CB		BC		BB	
Calf extra weaning, Kg	0	R$ 0,00	0,3	R$ 330,00	1,8	R$ 1.980,00	7,9	R$ 8.690,00
Anticipation of 1st P/AI, %	0	R$ 0,00	7,9	R$ 3.950,00	7,8	R$ 3.900,00	14,5	R$ 8.120,00
Pregnancy rate at the end of the BS, %	0	R$ 0,00	6,9	R$ 12.806,40	5,6	R$ 10.393,60	9,6	R$ 17.817,60
Reduction of resynchronization protocols, %	0	R$ 0,00	7,9	R$ 434,50	7,8	R$ 429,00	14,5	R$ 797,50
Total revenue		**R$ 0,00**		**R$ 17.520,90**		**R$ 16.702,60**		**R$ 35.425,10**
Economic gain				***R$ 4.413,60***		***R$ 4.803,75***		***R$ 22.049,32***
% of the amount invested				34%		40%		165%
Investment / return ratio				1,3		1,4		2,6

Block supplement= R$ 2.77; Control supplement= R$1.77; calves at weaning= R$1856.00/calf and R$10.00/kg; FTAI protocol= R$60.00

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