ALINE GOMES DA SILVA

SUPPLEMENTATION PLANS FOR DEVELOPMENT OF NELLORE HEIFERS, EFFECTS OF CREEP-FEEDING ON THE LACTATING DAM AND DIETARY STRATEGIES FOR NELLORE COWS IN THE LAST THIRD OF GESTATION

Doctoral thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of Doctor Scientiae.

Viçosa
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Approved: March 9, 2016.

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I dedicate this to my father and my mother, whose examples have guided my steps. They have given me the most valuable legacy parents can give to a child: love, education and the encouragement to pursue knowledge.

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"Success means having the courage, the determination, and the will to become the person you *believe you were meant to be."* – Dr. George Sheehan

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BIOGRAPHY

Aline Gomes da Silva, daughter of Arlindo Dávila da Silva and Ely Gomes da Silva, was born in Muqui/ES-Brazil on November 6, 1988.

She started the undergrad in Animal Science at Universidade Federal de Viçosa in 2006 and became a Bachelor of Science in Animal Science in 2010. At the same year she started the M.S. program with major on ruminant nutrition and beef cattle production.

In February of 2012 she became a M.S. in Animal Science. At the same year she started the doctorate program in Animal Science with major on ruminant nutrition and beef cattle production, with sandwich period at the West Central Research and Extension Center/University of Nebraska-Lincoln in North Platte/NE, USA. On March 9th of 2016 Ms. da Silva defended her doctoral thesis to obtain the Doctor Scientiae degree in Animal Science.

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ABSTRACT

SILVA, Aline Gomes da, D.Sc., Universidade Federal de Viçosa, March of 2016. **Supplementation plans for development of Nellore heifers, effects of creep-feeding on the lactating dam and dietary strategies for Nellore cows in the last third of gestation**. Adviser: Mário Fonseca Paulino. Co-Advisers: Edenio Detmann and Luciana Navajas Rennó.

Reproduction is the single most important economic component in any beef production system and the low offtake rate observed in the Brazilian herd reflects the low fertility rate of cows and the late age at puberty of replacement heifers. In order to provide more information about nutritional strategies to better develop Nellore heifers and to improve dams' performance, two studies were conducted and the results are here presented in three papers. In the first paper, fifty Nellore heifers with 131.8 ± 9.9 kg average body weight (BW) and 138 ± 19 d of age were supplemented from 4 to 14 months to evaluate the effects of high (H) and low (L) supplementation levels pre and post-weaning on performance, endocrine, metabolic and reproductive responses of heifers. Heifers were distributed in five supplementation plans: HH – animals received 6 g/kg of BW pre and post-weaning; HL – animals received 6 g/kg of BW of supplement pre-weaning and 3 g/kg of BW post-weaning; LH – animals received 3 g/kg of BW pre-weaning and 6 g/kg of BW post-weaning; LL – animals received 3 g/kg of BW pre and post-weaning; and CC – control, no supplement was fed. Interactions between level fed pre and post-weaning were not significant for any performance variables evaluated ($P > 0.10$). Level of supplement fed pre-weaning did not affect any of the performance variables evaluated at the end of the experiment ($P > 0.10$). There was a significant effect of supplementation and level of supplementation offered post-weaning on average daily gain (ADG) in the post-weaning phase and final BW ($P < 0.05$). Overall ADG was also affected only by supplementation and level of supplement fed post-weaning ($P < 0.05$) with animals receiving 6 g/kg of BW post-weaning gaining more. Follicular diameter was greater for animals receiving 6 g/kg of BW post-weaning ($P < 0.05$). Growth hormone tended to be higher for heifers receiving 6 g/kg of BW pre-weaning ($P < 0.10$) and in the post-weaning phase, growth hormone was lower for heifers receiving supplementation ($P < 0.05$). No difference in insulin levels was observed in the pre-weaning phase ($P > 0.10$) and insulin levels post-weaning were affected only by level of supplementation offered in that phase ($P < 0.05$). Most metabolites were not affected by pre-weaning treatment ($P > 0.10$). There was no difference in total cholesterol, HDL and LDL levels among treatments pre-weaning ($P > 0.10$). All metabolites related to fat metabolism reduced after weaning but only LDL was significantly different among treatments ($P < 0.05$). There was a positive effect of level of

supplementation post-weaning on serum total protein concentration ($P < 0.05$). Supplementation increased albumin levels ($P < 0.05$) and animals receiving high amount of supplement had higher albumin levels as well ($P < 0.05$). There was no significant difference in globulins concentrations ($P > 0.10$). In summary, performance, endocrine, metabolic and reproductive variables evaluated were improved by level of supplement fed post-weaning, with heifers receiving 6 g/kg of BW having greater responses, independently of level received pre-weaning. The same fifty pairs used in the study described for the previous paper were used in the second paper to evaluate the effects of high and low supplementation levels for Nellore heifer calves on performance, milk production and metabolic prolife of their dams. The same treatments evaluated during the pre-weaning phase of the previous paper were also here evaluated: 0 – control, no supplement was fed to calves; 3 - calves received supplement in the amount of 3 g/kg of BW; 6 - calves received supplement in the amount of 6 g/kg of BW. There was no significant effect level of supplementation offered to offspring on cow BW, BCS and subcutaneous fat thickness ($P > 0.10$). Level of supplementation of heifer calves did not significantly affect milk production corrected to 4% of fat ($P > 0.10$). Fat, protein, lactose and total solids of the milk also did not differ among supplementation strategies ($P > 0.10$). Level of supplement fed to calves had no effect on cows' glucose, total cholesterol, HDL, LDL, triglycerides, total protein and albumin levels ($P > 0.10$). In conclusion, creep-feeding calves in the amounts of 3 or 6 g/kg of BW daily has no major impact on dams' performance. In the second study, presented in the third paper, two experiments were conducted aiming to evaluate the effects of supplementation strategies for beef cows on the last third of gestation. In Experiment 1, to evaluate performance and reproductive responses, thirty-five pregnant Nellore cows were assigned to a completely randomized design with 4 treatments: control, which received no supplement; supplementation for the last 30 d of gestation (30d; 3.0 kg/d); supplementation for the last 60 d of gestation (60d; 1.5 kg/d); or supplementation for the last 90 d of gestation (90d; 1.0 kg/d). All supplemented treatments received the same total amount of supplement (20% of crude protein) throughout the experiment, 90 kg. Experiment 2 was delineated aiming to evaluate the effects of the amounts fed for cows in Experiment 1 on intake and metabolism. Four multiparous pregnant Nellore cows were assigned to a 4 × 4 Latin square design, with 4 experimental periods of 15 d each. There was a linear effect of days of supplementation on calving body weight (BW; $P < 0.05$) and a quadratic effect on BW change from parturition to d 31 post-calving ($P < 0.05$), with cows on the 60d strategy loosing less BW. No difference

was found in offspring birth BW ($P > 0.10$). The 60d strategy tended to have higher pregnancy rate in the first fixed time artificial insemination (FTAI; $P < 0.10$), but there was no difference in the overall pregnancy rates ($P > 0.10$). Level of supplementation did not affect forage intake or neutral detergent fiber digestibility ($P > 0.10$). Nitrogen excreted through urine tended to increase linearly with level of supplementation ($P < 0.10$). We conclude that providing 1.5 kg of supplement during the last 60 d of gestation improves cow reproductive performance in the following breeding season with no negative effect on forage intake.

RESUMO

SILVA, Aline Gomes da, D.Sc., Universidade Federal de Viçosa, março de 2016. **Planos de suplementação para desenvolvimento de novilhas Nelore, efeitos do creep-feeding sobre a matriz lactante e estratégias dietéticas para vacas Nelore no terço final de gestação.** Orientador: Mário Fonseca Paulino. Coorientadores: Edenio Detmann e Luciana Navajas Rennó.

A reprodução é o componente econômico individual de maior importância em sistemas de produção de carne bovina e a baixa taxa de desfrute observada no rebanho brasileiro reflete a baixa taxa de fertilidade de vacas e a elevada idade à puberdade das novilhas de reposição. Com o intuito de fornecer mais informações sobre estratégias nutricionais para desenvolver novilhas e melhorar o desempenho de matrizes, dois estudos foram realizados e são aqui apresentados na forma de três artigos. Cinquenta novilhas Nelore com 131,8 ± 9,9 kg de peso corporal (PC) médio e 138 ±19 dias de idade foram suplementadas dos 4 aos 14 meses para avaliar os efeitos de alto (A) e baixo (B) níveis de suplementação durante as fases de amamentação e recria sobre o desempenho e as respostas endócrina, metabólica e reprodutiva. Os animais foram distribuídos em cinco planos de suplementação: AA – as novilhas receberam suplemento na quantidade de 6 g/kg do PC durante a amamentação e recria; AB – as novilhas receberam 6 g/kg do PC na fase de amamentação e 3 g/kg do PC durante a recria; BA – as novilhas receberam 3 g/kg do PC durante a amamentação e 6 g/kg do PC durante a recria; BB – as novilhas receberam 3 g/kg do PC durante a amamentação e recria; e CC – controle, nenhum suplemento foi oferecido, apenas sal mineral. Interação entre os níveis fornecidos na amamentação e recria não foram significativos para nenhuma das variáveis de desempenho avaliadas ($P > 0,10$). O nível de suplementação oferecido durante a amamentação não apresentou efeito sobre as variáveis de desempenho avaliadas posteriormente, na fase de recria ($P > 0,10$). Houve efeito significativo da suplementação e do nível de suplementação na recria sobre o ganho médio diário (GMD) na recria e PC final ($P < 0,05$). O GMD total foi também afetado apenas pela suplementação e nível de suplementação utilizado na recria ($P < 0,05$), animais que receberam 6 g/kg do PC após o desmame tiveram maior GMD total. O diâmetro folicular foi também maior para os animais que receberam 6 g/kg do PC na recria ($P < 0,05$). Na fase de amamentação, houve uma tendência para maiores níveis de hormônio do crescimento (GH) nas novilhas que receberam 6 g/kg do PC ($P < 0,10$), e na recria os níveis de GH foram menores para as novilhas suplementadas ($P < 0,05$). Não houve diferença entre os tratamentos com relação aos níveis de insulina durante a amamentação ($P > 0,10$), entretanto, os níveis de insulina foram afetados pelo nível de

suplementação utilizado na recria (P < 0,05). A maioria dos metabólitos não foi afetada pelo nível de suplementação utilizado durante a amamentação (P > 0,10). Não houve diferença nos níveis de colesterol total, HDL e LDL entre os tratamentos durante a amamentação (P > 0,10). Todos os metabólitos relacionados com o metabolismo da gordura reduziram na recria em relação à fase de amamentação, mas apenas o LDL diferiu significativamente entre os tratamentos (P < 0,05). Houve um efeito positivo do nível de suplementação sobre a concentração de proteína total no soro durante a recria (P < 0,05). A suplementação também aumentou os níveis de albumina na recria (P < 0,05) e, entre os animais suplementados, animais que receberam alta quantidade de suplemento durante a recria apresentaram níveis mais elevados de albumina (P < 0,05). Mas não houve diferença significativa nas concentrações de globulinas (P > 0,10). Em resumo, o desempenho e variáveis endócrina, metabólica e reprodutiva foram incrementados pelo nível de suplementação fornecido na recria, novilhas que receberam suplemento na quantidade de 6 g/kg do PC na recria apresentaram melhores respostas de forma independente do nível recebido anteriormente, durante a amamentação. Os mesmos cinquenta pares vaca-bezerra utilizados no estudo descrito no primeiro artigo foram também utilizados para avaliar os efeitos de alto e baixo níveis de suplementação para bezerras Nelore sobre o desempenho, a produção de leite e o perfil metabólico de suas mães apresentado no segundo artigo. Os mesmos tratamentos avaliados durante a fase de amamentação do artigo anterior foram também aqui avaliados: 0 - controle, nenhum suplemento foi fornecido às bezerras; 3 – as bezerras receberam suplemento em quantidade de 3 g/kg do PC; 6 - as bezerras receberam suplemento em quantidade de 6 g/kg do PC. Não houve efeito significativo do nível de suplementação fornecido à prole sobre o PC, escore de condição corporal e espessura de gordura subcutânea da vaca ao desmame (P > 0,10). O nível de suplementação das bezerras não afetou significativamente a produção de leite corrigida para 4% de gordura (P > 0,10). Os teores de gordura, proteína, lactose e sólidos totais do leite também não diferiram entre os níveis de suplementação (P > 0,10). O nível de suplemento fornecido à prole não influenciou os teores de glicose, colesterol total, HDL, LDL, triglicerídeos, proteínas totais, albumina e globulinas (P > 0,10). Em conclusão, a suplementação de bezerras lactentes nas quantidades de 3 ou 6 g/kg do PC diariamente não afeta o desempenho da matriz lactante. No segundo estudo, apresentado no terceiro artigo, foram realizados dois experimentos com o objetivo de avaliar os efeitos de estratégias de fornecimento de suplemento para vacas de corte no terço final da gestação. No Experimento 1, delineado para avaliar o desempenho e as respostas reprodutivas, 35 vacas Nelore gestantes

foram distribuídas em delineamento experimental inteiramente casualizado com quatro tratamentos: controle - os animais receberam apenas sal mineral durante todo o experimento; 30d – os animais receberam suplementação durante os últimos 30 dias de gestação na quantidade de 3.0 kg/d; 60d – os animais receberam suplementação durante os últimos 60 dias de gestação na quantidade de 1.5 kg/d e; 90d – os animais receberam suplementação durante os últimos 90 dias de gestação na quantidade de 1.0 kg/d. Todos os tratamentos suplementados receberam a mesma quantidade total de suplemento ao longo do experimento (90 kg; 20% de proteína bruta). O Experimento 2 do terceiro artigo foi delineado com o objetivo de avaliar os efeitos das quantidades que foram diariamente fornecidas para as vacas do Experimento 1 sobre o consumo e metabolismo. Para tanto, quatro vacas Nelore multíparas e gestantes foram utilizadas em delineamento quadrado latino 4 × 4, com 4 períodos experimentais de 15 dias cada. Houve efeito linear de estratégia de suplementação sobre o PC ao parto ($P < 0,05$) e efeito quadrático sobre a variação de peso pós-parto ($P < 0,05$), vacas na estratégia 60d perderam menos peso do parto até os 31 dias pós-parto. Nenhuma diferença significativa foi encontrada no PC da prole ao nascimento ($P > 0,10$). Houve uma tendência quadrática sobre a taxa de gestação na primeira inseminação artificial em tempo fixo (IATF; $P < 0,10$), mais vacas da estratégia 60d conceberam na primeira IATF, embora nenhuma diferença estatística nas taxas de gestação total tenha sido observada ($P > 0,10$). O nível de suplementação utilizado não afetou o consumo de forragem ou a digestibilidade da fibra em detergente neutro ($P > 0,10$). A excreção de N através da urina tendeu a aumentar linearmente com o nível de suplementação ($P < 0,10$). Conclui-se, portanto, que o fornecimento de 1,5 kg de suplemento durante os últimos 60 dias de gestação melhora o desempenho reprodutivo de vacas de corte na estação de monta subsequente sem nenhum efeito negativo sobre o consumo de forragem.

INTRODUCTION

Reproduction is the single most important economic component in any beef production system (Willham, 1973). The low offtake rate observed in the Brazilian herd reflects the low fertility rate of cows and the late puberty of replacement heifers.

Nellore breed represents 90% of the Brazilian beef herd. Although well adapted to tropical conditions, Bos indicus heifers are older and have higher body weight at puberty when compared to European breeds, even when raised in similar conditions (Rodrigues et al., 2002).

Age at puberty varies depending on numerous factors including body weight, genetics, nutrition, and management. Reproduction appears to be more sensitive than the growth axis in relation to the availability of nutrients and a particular metabolic state may be required for the onset of puberty (Steiner et al., 1983). Several hormones and metabolites have been studied as nutritional signals to reproduction, for example, IGF-I, insulin, GH, leptin, glucose and others (Steiner, 1987; Schillo et al., 1992).

Understanding how nutrition affects the central nervous system and modulates the frequency of release of LH pulses from the pituitary gland has been researched for decades. Fluctuations of the intermediary metabolism associated with changes in both body mass and body fat may modulate the release of LH pulses (Schillo et al., 1992; Hiney et al., 1996).

Reserves of body fat have been related to the maintenance of the estrous cycle in cattle (Imakawa et al., 1986; Richards et al., 1989) and may act as a marker of energy available for reproductive activity (Hall et al., 1995). According to Frish (1976), there is a minimum body fat at which puberty happens. Hall et al. (1995) found greater fat thickness, higher amount of total body fat, higher body mass, and lower age at puberty in animals fed to achieve high

average daily gain (1 kg/d). On other hand, Bronson and Manning (1991) discussed that body composition has little direct physiological or biological relationship with the beginning of the estrous cycle.

The occurrence of puberty depends on the growth rate and development of the animal to support the endocrine mechanisms that result in first ovulation (Maquivar and Day, 2009). Studies have shown different results on when is the best time to accelerate growth in bovine females. Some authors have reported the occurrence of early puberty with increased rate of gain in early stages of development (Wiltbank et al., 1969; Arije and Wiltbank, 1971; Buskirk et al., 1995; Patterson et al., 1992; Gasser et al., 2006; Cardoso et al., 2014; Rodríguez-Sánchez et al., 2015), while others observed a reduction in age at puberty with higher weight gain post-weaning (Buskirk et al., 1955; Hall et al., 1995; Lammers et al., 1999; Gojjan et al., 2011; Barcellos et al., 2014; Rodríguez-Sánchez et al., 2015).

The early phase of development may be critical for the establishment of many components of the reproductive axis, thus the possibility that pre-weaning nutritional status have more influence on puberty is consistent with the dynamic changes of this period. However, establishing a phase-specific target ADG is not so simple when the existence of complex interactions between genotype and environment variability are considered.

The biological foundation of beef systems is the cow-calf pair, understanding how nutritional strategies applied to offspring can impact dams' performance is of paramount importance. It is estimated that 50% of the cows in extensive beef systems do not receive adequate nutritional management and that is one of the major reasons for low fertility rates in tropical herds (Madureira et al., 2014). Body condition score at parturition and weaning can greatly influence pregnancy rates of dams in the following breeding season. In fact, factors

that affect milk production, such as creep-feeding, can impact cows' requirements and consequently performance.

Some authors have found that creep-feeding can reduce milk production and consequently improve cows' performance (Kress et al., 1990; Fordyce et al., 1996; Henriques et al., 2011) while several others have found that supplementing calves have no effect on their mothers' performance (Valente el al., 2012; Valente et al., 2013; Moriel and Arthington, 2013).

The use of protein-energetic supplement for cows in the last third of gestation is a strategy that can be adopted in order to accumulate energy in body tissues and lessen the post calving negative energy balance. According to Baruselli et al. (2004) inadequate nutritional status at calving is the main factor limiting adequate response to synchronization protocols. Meeting nutritional requirements for pregnant cows is important to maintain an adequate supply of nutrients for development of the fetus and to ensure appropriate cow's body condition score at calving. In this context, finding the best strategy of supplementation for pregnant cows is necessary to reduce feeding costs related to gas, labor and machinery, without compromising performance.

With the information above in mind, studies were conducted aiming to:

1- Evaluate the effects of high and low supplementation levels in the pre and post-weaning phases of Nellore heifers grazing tropical pastures on performance, endocrine and metabolic responses and characteristics related to reproduction;
2- Evaluate the effects of high and low supplementation levels for Nellore heifer calves on performance, milk production and metabolic profile of their dams; and

3- Evaluate the effect of supplement delivery strategies for pregnant beef cows on the last third of gestation.

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Performance, endocrine, metabolic, and reproductive responses of Nellore heifers submitted to different supplementation levels pre and post-weaning

Abstract. Literature presents mixed results on when is the best time to accelerate growth in bovine females in order to induce precocious puberty. The present study was conducted to evaluate the effects of high and low supplementation levels pre and post-weaning on performance, endocrine, metabolic and reproductive responses of Nellore heifers. Fifty Nellore heifers with 131.8 ± 9.9 kg average body weight (BW) and 138 ± 19 d of age were supplemented from 4 to 14 months. Heifers were distributed in five supplementation plans: HH – animals received 6 g/kg of BW pre and post-weaning; HL – animals received 6 g/kg of BW of supplement pre-weaning and 3 g/kg of BW post-weaning; LH – animals received 3 g/kg of BW pre-weaning and 6 g/kg of BW post-weaning; LL – animals received 3 g/kg of BW pre and post-weaning; and CC – control, no supplement was fed. Interaction between level of supplementation offered in pre and post-weaning phases were not significant for any performance variables evaluated ($P > 0.10$). Level of supplement fed pre-weaning did not affect any of the performance variables evaluated at the end of the experiment ($P > 0.10$). There was a significant effect of supplementation and level of supplementation fed post-weaning on average daily gain (ADG) and final BW ($P < 0.05$). Overall ADG was also affected only by supplementation and level of supplement fed post-weaning ($P < 0.05$) with animals receiving 6 g/kg of BW post-weaning gaining more. Follicular diameter was greater for animals receiving 6 g/kg of BW post-weaning ($P < 0.05$). Pre-weaning, growth hormone (GH) tended to be higher for heifers receiving 6 g/kg of BW ($P < 0.10$) and GH was lower for heifers receiving supplement post-weaning ($P < 0.05$). No difference in insulin levels was observed during the pre-weaning phase ($P > 0.10$) and insulin levels post-weaning were

affected only by level of supplementation fed post-weaning ($P < 0.05$). During the pre-weaning phase, most metabolites were not affected by treatment ($P > 0.10$). There was no difference in total cholesterol, HDL and LDL levels among treatments pre-weaning ($P > 0.10$). All metabolites related to fat metabolism reduced post-weaning but only LDL was significantly different among treatments ($P < 0.10$). There was a positive effect of level of supplementation post-weaning on serum total protein concentration ($P < 0.05$). Supplementation increased albumin levels ($P < 0.05$) and animals receiving high amount of supplement had higher albumin levels as well ($P < 0.05$). There was no significant difference in globulins concentrations ($P > 0.10$). In summary, performance, endocrine, metabolic and reproductive variables evaluated in the current study were improved by level of supplement fed post-weaning, heifers receiving 6 g/kg of BW post-weaning had greater responses, independently of level received during the pre-weaning phase.

Additional keywords: body measures, GH, insulin, metabolism

Introduction

Nellore animals represent approximately 90% of the Brazilian beef herd. Although well adapted to tropical conditions, Bos indicus heifers are older and heavier at puberty when compared to European breeds, even when raised in similar conditions (Rodrigues et al., 2002). Age at puberty has an important impact on production, economic and reproductive efficiency of the future dam and varies depending on numerous factors, including body weight, genetics, nutrition, and management.

The occurrence of puberty depends on the growth rate and development of the animal to support the endocrine mechanisms that result in first ovulation (Maquivar & Day, 2009). Studies have shown different results on when is the best time to accelerate growth in bovine

females. Some authors have reported the occurrence of early puberty with increased rate of gain in early stages of development (Wiltbank et al., 1969; Arije and Wiltbank, 1971; Buskirk et al., 1995; Patterson et al., 1992; Gasser et al., 2006; Cardoso et al., 2014; Rodríguez-Sánchez et al., 2015), while others observed a reduction in age at puberty with higher weight gain post-weaning (Buskirk et al., 1955; Hall et al., 1995; Lammers et al., 1999; Gojjan et al., 2011; Barcellos et al., 2014; Rodríguez-Sánchez et al., 2015).

However, a target average daily gain (ADG) should not be established based solely on animal's physiological response, the existence of complex interactions between genotype and environment, seasonality in forage production and economic efficiency might also be considered when planning a nutritional strategy to develop heifers.

This study was conducted to evaluate the effects of high and low supplementation levels pre- and post-weaning on performance, endocrine and metabolic responses and characteristics related to reproduction of Nellore heifers.

Material and Methods

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV 0011).

Experimental Design and Treatments

The experiment was conducted at the facilities of the Department of Animal Science – Universidade Federal de Viçosa, Brazil, from February to November of 2013. Fifty Nellore heifers with 131.8 ± 9.9 kg average body weight (BW) and 138 ± 19 d of age were used (10 experimental units per treatment).

The experimental design was completely randomized in a 2 × 2 + 1 factorial design with two levels of supplementation, 6 or 3 g/kg of BW – high (H) and low (L), respectively – in two phases, pre and post-weaning. In this way, heifers were distributed in five supplementation plans: HH – animals received 6 g/kg of BW pre and post-weaning; HL – animals received 6 g/kg of BW of supplement pre-weaning and 3 g/kg of BW post-weaning; LH – animals received 3 g/kg of BW pre-weaning and 6 g/kg of BW post-weaning; LL – animals received 3 g/kg of BW pre and post-weaning; and CC – control, no supplement was fed. The supplement was composed of corn, sorghum and soybean meal and formulated to contain approximately 25% crude protein (CP) as presented in Table 1. Animals from all treatments had unlimited access to mineral salt throughout the experiment. The mineral salt was composed of 8.7% calcium, 9.0% phosphor, 18.7% sodium, 9.0% sulfur, 2400 mg/kg of zinc, 800 mg/kg of copper, 1600 mg/kg of manganese, 40.0 mg/kg of iodine, 8.00 mg/kg of cobalt, 8.16 mg/kg of selenium.

The pre-weaning phase lasted 120 d divided into three experimental periods of 40 d and the post-weaning phase lasted 180 d divided into five experimental periods of 36 d.

In the pre-weaning phase, heifer calves with their respective dams were placed in an experimental area of Brachiaria decumbens, divided in 5 paddocks of 7.0 ha each with free access to water and creep-feeders. Heifer calves were weaned at 240 d of age. After weaned, calves were transferred to another area of Brachiaria decumbens and distributed in five paddocks of 2.5 ha each with water dispensers and feeders.

Performance, Body Measures and Carcass Characteristics

After 14h of solids fasting, animals were weighted in the beginning and in the end of each phase.

Body measures (BM) were taken at weaning and end of the experiment. The rump width (the maximum distance between iliac tuberosities), rump length (from the ischial tuberosity to the iliac tuberosity), rib depth (vertically from the highest point over the scapulae to the end point of the rib), body length (from the anterior point of the scapulae vertically to the posterior midline), height at withers (from the highest point of the shoulder blade to the ground) and rump height (from the iliac tuberosity vertically to the ground) were recorded with a height stick. The heart girth (the body circumference immediately posterior to the front legs) was measured with a flexible tape.

At weaning and at the end of the experiment, Longissimus muscle area (LMA) and fat thickness over the Longissimus muscle were measured by ultrasound scan (Aloka SSD 500; 3.5 MHz linear probe) of the area between the 13th-14th ribs. Vegetable oil was used to ensure adequate acoustic contact.

Follicle Diameter

At the end of the experiment, an ultrasound Aloka SSD500 with trans-rectal transducer of 5MHz was used to measure the diameter of the dominant follicle.

Forage Analysis and Intake Trial

Pasture chemical composition was assessed by samples hand-plucked every 2 weeks. In the middle of every experimental period, a second pasture sample was also collected to estimate forage potentially digestible dry matter (pdDM) as proposed by Paulino et al. (2008), 4 subsamples were randomly collected in each plot by cutting it close to the ground using a metal square (0.5 × 0.5 m). Samples were weighed and oven dried at 60ºC for 72 h. After that, mill grounded to pass through a 2 mm screen for indigestible neutral detergent fiber (iNDF) analysis (Casali et al., 2008). A sub portion of 20 g of each sample was grounded to pass

through a 1 mm screen for analyses of dry matter (DM), ash, crude protein (CP) and neutral detergent fiber (NDF).

A 9 d intake trial was carried out in each phase. Chromium oxide (Cr_2O_3) was used as external maker to estimate fecal excretion (in the amount of 10 and 15 g per animal pre and post-weaning, respectively). The chromium oxide was packed in paper cartridges and delivered via esophagus with a metal probe once daily, at 10 a.m. Individual intake of supplement was estimated using titanium dioxide (TiO_2) mixed in the supplement at the proportion of 10 g/kg of supplement. Finally, iNDF was used as internal marker to estimate DM intake. Six days were allowed for stabilization of markers excretion, after that, fecal samples were collected at 3 p.m. on the 7th d, at 11 a.m. on the 8th d, and at 7 a.m. on the 9th d of the intake trial.

Feces samples were collected immediately after defecation or directly into the rectum of animals at amounts of approximately 200 g, identified, dried (60°C/72 h) and mill grounded to pass through a 2 mm screen for indigestible neutral detergent fiber (iNDF) analysis (Casali et al., 2008). A sub portion of 20 g of each sample was grounded to pass through a 1 mm screen for analyses of dry matter (DM), ash, crude protein (CP) and neutral detergent fiber (NDF) as described for forage samples. Grounded samples were proportionally sub-sampled to a pooled 3 d sample per animal per phase.

Milk intake by calves was estimated on d 20, 60 and 100 of the experimental period (pre-weaning phase), the milk production of the three sampling days were averaged by cow. Aiming to empty the udder, calves were separated from their mothers from 3 p.m. to 5:45 p.m, when they were reunited to the dams and allowed to suckle. At 6 p.m. calves were again separated from their dams until the next morning. At 6 a.m. of the next day, cows were milked immediately after an injection of 20 IU of oxytocin (Ceva Ocitovet, Paulínia, Brazil)

in the mammary vein and the produced milk was weighted. The milking was planned to do not occur time longer than 2 h from the first and the last cow milked. The exact time when each cow was milked was recorded and the milk production was converted into a 24h production. The milk produced was corrected to 4% of fat ($Milk_{4\%}$) calculated by the following equation (NRC, 2001):

$Milk_{4\%}$ (kg) = 0.4 × (milk production) + [15 × (fat production × milk production/100)]

Samples of forage, feces and supplement were analyzed following procedures described by Detmann et al. (2012) for DM (index INCT-CA G-003/1), CP (index INCT-CA N-001/1), ash (index INCT-CA M-001/1), NDF (index INCT-CA F-002/1) corrected for ash residue (index INCT-CA M-002/1) and residual nitrogen compounds (index INCT-CA N-004/1), the iNDF (index INCT-CA F-009/1) was evaluated using F57 (Ankon®) bags incubated in rumen by 288 h. Fecal samples were also analyzed for levels of chromium by atomic absorption spectrophotometry (index INCT-CA M-005/1) and titanium dioxide by colorimetry (index INCT-CA M-007/1) as recommended by Detmann et al. (2012). Milk was analyzed for protein, fat, lactose, and total solids content, using spectroscopy (Foss MilkoScan FT120, Hillerød, Denmark).

The potentially digestible dry matter (pdDM) was estimated using the pasture sample collected in each period using a metal square as described previously, using the following equation (Paulino et al., 2008):

pdDM (%; dry matter basis) = 0.98 × (100 – apNDF) + (apNDF – iNDF)

Where: 0.98 is the true digestibility coefficient of intracellular content; apNDF is forage content of neutral detergent fiber corrected for residual ash and nitrogen; iNDF is forage content of indigestible neutral detergent fiber.

Fecal excretion (FE) was estimated by ratio of chromium oxide and its concentration in the feces. The estimate of individual supplement intake (SI) was obtained by using the following equation:

$$SI\ (kg/d) = (FE \times TCF)/TCS$$

Where: SI is the dry matter supplement intake (kg/d); FE is the fecal excretion (kg/d); TCF is the concentration of titanium dioxide in the feces (kg/kg); TCS is the concentration of titanium dioxide in the supplement (kg/kg).

Dry matter intake (DMI) was estimated by using the iNDF as an internal marker and calculated by the following equation:

$$DMI\ (kg/d) = [((FE \times iNDF\ feces) - iNDF\ supplement) \div iNDF\ forage] + SI + MI$$

Where: FE is the fecal excretion (kg/d); iNDF feces is the concentration of iNDF in the feces (kg/kg); iNDF supplement is the iNDF in the supplement (kg); iNDF forage is the concentration of iNDF in forage (kg/kg); SI is the supplement intake (kg/d) and MI is the milk intake corrected to 4% of fat (kg/d).

Blood Sampling and Analysis

Blood samples were collected each 40 d during the pre-weaning phase and each 36 d in the post-weaning phase, at 8:00 am, to measure levels of growth hormone (GH), insulin, glucose, total cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides, total protein, albumin, globulins and urea. Two samples of each heifer were collected from the jugular vein with vacuum tubes. One of the samples was collected in tubes with clot activator and gel for serum separation (BD Vacuntainer® SST II Plus, São Paulo, Brazil) for analyses of GH, insulin, total cholesterol, HDL, triglycerides, total protein,

albumin and urea. The sample collected in the second tube, with EDTA and sodium fluoride (BD Vacutainer® Fluoreto/EDTA, São Paulo, Brazil), was used for glucose analysis. After collected, samples were centrifuged at 3600 × g for 20 min, serum and plasma were immediately frozen at -20°C in triplicates until further analysis.

Growth hormone and insulin were analyzed by chemiluminescent method using Access Ultrasensitive hGH Reagent (Ref. Number 33580, Beckman Coulter®, Brea, USA) and Access Ultrasensitive Insulin Reagent (Ref. Number 33410, Beckman Coulter®, Brea, USA) in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA). Glucose (Ref. Number K082, Bioclin® Quibasa, Belo Horizonte, Brazil), total cholesterol (Ref. Number K083, Bioclin® Quibasa, Belo Horizonte, Brazil), HDL (Ref. Number K071, Bioclin® Quibasa, Belo Horizonte, Brazil), triglycerides (Ref. Number K117, Bioclin® Quibasa, Belo Horizonte, Brazil), and urea (Ref. Number K056, Bioclin® Quibasa, Belo Horizonte, Brazil) were quantified by enzymatic-colorimetric method and total protein (Ref. Number K031, Bioclin® Quibasa, Belo Horizonte, Brazil) and albumin (Ref. Number K040, Bioclin® Quibasa, Belo Horizonte, Brazil) by colorimetric method. The LDL levels were estimated according to the Friedewald equation (Tietz, 1986). Globulins were calculated subtracting the albumin quantified from the total protein level. Serum urea N (SUN) was estimated as 46.67% of total serum urea. Metabolites were analyzed in accordance with manufacturer's instructions in an automatic biochemistry analyzer (Mindray BS200E, Shenzhen, China). Results were averaged by phase, resulting in the serum and plasma concentrations of hormones and metabolites for each animal per phase (pre and post-weaning).

On the last day of experiment, a blood sample was taken from the jugular vein, using vacuum tubes with clot accelerator and gel for serum separation (BD Vacuntainer® SST II

Plus, São Paulo, Brazil). Immediately after collection the samples were centrifuged at 3600 × g for 20 min, the serum was frozen at -20°C and subsequently analyzed for progesterone levels by chemiluminescent method using Access Progesterone Reagent (Ref. Number 33550, Beckman Coulter®, Brea, USA) in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA).

Statistical Analysis

Statistical analyzes were performed using PROC GLIMMIX in SAS 9.4. Treatments were compared using orthogonal contrasts (Steel et al., 1997). Within the pre-weaning, phase linear and quadratic effects of level were evaluated. For the whole experimental period, contrasts were constructed in order to evaluate the effects of supplementation (CC vs HH, HL, LH and LL) and level of supplementation fed pre-weaning (HH and HL vs LH and LL) and post-weaning (HH and LH vs HL and LL). Initial body weight was used as covariate for performance data analysis. Significant difference was considered at $P < 0.05$ and tendency at $0.05 < P < 0.10$, interaction was considered significant when $P < 0.10$.

Results

Potentially digestible forage mass and CP content of forage throughout the experiment are presented in Table 2. Average pdDM and CP content in forage were 3984 kg/ha and 105 g/kg in pre-weaning phase and 4070 kg/ha and 77 g/kg in post-weaning phase.

No significant difference was observed in BW at weaning, ADG, and LMA according to supplementation level offered pre-weaning (Table 3; $P > 0.10$). Heifer calves were weaned with average of 219 kg of BW across treatments. Despite no difference in BW and ADG, there was a positive linear effect of supplementation level on fat thickness at weaning ($P < 0.01$).

Iteration between L-Pre-weaning × L-Post-weaning was not significant for any performance variables evaluated ($P > 0.10$). Level of supplement fed pre-weaning had no significant effect afterwards, and did not influence any of the performance variables evaluated at the end of the experiment ($P > 0.10$). There was a significant effect of supplementation and level of supplementation offered post-weaning on post-weaning ADG ($P < 0.01$). Post-weaning ADG was 410 g/d for heifers receiving high level of supplementation, 315 g/d for heifer receiving low level and 157 g/d for control heifers. Overall ADG was also affected only by supplementation and level of supplementation offered post-weaning ($P < 0.01$), with animals receiving 6 g/kg of BW after weaning gaining more. The average BW at the beginning of the breeding season (at the ending of the experiment) differed among strategies ($P < 0.01$) and was 297 kg for heifers in supplementation strategies HH and LH, 275 for heifers in HL and LL and 244 kg for control (CC) heifers.

At the end of the experiment, supplemented animals had higher LMA ($P < 0.05$). Animals receiving 6 g/kg of supplement also had higher fat thickness ($P < 0.01$), 3.43 mm and 2.98 mm for HH and LH heifers, respectively, while HL and LL heifers had fat thickness comparable to the control heifers (2.54 for HL and 2.27 for LL vs. 2.52 for control). Despite a linear effect of level of supplementation on fat thickness at weaning, supplementation level offered in the previous phase (pre-weaning) did not affect fat thickness at the end of the experiment ($P > 0.10$).

Follicular diameter was greater for animals receiving 6 g/kg of BW post-weaning ($P < 0.01$) and although only two animals have reached puberty before the breeding season, both were receiving high level of supplementation in post-weaning, one from HH and one from LH (pubertal status evaluated via ultrasonography, data no presented). No difference in progesterone concentrations was observed ($P > 0.05$).

Body measures (BM) of heifers at weaning and beginning of breeding season are presented in Table 4. Height at withers and rump height were not different among treatments at weaning or breeding season ($P > 0.05$). At weaning, only rib depth and body length were linearly and positively affected by level of supplementation ($P < 0.05$). At the end of the experiment important BM were improved by supplementation and level of supplementation offered post-weaning ($P < 0.05$). Rump width, rump length, rib depth, body length, heart girth, and BW:height ratio were greater for supplemented animals. Heart girth tended to be higher for animals receiving high level of supplementation post-weaning ($P = 0.09$) and BW:height ratio was also greater for animals receiving high level of supplementation post-weaning ($P < 0.05$).

Supplementation linearly increased intake of DM, OM and CP (Table 5; $P < 0.05$) in the pre-weaning phase. Compared to control, supplemented animals had higher intake of DM, OM and CP post-weaning, ($P < 0.05$), level of supplementation positively affected intake of DM, OM and CP post-weaning as well ($P < 0.01$).

Endocrine and metabolic profile of heifers in pre and post-weaning phases are presented in Table 6. Pre-weaning, growth hormone tended to be higher for heifers receiving 6 g/kg of BW ($P = 0.06$). Post-weaning, growth hormone was lower for heifers receiving supplement (30.2 ng/mL) compared to control heifers (35.2 ng/mL; $P < 0.05$) and tended to be affected by level of supplement fed previously, in the pre-weaning phase, with heifers that received high level pre-weaning having higher serum concentrations of GH ($P = 0.09$).

No difference in insulin levels was observed pre-weaning ($P > 0.10$). Insulin level post-weaning was affected only by level of supplementation fed post-weaning ($P < 0.01$), animals receiving high level of supplement post-weaning had greater insulin serum concentration (1.52 μIU/mL) in comparison to heifers receiving low level (1.19 μIU/mL).

Pre-weaning, most metabolites were not affected by treatment ($P > 0.10$). Exception was SUN, that linearly increased with level of supplement offered ($P < 0.01$), and triglycerides that tended to be higher for control heifers ($P < 0.10$).

Glucose level was not different among treatments pre-weaning ($P > 0.10$) and reduced for all treatments post-weaning, when animals receiving supplementation tended to have higher levels of glucose compared to control animals, 70 vs. 65 for supplemented and not supplemented, respectively ($P = 0.06$).

There was no difference in total cholesterol, HDL and LDL levels among treatments pre-weaning ($P > 0.10$). All metabolites related to fat metabolism (cholesterol, HDL, LDL and triglycerides) reduced post-weaning, but only LDL was significantly different among treatments (supplementation effect; $P < 0.01$), with control heifers (CC) having the highest LDL levels (37 mg/dL).

Total protein in serum was not different among treatments pre-weaning ($P > 0.10$) but there was a positive effect of level of supplementation offered post-weaning on total protein concentrations ($P < 0.05$). Supplementation increased albumin levels ($P < 0.05$) and animals receiving high amount of supplement had higher albumin levels as well ($P < 0.05$). The difference observed in total protein in serum was due to albumin, whereas no significant difference was observed in globulins concentrations ($P > 0.10$).

Concentrations of SUN were higher for supplemented heifers post-weaning ($P < 0.01$) and were also affected by level of supplement received pre-weaning ($P < 0.05$), heifers that have received high level of supplementation pre-weaning had higher levels of SUN post-weaning (means were 15.1 for HH and HL vs 13.4 for LH and LL). Level of supplementation

offered post-weaning also affected SUN levels ($P < 0.01$), HH and LH heifers had average 15.6 mg/dL of SUN vs 12.8 mg/dL in HL and LL heifers.

Discussion

Important performance, endocrine and metabolic variables evaluated in the present study were affected by supplementation level offered post-weaning, with level of supplementation offered pre-weaning having low or no effect on animals' performance at weaning and at the end of the experiment, corresponding to the breeding season.

Roberts et al. (2009) demonstrated that puberty was much more affected by variation in rate of growth up to approximately 8 months of age than subsequent growth up to the start of breeding. Together, results from Gasser et al. (2006) and Cardoso et al. (2014) indicate that during early calfhood development, plausibly between 4 and 6.5 months of age, heifers are more sensitive to nutritional programming that can accelerate puberty. Most of studies proving this positive effect of early nutrition on heifer development were conducted using level of feeding in order to change ADG, most of them using heifers weaned early (Gasser et al., 2006; Cardoso et al., 2014) or in controlled pre-weaning regimen (Rodríguez-Sánchez et al., 2015).

Results from those studies raised our hypothesis that creep-feeding heifers accompanied of their dams with high level of supplementation could improve heifer performance. This hypothesis was refuted in the present study; creep-feeding heifer calves in ad libitum suckling was not efficient to improve their performance at weaning and had no effect on performance variables evaluated later in their lives, in the post-weaning phase.

There is an inverse association between genetic potential for milk production and age of puberty (Notter et al., 1978; Martin et al., 1992), indicating that inherent differences,

especially genetics and milk supply, contribute to the influence of pre-weaning growth rate on subsequent attainment of puberty. As such, it would not be advantageous to implement management strategies to increase pre-weaning growth in attempt to increase proportion pubertal as this would result in retention of more heifers with less desirable genetic characteristics for growth and reproduction (Roberts et al., unpublished data).

When forages CP content is less than about 70 g/kg, feeding a protein supplement generally improves animal performance by improving rumen microbial activity, forage intake, and digestibility. As we can observe in Table 2, average CP content of forage during the pre-weaning phase was 105 g/kg of forage and combined with milk ingested ad libitum was probably enough to attend heifers requirement of CP for maintenance and genetic programed growth in all treatments. The linear increase in intake of DM, OM and CP pre-weaning reflected in more fat being deposited and increased fat thickness at weaning instead of muscle deposition, as no difference in LMA or ADG was observed at weaning.

Optimal SUN concentrations in beef heifers range between 11 and 15 mg/dL (Byers and Moxon, 1980), indicating that heifers in the high level were consuming CP in excess. Given that energy is also required to metabolize ruminal ammonia into urea by the liver (Reynolds, 1992), the lack of differences on pre-weaning ADG among treatments may also be associated with a greater amount of energy being partitioned towards N recycling instead of growth in supplemented heifers (Moriel et al., 2012).

Nutritional management of replacement heifers from weaning to breeding is critical to their lifetime productivity (Eborn et al., 2013). Improved forage intake increases total dietary energy intake; in mature forage-based diets, as during the dry season, inadequate supply of nitrogen to the rumen would be the main factor limiting intake, which would in turn lead to limited energy intake by the animal (Detmann et al., 2010). In the post-weaning phase, CP

content of forage was lower than in the pre-weaning phase, in this way, providing supplementation in higher level efficiently improved heifers' post-weaning ADG, and consequently overall ADG and final BW.

Rodríguez-Sánchez et al. (2015) showed that heifers compensated for the lower pre-weaning ADG during the post-weaning phase and for body size as well, except for heifers that remain in low feed treatment, although animals are more able to compensate for BW than for skeletal growth (Swali et al., 2008; Rodríguez-Sánchez et al., 2015). In this way, severe nutritional restriction can cause permanent impairment to heifers' growth.

The height at withers in cattle is primarily a composite of the long bone measurement of the forelimb and is a good indicator of skeletal development. While other authors have reported difference in height due to nutritional treatment applied (Roberts et al., 2009; Rodríguez-Sánchez et al., 2015), the lack of difference in height indicates that level of feed or nutrient restriction applied in the present study was not detrimental for skeletal development and subsequent mature size, reflected by height.

The BW:height ratio reflects animals' body condition (Eborn et al., 2013). Supplemented heifers had greater BW:height ratio and within supplemented heifers, heifers receiving 6 g/kg of BW had grater BW:height ratio, showing that higher level of supplementation post-weaning improves animals' capacity of muscle and fat deposition.

The rump width and length provide an estimate of the internal pelvic area, which can influence the incidence and degree of calving difficulty in heifers calving for the first time. Rump width and length were both higher for supplemented heifers.

The heart girth is the BM that most correlates to BW. In the current study, heart girth was influenced only by feeding treatment applied post-weaning, with heifers receiving higher level of supplementation after weaning tending to have higher heart girth.

Reserves of body fat have been related to the maintenance of the estrous cycle in cattle (Imakawa et al., 1986; Richards et al., 1989) and may act as a marker of energy available for reproductive activity (Hall et al., 1995), acting as a permissive signal allowing ovulation and pregnancy. In the present study, animals receiving high level of supplementation post-weaning had greater fat thickness at the end of the experiment.

Concentrations of GH are increased during feed restriction (Bossis et al., 1999), when GH plays a catabolic role mobilizing lipid from adipose tissue in order to conserve glucose (Lawrence et al., 2012). The post-weaning phase of heifers development is characterized by annual drought that usually takes place in Southeast and Midwest Brazil. In the present study, not supplemented animals had higher GH and lower insulin concentrations compared to animals receiving high amount of supplement (Table 6). Endocrine profile of control heifers is in accordance with their lower feeding and nutritional level, which in turn is reflex of lower DM, OM and CP intakes (Table 5). The higher GH concentration was not enough to equalize glucose levels of control heifers to the supplemented heifers thought, and not supplemented heifers had lower glucose serum concentrations in the post-weaning period.

High-starch diets increase propionate production, and propionate is converted to glucose in the liver and stimulates release of insulin, increased insulin concentration can be the result of aminoacids stimulation of insulin secretion as well (Harmon, 1992). Heifers receiving high supplementation level post-weaning had greater intake of CP and OM, and consequently energy, due better quality of supplement provided in comparison with forage; as a consequence, supplemented heifers had higher glucose levels.

The insulin like growth factor-1 (IGF-1) is a potent stimulus to cell proliferation and cell hypertrophy (Lawrence et al., 2012). Insulin plays an important role in regulating the ability of GH to stimulate IGF-1 production; during periods of fasting, the lack of insulin uncouples GH from IGF-1 production and GH plays an important role in shifting metabolism to metabolize lipids (Lawrence et al., 2012). Due to GH and insulin pattern among strategies, not supplemented heifers probably had lower IGF-1 concentration and therefore the reduced corporal growth and follicular size observed.

Expression of glucose transporters (GLUT) 1, 3 and 4 have been reported in the bovine ovary (Nishimoto et al., 2006). The same authors found that expression of GLUT1 and 3 in ovarian and in all bovine tissues and organs examined at substantial levels, but GLUT4, an insulin-dependent transporter, is lower in ovary than in muscle and adipose tissue, indicating that insulin-activated glucose uptake by ovarian cells have lower priority in comparison to muscular cells, for example. Therefore, GLUT4 may play a supporting role in the bovine follicle (Nishimoto et al., 2006), providing more glucose to follicle metabolism when more glucose is available in the blood steam. In accordance with these findings, in our study, animals receiving high level of supplementation post-weaning had higher insulin concentrations and follicle diameter.

Availability of metabolic fuels seems to be the most important factor influencing reproduction (Hall et al., 1995); cholesterol, for example, is substrate for progesterone and other steroidal hormones production. Quantities of circulating lipoproteins in blood, main source of cholesterol for tissues, changes according to physiological and nutritional status in cattle. High density lipoproteins are the primary source of cholesterol for luteal progesterone biosynthesis (Bao et al., 1995; Bao et al., 1997). In agreement with Bao and coworkers'

findings, in our study no difference was observed for total cholesterol or HDL concentrations, in accordance with equal progesterone concentrations among treatments.

Serum proteins are constituted mainly by albumin and globulins. Serum proteins are nutrient, hormone and growth factor transporters and play a role on determining degradation rate and buffering hormone concentrations (Lawrence et al., 2012). The difference in serum protein observed between supplemented and not supplemented animals post-weaning is due higher albumin levels in supplemented heifers, as there was no difference in globulin concentration among treatments.

Albumin is the main serum protein synthetized by the liver and its concentrations can be related to aminoacids and nutrient availability, the lower values for albumin concentrations in control heifers and heifers receiving low level of supplementation post-weaning further indicates that animals from high supplementation strategy were in greater nutritional status at the end of the experiment.

In summary, performance, endocrine, metabolic and reproductive variables evaluated in the current study were improved by level of supplementation applied post-weaning. Due to higher nutrient intake provided via concentrate supplement, heifers receiving 6 g/kg of BW post-weaning had greater responses, independently of level received pre-weaning. Further research is needed to evaluate the effects of pre and post-weaning supplementation strategies on development of grazing Nellore heifers.

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Table 1. Ingredients and composition of supplements provided to heifers pre and post-weaning

[A] OM – organic matter; CP – crude protein; NDF – neutral detergent fiber corrected for ash and protein residue.

Item[A]	Supplement	
	Pre-weaning	Post-weaning
Ingredients % (as-fed basis)		
Corn	26.0	27.5
Sorghum	26.0	27.5
Soybean Meal	45.0	45.0
Molasses	3.0	-
Chemical Composition (g/kg; dry matter basis)		
OM	961	967
CP	298	270
apNDF	139	136

Table 2. Potentially digestible forage mass and chemical composition of forage during the experiment

[A] pdDM – potentially digestible forage dry matter; OM – organic matter; CP – crude protein; NDIN – neutral detergent insoluble N; apNDF – neutral detergent fiber corrected for ash and protein residue; iNDF – indigestible neutral detergent fiber.

[B] pdDM was estimated for forage sampled in the area delimited by a metal square 0.5 × 0.5; chemical composition was evaluated in the hand-plucked forage sample.

Item[A,B]	Phase							
	Pre-weaning			Post-weaning				
	Experimental Period							
	1	2	3	4	5	6	7	8
pdDM (kg/ha)	3730	5071	3152	6351	3355	3224	2239	5083
OM (g/kg)	926	914	921	913	920	908	910	926
CP (g/kg)	102.9	118.4	93.8	81.4	97.8	74.8	59.6	71.5
NDIN (% of total N)	31.9	26.1	30.2	36.3	37.1	34.2	17.2	16.0
apNDF (g/kg)	558	581	603	608	588	625	630	610
iNDF (g/kg)	172	183	236	232	198	278	284	214

Table 3. Performance, carcass characteristics and reproductive performance of heifers according to supplementation plan applied pre and post-weaning

[A] BW – body weight; ADG – average daily gain; LMA – Longissimus muscle area; Fat thickness– fat thickness over the Longissimus muscle.

[B] Carcass characteristics accessed by ultrasonography.

[C] H – animals received 6 g/kg of BW; L – animals received 3 g/kg of BW; and C – control, no supplement offered.

[D] L – linear effect of level of supplementation offered in the pre-weaning phase; Q – quadratic effect of level of supplementation offered in pre-weaning phase; S – Supplementation effect in the pre-weaning phase; L-Pre-weaning– effect of supplementation level in the pre-weaning phase; L-Post-weaning – effect of supplementation level in the post-weaning phase; L-Pre-weaning × L-Post-weaning – interaction between supplementation level pre and post-weaning.

Item[A,B]	Pre-weaning[C]					SEM	P-value[D]					
	H		L		C		Pre-weaning			Overall		
	Post-weaning											
	H	L	H	L	C		L	Q	S	L-Pre-weaning	L-Post-weaning	L-Pre-weaning × L-Post-weaning
8 months – Weaning												
BW (kg)	218		221		216	3.53	0.50	0.31	-	-	-	-
Pre-weaning ADG (g/d)	706		727		678	29.4	0.45	0.27	-	-	-	-
LMA (cm²)	38.7		39.1		36.9	1.68	0.38	0.53	-	-	-	-
Fat thickness (mm)	2.06		1.48		0.51	0.30	<0.01	0.83	-	-	-	-
14 months - Breeding Season												
BW (kg)	296	275	297	274	244	6.60	-	-	<0.01	0.98	<0.01	0.88
Post-weaning ADG (g/d)	416	323	406	307	157	23.8	-	-	<0.01	0.58	<0.01	0.89
Overall ADG (g/d)	537	470	541	467	364	21.7	-	-	<0.01	0.97	<0.01	0.87

LMA (cm^2)	49.8	46.7	49.0	46.4	42.4	2.10	-	-	0.02	0.75	0.11	0.90
Fat thickness (mm)	3.43	2.54	2.98	2.27	2.52	0.33	-	-	0.43	0.21	<0.01	0.75
Follicular Diameter (mm)	11.5	9.5	11.3	7.7	9.9	0.78	-	-	0.91	0.24	<0.01	0.38
Progesterone (ng/mL)	1.97	1.37	1.04	1.49	0.93	0.35			0.19	0.28	0.83	0.16

Table 4. Body measurements of heifers at 8 and 14 months of age according to supplementation plan applied in pre and post-weaning phases

[A] BW – body weight.

[B] H – animals received 6 g/kg of BW; L – animals received 3 g/kg of BW; and C – control, no supplement offered.

[C] L – linear effect of level of supplementation offered in the pre-weaning phase; Q – quadratic effect of level of supplementation offered in pre-weaning phase; S – Supplementation effect in the pre-weaning phase; L-Pre-weaning– effect of supplementation level in the pre-weaning phase; L-Post-weaning – effect of supplementation level in the post-weaning phase; L-Pre-weaning × L-Post-weaning – interaction between supplementation level pre and post-weaning.

Item[A]	Pre-weaning[B]					SEM	P-value[C]					
	H		L		C		Pre-weaning			Overall		
	Post-weaning											
	H	L	H	L	C		L	Q	S	L-Pre-weaning	L-Post-weaning	L-Pre-weaning × L-Post-weaning
8 months – Weaning												
Rump Width (cm)	33.1		32.2		32.8	0.64	0.75	0.21	-	-	-	-
Rump Length (cm)	36.1		36.0		35.5	0.66	0.46	0.87	-	-	-	-
Rib Depth (cm)	51.3		49.7		48.7	0.75	0.01	0.30	-	-	-	-
Body Length (cm)	113		112		109	1.87	0.05	0.85	-	-	-	-
Heart Girth (cm)	135		134		136	2.07	0.51	0.58	-	-	-	-
Height at Withers (cm)	113		113		112	1.48	0.71	0.69	-	-	-	-
Rump Height (cm)	119		120		120	1.59	0.85	0.65	-	-	-	-
BW:Height at Withers (kg/cm)	2.01		2.03		1.93	0.07	0.30	0.50	-	-	-	-
14 months – Breeding Season												
Rump Width (cm)	39.1	38.7	38.9	38.6	36.4	0.80	-	-	0.01	0.85	0.66	0.90
Rump Length (cm)	41.2	40.4	40.7	40.8	38.3	0.63	-	-	<0.01	0.94	0.58	0.53

Rib Depth (cm)	56.3	55.7	55.9	55.6	51.4	0.82	-	-	<0.01	0.76	0.59	0.81
Body Length (cm)	124	124	128	123	118	1.78	-	-	<0.01	0.42	0.19	0.21
Heart Girth (cm)	153	150	154	150	141	2.07	-	-	<0.01	0.89	0.09	0.99
Height at Withers (cm)	125	123	124	124	121	1.81	-	-	0.12	0.99	0.56	0.86
Rump Height (cm)	131	129	132	130	127	1.73	-	-	0.10	0.39	0.25	0.98
BW:Height at Withers (kg/cm)	2.40	2.28	2.48	2.29	2.08	0.07	-	-	<0.01	0.55	0.04	0.58

Table 5. Intake according to supplementation plan applied to heifers in pre and post-weaning phases

[A] DM – dry matter; OM – organic matter; CP – crude protein.

[B] Milk intake is presented as natural basis; all other variables as dry matter basis.

[C] H – animals received 6 g/kg of BW; L – animals received 3 g/kg of BW; and C – control, no supplement offered.

[D] L – linear effect of level of supplementation offered in the pre-weaning phase; Q – quadratic effect of level of supplementation offered in pre-weaning phase; S – Supplementation effect in the pre-weaning phase; L-Pre-weaning– effect of supplementation level in the pre-weaning phase; L-Post-weaning – effect of supplementation level in the post-weaning phase; L-Pre-weaning × L-Post-weaning – interaction between supplementation level pre and post-weaning.

Item[A,B]	Pre-weaning[C]					SEM	P-value[D]					
	H		L		C		Pre-weaning			Overall		
	Post-weaning											
	H	L	H	L	C		L	Q	S	L-Pre-weaning	L-Post-weaning	L-Pre-weaning × L-Post-weaning
Pre-weaning												
$Milk_{4\%}$ (kg/d)	6.70		6.60		7.60	0.69	0.29	0.35	-	-	-	-
DM (kg/d)	4.22		3.88		3.12	0.4	0.03	0.94	-	-	-	-
Forage DM (kg/d)	2.78		2.61		2.19	0.34	0.15	0.95	-	-	-	-
OM (kg/d)	3.90		3.57		2.87	0.37	0.03	0.97	-	-	-	-
CP (g/d)	743		687		499	63.6	<0.01	0.67	-	-	-	-
Post-weaning												
DM (kg/d)	4.01	3.31	4.25	3.28	2.68	0.32	-	-	<0.01	0.74	0.01	0.68
Forage DM (kg/d)	2.43	2.54	2.66	2.51	2.68	0.22	-	-	0.58	0.64	0.94	0.56
OM (kg/d)	2.40	1.68	2.51	1.64	1.06	0.20	-	-	<0.01	0.85	<0.01	0.70
CP (g/d)	595	335	560	315	118	44.2	-	-	<0.01	0.54	<0.01	0.86

Table 6. Endocrine and metabolic profile of heifers according to supplementation plan applied in pre and post-weaning phases

[A] H – animals received 6 g/kg of BW; L – animals received 3 g/kg of BW; and C – control, no supplement offered.

[B] L – linear effect of level of supplementation offered in the pre-weaning phase; Q – quadratic effect of level of supplementation offered in pre-weaning phase; S – Supplementation effect in the pre-weaning phase; L-Pre-weaning– effect of supplementation level in the pre-weaning phase; L-Post-weaning – effect of supplementation level in the post-weaning phase; L-Pre-weaning × L-Post-weaning – interaction between supplementation level pre and post-weaning.

Item	Pre-weaning[A]					SEM	P-value[B]					
	H		L		C		Pre-weaning		Overall			
	Post-weaning											
	H	L	H	L	C		L	Q	S	L-Pre-weaning	L-Post-weaning	L-Pre-weaning × L-Post-weaning
Pre-weaning												
GH (ng/mL)	29.8		25.3		25.9	1.62	0.06	0.10	-	-	-	-
Insulin (µIU/mL)	1.45		1.43		1.37	0.14	0.66	0.87	-	-	-	-
Glucose (mg/dL)	84.3		80.6		80.2	3.99	0.40	0.66	-	-	-	-
Cholesterol (mg/dL)	172		160		175	6.80	0.76	0.13	-	-	-	-
HDL (mg/dL)	103.8		98.5		97.9	3.53	0.18	0.48	-	-	-	-
LDL (mg/dL)	61.3		54.6		69.4	7.83	0.40	0.15	-	-	-	-
Triglycerides (mg/dL)	34.0		34.4		40.6	2.98	0.07	0.29	-	-	-	-
Total Protein (g/dL)	6.46		6.32		6.44	0.11	0.84	0.22	-	-	-	-
Albumin (g/dL)	3.28		3.17		3.20	0.08	0.40	0.34	-	-	-	-
Globulins (g/dL)	3.19		3.15		3.24	0.12	0.72	0.57	-	-	-	-
SUN (mg/dL)	19.4		15.4		11.3	0.80	<0.01	0.90	-	-	-	-
Post-weaning												
GH (ng/mL)	30.5	33.0	29.9	27.4	35.2	1.80	-	-	0.02	0.09	0.97	0.17

Insulin (µIU/mL)	1.51	1.22	1.52	1.16	1.29	0.11	-	-	0.57	0.82	<0.01	0.78
Glucose (mg/dL)	74.3	70.2	67.1	70.5	65.1	2.51	-	-	0.06	0.17	0.87	0.14
Cholesterol (mg/dL)	102	102	91	103	107	4.12	-	-	0.11	0.26	0.14	0.13
HDL (mg/dL)	72.1	70.3	66.3	70.5	65.5	2.73	-	-	0.17	0.31	0.68	0.28
LDL (mg/dL)	24.8	25.3	18.6	27.4	37.0	3.16	-	-	<0.01	0.52	0.15	0.19
Triglycerides (mg/dL)	26.6	30.8	31.2	30.3	29.0	1.75	-	-	0.70	0.24	0.36	0.16
Total Protein (g/dL)	6.31	5.91	6.10	5.97	5.90	0.11	-	-	0.18	0.51	0.02	0.24
Albumin (g/dL)	3.27	2.91	3.16	2.93	2.83	0.06	-	-	<0.01	0.47	<0.01	0.31
Globulins (g/dL)	3.04	3.00	2.94	3.04	3.07	0.12	-	-	0.62	0.80	0.79	0.55
SUN (mg/dL)	16.9	13.2	14.4	12.4	11.4	0.69	-	-	<0.01	0.02	<0.01	0.21

Performance, milk production, and metabolism of Nellore cows when their calves are submitted to different supplementation levels

Abstract. Creep-feeding has been used to reduce calves nutritional dependence on the cow, but research results under tropical conditions have not been conclusive about the positive effects on the cow. Therefore, this study was conducted to evaluate the effects of high and low supplementation levels for Nellore heifer calves on performance, milk production and metabolic prolife of their mothers. Fifty multiparous Nellore cows and their respective calves were used. The following treatments were evaluated: 0 – control, no supplement was fed to calves; 3 - calves received supplement in the amount of 3 g/kg of body weight (BW); 6 - calves received supplement in the amount of 6 g/kg of BW. There was no significant effect level of supplementation offered to offspring on cow BW, body condition score (BCS) and subcutaneous fat thickness ($P > 0.05$). Level of supplementation of heifer calves did not significantly affect milk production corrected to 4% of fat ($P > 0.10$). Fat, protein, lactose and total solids of the milk also did not differ among supplementation strategies ($P > 0.10$). Level of supplement fed to calves had no effect on cows' glucose, total cholesterol, HDL, LDL, triglycerides, total protein and albumin levels ($P > 0.10$), but cows nursing calves that did not received supplement had lower level of serum urea N (SUN; $P < 0.05$). We conclude that creep-feeding in the amounts of 3 or 6 g/kg of BW daily has no major impact on dams' performance.

Additional keywords: fat thickness, grazing dams, cow metabolism

Introduction

The foundation of beef systems is the cow-calf pair, so understanding how nutritional strategies applied to offspring can improve dams' performance is of paramount importance. Factors that affect milk production, such as creep-feeding, can impact cows' requirements and performance.

Creep feeding is used with the objective of increasing calves' body weight at weaning. However, as supplement intake may influence pre-weaning behavior, it may also influence milk production, and consequently the strategy can have an indirect effect on the cow. Some authors have found that creep-feeding can reduce milk production and consequently improve cows' performance (Kress et al., 1990; Fordyce et al., 1996; Henriques et al., 2011) while others have found that supplementing calves have no effect on their mothers' performance (Valente el al., 2012; Valente et al., 2013; Moriel and Arthington, 2013).

The present study was conducted to evaluate the effects of high and low supplementation levels for Nellore heifer calves on performance, milk production and metabolic prolife of their dams.

Material and Methods

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV 0011).

Experimental Design and Treatments

The experiment was conducted at the facilities of the Department of Animal Science - Universidade Federal de Viçosa, Brazil, from February to June of 2013. Fifty multiparous Nellore cows and their respective calves were used. Cows' body weight (BW) and body condition score (BCS) were 450 ± 20 kg and 4.6 ± 0.02, respectively, at beginning of the experiment. Initially, heifer calves were 138 ± 19 d old and weighted 131.8 ± 9.9 kg.

Pairs were placed in an experimental area of Brachiaria decumbens, divided in 5 paddocks of 7.0 ha each with free access to water and feeders.

The effects of level of supplementation applied to the calves in creep-feeding on the dam were evaluated using the following treatments: 0 – control, no supplement was fed to calves; 3 - calves received supplement in the amount of 3 g/kg of BW; 6 - calves received supplement in the amount of 6 g/kg of BW. Mineral salt (8.7% calcium, 9.0% phosphor, 18.7% sodium, 9.0% sulfur, 2400 mg/kg of zinc, 800 mg/kg of copper, 1600 mg/kg of manganese, 40.0 mg/kg of iodine, 8.00 mg/kg of cobalt, 8.16 mg/kg of selenium) was provided ad libitum for dams and calves from all treatments. The experiment was conducted in a completely randomized design, with 10 experimental units for treatment 0, and 20 experimental units for treatments 3 and 6. The experiment lasted 120 d divided into 3 experimental periods of 40 d.

Dam's Performance

After 14 h of solids fasting, cows were weighted in the beginning and in the end of the experiment. Fat thickness over the Longissimus muscle were measured by ultrasound scan (Aloka SSD 500; 3.5 MHz linear probe) between the 13th-14th ribs at the end of the experiment. Vegetable oil was used to ensure adequate acoustic contact. In the morning of the same day when ultrasound was performed, body condition scores (BCS) were also recorded on a scale ranging from 1 to 9 as recommended by NRC (1996) by 2 experienced technicians.

Forage Analysis

Pasture chemical composition was assessed by samples hand-plucked every 2 weeks. In the middle of every experimental period a second pasture sample was also collected to estimate forage potentially digestible dry matter (pdDM) as proposed by Paulino et al. (2008), 4

subsamples were randomly collected in each plot by cutting it close to the ground using a metal square (0.5 × 0.5 m). Samples were weighed and oven dried at 60ºC for 72 h. After that, mill grounded to pass through a 2 mm screen for indigestible neutral detergent fiber (iNDF) analysis (Casali et al., 2008). A sub portion of 20 g of each sample was grounded to pass through a 1 mm screen for analyses of dry matter (DM), ash, crude protein (CP) and neutral detergent fiber (NDF).

Samples of forage were analyzed following procedures described by Detmann et al. (2012) for DM (index INCT-CA G-003/1), CP (index INCT-CA N-001/1), ash (index INCT-CA M-001/1), NDF (index INCT-CA F-002/1) corrected for ash residue (index INCT-CA M-002/1) and residual nitrogen compounds (index INCT-CA N-004/1), and iNDF (index INCT-CA F-009/1) was evaluated using F57 (Ankon®) bags incubated in rumen by 288 h.

The potentially digestible dry matter (pdDM) was estimated using the second pasture sample collected in each period as described previously, using the following equation (Paulino et al., 2008):

pdDM (%; dry matter basis) = 0.98 × (100 – apNDF) + (apNDF – iNDF)

Where: 0.98 is the true digestibility coefficient of intracellular content; apNDF is forage content of neutral detergent fiber corrected for residual ash and nitrogen; iNDF is forage content of indigestible neutral detergent fiber.

Milk Production

Milk production was estimated on d 20, 60 and 100 of the experiment, milk production of the three sampling days were averaged by cow. Aiming to empty the udder, calves were separated from their mothers from 3 p.m. to 5:45 p.m, when they were reunited to the dams and allowed to suckle. At 6 p.m. calves were again separated from their dams until

the next morning. At 6 a.m. of the next day, cows were milked immediately after an injection of 20 IU of oxytocin (Ceva Ocitovet, Paulínia, Brazil) in the mammary vein and the produced milk was weighted. The milking was planned to do not occur time longer than 2 h from the first and the last cow milked. The exact time when each cow was milked was recorded and the milk production was converted into a 24h production. The milk produced was corrected to 4% of fat ($Milk_{4\%}$) calculated by the following equation (NRC, 2001):

$Milk_{4\%}$ (kg) = 0.4 × (milk production) + [15 × (fat production × milk production/100)]

Milk was analyzed for protein, fat, lactose, and total solids content, using spectroscopy (Foss MilkoScan FT120, Hillerød, Denmark).

Blood Sampling and Analysis

Blood samples were collected at the beginning of the experiment and each 40 d at 8:00 am, to measure the levels of glucose, total cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides, total protein, albumin, globulins and urea. Two samples of each dam were collected from the jugular vein with vacuum tubes. One of the samples was collected in tubes with clot activator and gel for serum separation (BD Vacuntainer® SST II Plus, São Paulo, Brazil) for analyses of total cholesterol, HDL, triglycerides, total protein, albumin and urea. The sample collected in the second tube with EDTA and sodium fluoride (BD Vacutainer® Fluoreto/EDTA, São Paulo, Brazil) was used for glucose analysis. After collected, the samples were centrifuged at 3600 × g for 20 min, serum and plasma were immediately frozen at -20°C in duplicates until further analysis.

Glucose (Ref. Number K082, Bioclin® Quibasa, Belo Horizonte, Brazil), total cholesterol (Ref. Number K083, Bioclin® Quibasa, Belo Horizonte, Brazil), HDL (Ref. Number K071, Bioclin® Quibasa, Belo Horizonte, Brazil), triglycerides (Ref. Number K117,

Bioclin® Quibasa, Belo Horizonte, Brazil), and urea (Ref. Number K056, Bioclin® Quibasa, Belo Horizonte, Brazil) were quantified by enzymatic-colorimetric method and total protein (Ref. Number K031, Bioclin® Quibasa, Belo Horizonte, Brazil) and albumin (Ref. Number K040, Bioclin® Quibasa, Belo Horizonte, Brazil) by colorimetric method. The LDL levels were estimated according to the Friedewald equation (Tietz, 1986). Globulins were calculated subtracting the albumin quantified from the total protein level. Serum urea N (SUN) was estimated as 46.67% of total serum urea. Metabolites were analyzed in accordance with manufacturer's instructions in an automatic biochemistry analyzer (Mindray BS200E, Shenzhen, China).

Statistical Analysis

Statistical analyzes were performed using PROC GLIMMIX in SAS 9.4. Orthogonal contrasts (Steel et al., 1997) were used to evaluate the linear and quadratic effects of level of supplementation. Initial BW and BCS were used as covariate for performance data analysis. Significant difference was considered at $P < 0.05$.

Results

Average forage mass potentially digestible was 3730, 5071 and 3152 kg/ha in each experimental period (Table 1) and CP content 103, 118 and 94 g/kg of forage.

There was no significant effect of level of supplementation offered to offspring on cow BW, BCS, subcutaneous fat thickness and average daily gain (ADG - Table 2; $P > 0.05$). Cows in all strategies gained BW during the experiment, approximately 217 g/d, and at weaning had an average BW of 480 kg. Cows from all treatments also gained BCS throughout the experiment and at weaning had BCS of 4.98 on average across all strategies. The same pattern observed for BCS at weaning was also observed for fat thickness at

weaning, there was no significant difference in fat thickness at weaning among strategies ($P > 0.05$).

The ADG and BW at weaning of their calves also did not differ among strategies, calves ADG was 704 g/d and weaning average BW was 218 kg (data not presented).

Level of supplementation of heifer calves did not significantly affect milk production corrected to 4% of fat ($P > 0.05$; Table 3). Milk composition of cows among supplementation strategies was similar as well ($P > 0.05$).

Level of supplement fed to calves had no effect on cows' glucose, total cholesterol, HDL, LDL, triglycerides, HDL:LDL ratio, total protein and albumin levels (Table 4; $P > 0.05$), but there was positive linear effect of level of supplementation offered to calves on cows' SUN levels ($P < 0.05$).

Discussion

Suckling can negatively affect cow reproduction by increasing milk production, and consequently reducing cow's BCS (Wettemann et al., 2002). Creep-feeding has been successfully used to progressively reduce calves nutritional and social dependence on the cow (Enríquez et al., 2011), with positive results on both, cow and calf performance.

In the present study no significant effect of creep-feeding was observed on cows' BW, ADG or BCS (Table 2). In agreement with our results, several authors also did not observe improvement in cow's performance when their calves were creep-fed (Valente el al., 2012; Valente et al., 2013; Moriel and Arthington, 2013).

Average daily milk production across treatments in the present study, approximately 7.0 kg (Table 3), was higher than the previously reported for cows from the same herd, 5.8 kg

when nursing heifer calves (Valente et al., 2012) and 6.2 kg when nursing male calves (Valente el al., 2013), probably due the higher forage quality (Table 1) during the present study compared to the previous studies cited.

Strategies applied to calves were not capable of modifing cows' metabolism; with exception to serum urea N (SUN), the levels of all metabolites evaluated were not different among treatments. Metabolites concentrations in the blood steam are strongly related to diet quality and nutrient availability. No difference in most metabolites evaluated is explained by the fact that diet was equal for cows from all treatments (pasture plus mineral salt ad libitum), as only their calves received supplement.

Average glucose serum levels of cows in the present study, approximately 58 mg/dL, are lower than previously reported by Hart et al. (1978) and Lake et al. (2006) for low yield lactating cows, of 68.1 and 71.2 mg/dL, respectively. This lower glucose level is probably due the fact that no concentrate was fed to cows in the present study as opposed to those studies of Hart et al. (1978) and Lake et al. (2006).

Grummer and Carroll (1988) reviewing lipoproteins cholesterol in different species reported that in ruminants HDL accounts for the majority of blood cholesterol, whereas in most species LDL prenominates as main source of cholesterol for tissues. Furthermore, Williams and Stanko (1999) reported that about 80% of bovine lipoproteins cholesterol are high density lipoproteins. This pattern was not observed in the present study, where cows had a HDL:LDL ratio close to one, indicating that proportions of HDL and LDL were fairly similar. The high levels of total serum cholesterol (200 mg/dL) of cows in the present study are consistent with the reported range for lactating cows (up to 300 mg/dL; reviewed by Williams and Stanko, 1999).

While urea varies widely among days and even within the day, albumin is good long term indicator of protein status, since no large variation occurs within a short period of time (Fuhrman at al., 2004). Although control cows had reduced SUN levels, no difference was observed for total protein and albumin, providing evidence that control cows were not in a detrimental protein and nutritional status. There is no biological explanation for the linear effect of calves supplementation in cows' SUN level's found in the present study.

We conclude that creep-feeding heifer calves in the amounts of 3 or 6 g/kg of BW daily has no major effect on performance or metabolism of the lactating dam.

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Table 1. Potentially digestible forage mass and chemical composition of forage in each experimental period

[A] pdDM – potentially digestible forage dry matter; OM – organic matter; CP – crude protein; NDIN – neutral detergent insoluble N; apNDF – neutral detergent fiber corrected for ash and protein residue; iNDF – indigestible neutral detergent fiber.

[B] pdDM was estimated for forage sampled in the area delimited by a metal square 0.5 × 0.5; chemical composition was evaluated in the hand-plucked forage sample.

Item[A,B]	Experimental Period		
	1	2	3
pdDM (kg/ha)	3730	5071	3152
OM (g/kg)	926	914	921
CP (g/kg)	102.9	118.4	93.8
NDIN (% of total N)	31.9	26.1	30.2
apNDF (g/kg)	558	581	603
iNDF (g/kg)	172	183	236

Table 2. Performance of lactating dams according to treatment applied to offspring

[A] BW – body weigh; BCS – body condition score; ADG – average daily gain;

[B] Treatments applied to offspring: 0 – no supplementation; 3 – supplementation of 3 g/kg of BW; 6 – supplementation of 6 g/kg of BW;

[C] L and Q – linear and quadratic effects of calves' supplementation.

Item[A]	Treatment[B]			SEM	P-value[C]	
	0	3	6		L	Q
Initial BW (kg)	441	454	453	19.6	0.61	0.71
Initial BCS	4.60	4.63	4.71	0.21	0.66	0.87
BW at Weaning (kg)	480	474	477	4.62	0.62	0.30
BCS at Weaning	4.80	5.13	5.03	0.21	0.39	0.29
Fat Thickness at Weaning (mm)	3.69	4.38	4.57	0.95	0.45	0.78
ADG (g/d)	242	192	218	41.4	0.61	0.30

Table 3. Milk production and milk composition of dams according to treatment applied to offspring

[A] $Milk_{4\%}$ – milk production corrected to 4% of fat; TS – total solids.

[B] Treatments applied to offspring: 0 – no supplementation; 3 – supplementation of 3 g/kg of BW; 6 – supplementation of 6 g/kg of BW;

[C] L and Q – linear and quadratic effects of calves' supplementation.

Item[A]	Treatment[B]			SEM	P-value[C]	
	0	3	6		L	Q
$Milk_{4\%}$ (kg)	7.63	6.58	6.74	0.69	0.29	0.35
Fat (%)	4.42	4.63	4.80	0.26	0.23	0.95
Protein (%)	3.63	3.46	3.42	0.10	0.09	0.50
Lactose (%)	4.49	4.35	4.43	0.07	0.50	0.14
TS (%)	13.7	13.6	13.8	0.34	0.89	0.63

Table 4. Metabolic profile of dams according to treatment applied to offspring

[A] Treatments applied to offspring: 0 – no supplementation; 3 – supplementation of 3 g/kg of BW; 6 – supplementation of 6 g/kg of BW;

[B] L and Q – linear and quadratic effects of calves' supplementation.

Item	Treatment[A]			SEM	P-value[B]	
	0	3	6		L	Q
Glucose (mg/dL)	58.0	57.8	58.4	1.19	0.81	0.70
Cholesterol (mg/dL)	214	191	194	11.8	0.18	0.24
HDL (mg/dL)	94.1	92.9	94.1	2.67	0.99	0.65
LDL (mg/dL)	114.4	91.3	95.6	11.2	0.18	0.20
Triglycerides (mg/dL)	25.9	25.6	26.1	1.51	0.94	0.76
HDL:LDL ratio	0.98	1.14	1.19	0.18	0.35	0.74
Total Protein (g/dL)	7.40	7.42	7.56	0.12	0.29	0.58
Albumin (g/dL)	3.40	3.39	3.37	0.06	0.68	0.96
Globulins (g/dL)	3.99	4.03	4.19	0.08	0.21	0.57
SUN (mg/dL)	12.0	13.4	13.8	0.52	0.01	0.29

Supplement delivery strategies for Nellore cows during the last third of gestation

Abstract. Two experiments were conducted aiming to evaluate the effect of supplement delivery strategies for beef cows on the last third of gestation. In Experiment 1, thirty-five pregnant Nellore cows were assigned to a completely randomized design with 4 treatments: control, which received no supplement; supplementation for the last 30 d of gestation (30d; 3.0 kg/d); supplementation for the last 60 d of gestation (60d; 1.5 kg/d); or supplementation for the last 90 d of gestation (90d; 1.0 kg/d). All supplemented treatments received the same total amount of supplement (20% of crude protein; CP) throughout the experiment, 90 kg. A second experiment (Experiment 2) was delineated aiming to evaluate the effects of the amounts offered in Experiment 1 on intake and metabolism. Four multiparous pregnant Nellore cows were assigned to a 4 × 4 Latin square design, with 4 experimental periods of 15 d each. There was a linear effect of days of supplementation on calving body weight (BW; $P < 0.05$) and a quadratic effect on BW change from parturition to d 31 post-calving ($P < 0.05$), with cows on the 60d strategy loosing less BW. No difference was found in offspring birth BW ($P > 0.10$). The 60d strategy had the highest pregnancy rate in the first fixed time artificial insemination (FTAI; $P = 0.08$), but there was no difference in the overall pregnancy rates ($P > 0.10$). Level of supplementation did not affect forage intake or neutral detergent fiber digestibility ($P > 0.10$). Nitrogen excreted through urine tended to increase linearly with level of supplementation ($P < 0.10$). We conclude that providing 1.5 kg of supplement during the last 60 d of gestation improves cow reproductive performance in the following breeding season with no negative effect on forage intake.

Additional keywords: calving, nutrition, flushing, reproduction

Introduction

Among the factors affecting the reproductive performance of beef cattle, nutrition is perhaps the one that has the highest impact (Wettemann et al., 2002). It is estimated that 50% of the cows in extensive beef systems do not receive adequate nutritional management and that is one of the major reasons for low fertility rates in tropical herds (Madureira et al., 2014).

Supplementation to grazing animals is a practice that can be adopted in pasture management under tropical conditions to increase supporting capacity of the pastures and animal performance and, when applied in the last third of gestation, is essential to accumulate energy in body tissues in order to lessen the post calving negative energy balance. According to Baruselli et al. (2004), inadequate nutritional status at calving is the main factor limiting adequate response to synchronization protocols.

In this context, finding the best strategy of supplementation is necessary to improve performance, reduce labor and consequently feeding costs. Therefore, we conducted a study to evaluate the effects of supplementation strategies for pregnant beef cows in the last third of gestation.

Material and Methods

The 2 experiments were conducted at the facilities of Department of Animal Science – Universidade Federal de Viçosa, Brazil, from July to December of 2012. All animal care and handling procedures were ethically standardized and approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil.

Experiment 1 – Performance

Thirty-five multiparous, average 5 yr old, single pregnant Nellore cows with 491.88 ± 55 kg average body weight (BW), 4.7 ± 0.58 average body condition score (BCS) and 200 ± 15 d of gestation were used. Cows were housed in an experimental area of Brachiaria decumbens divided in 4 paddocks of 5.0 ha. Cows had unlimited access to water, feeders and mineral salt (8.7% calcium, 9.0% phosphor, 18.7% sodium, 9.0% sulfur, 2400 mg/kg of zinc, 800 mg/kg of copper, 1600 mg/kg of manganese, 40.0 mg/kg of iodine, 8.00 mg/kg of cobalt, 8.16 mg/kg of selenium).

The strategies evaluated were: 30d – cows received 3.0 kg of supplement beginning 30 d prior to calving; 60d – cows received 1.5 kg of supplement beginning 60 d prior to calving; 90d – cows received 1.0 kg of supplement beginning 90 d prior to calving; and control – no supplement was fed. All supplemented treatments received the same total amount of supplement throughout the experiment, 90 kg/d per head.

Animals were assigned to a completely randomized design with 4 treatments. Due the miscarriage of one cow during the experimental period, there were 9 replicates for treatments control, 60d and 90d, and 8 replicates for treatment 30d. Supplement was composed of corn, sorghum and soybean meal, and formulated to contain approximately 20% crude protein (CP; Table 1).

Cow BW was recorded at the beginning of the experiment, the week before the expected date of parturition (Calving BW), and 31 d after parturition. Cow BCS was recorded at the beginning of experiment, prior to calving and in the first day of the breeding season on a scale ranging from 1 to 9, as recommended by NRC (1996) by 2 experienced technicians. Calf BW was also recorded at birth. Shrunk BW (SBW) was calculated using adjustments proposed by Gionbelli et al. (2015) for Nellore cows as follows:

$SBW = 0.8084 \times BW^{1.0303}$

After calving cows were managed as a single herd until the pregnancy diagnosis.

Twenty-one and 31 days after calving a blood sample was taken from the jugular vein, using vacuum tubes with clot accelerator and gel for serum separation (BD Vacuntainer® SST II Plus, São Paulo, Brazil). Immediately after collection the samples were centrifuged at 3600 × g for 20 min, the serum was frozen at -20°C and subsequently analyzed for progesterone by chemiluminescent method using Access Progesterone Reagent (Ref. Number 33550, Beckman Coulter®, Brea, USA) in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA).

Pasture chemical composition (Table 2) was assessed by samples hand-plucked every 2 weeks. In the middle of every experimental period a second pasture sample was also collected to estimate forage potentially digestible dry matter (pdDM) as proposed by Paulino et al. (2008), 4 subsamples were randomly collected in each plot by cutting it close to the ground using a metal square (0.5 × 0.5 m). Samples were weighed and oven dried at 60ºC for 72 h. After that, mill grounded to pass through a 2 mm screen for indigestible neutral detergent fiber (iNDF) analysis (Casali et al., 2008). A sub portion of 20 g of each sample was grounded to pass through a 1 mm screen for analyses of dry matter (DM), ash, crude protein (CP) and neutral detergent fiber (NDF).

Forage samples were analyzed following procedures described by Detmann et al. (2012) for DM (index INCT-CA G-003/1), CP (index INCT-CA N-001/1), ash (index INCT-CA M-001/1), NDF (index INCT-CA F-002/1) corrected for ash residue (index INCT-CA M-002/1) and residual nitrogen compounds (index INCT-CA N-004/1), and iNDF (index INCT-CA F-009/1) was evaluated using F57 (Ankon®) bags incubated in rumen by 288 h.

The potentially digestible dry matter (pdDM) was estimated using the second sample collected in each period as described previously using the following equation (Paulino et al., 2008):

pdDM (%; dry matter basis) = 0.98 × (100 – apNDF) + (apNDF – iNDF)

Where: 0.98 is the true digestibility coefficient of intracellular content; apNDF is forage content of neutral detergent fiber corrected for residual ash and nitrogen; iNDF is forage content of indigestible neutral detergent fiber.

In the breeding season cows were synchronized using the following protocol: on d 0 a intravaginal device of progesterone release (Tecnopec Primer®, São Paulo, Brazil) was inserted and injection of 2.0 mg of estradiol benzoate (Tecnopec RIC-BE®, São Paulo, Brazil) i.m. was performed. On d 7 the intravaginal device was removed and cows received a 2 mL injection of cloprosterol sodium (MSD Saúde Animal Ciosin®, São Paulo, Brazil). Finally, on d 8 cows received 0.5 mL of estradiol cypionate i.m. (Zoetis-Pfizer E.C.P.®, Campinas, Brazil). Fixed time artificial insemination (FTAI) was preformed 46 to 52 h following intravaginal device removal (d 10). Semen from 5 Nellore sires were randomly assigned to each cow. The protocol was repeated once more in a way that cows that did not conceive in the first FTAI were inseminated again 30 d after the first one. Pregnancy diagnosis was determined via trans-rectal ultrasonography 30 d after each FTAI. Overall pregnancy rate was calculated by considering the cows that conceived in the first and second FTAI.

Response variables were analyzed using GLIMMIX in SAS 9.4. Initial BW of cows was used as covariate for performance data analysis. Treatments were compared using orthogonal contrasts (Steel et al., 1997). Contrasts were constructed in order to evaluate the

effects of supplementation and the linear and quadratic effects of days of supplementation (30, 60 and 90 days). Significant difference was considered at $P < 0.05$.

Experiment 2 – Intake and Metabolism

In order to evaluate the effects of the amounts of supplement offered daily in Experiment 1 on intake and metabolism, a second experiment was conducted simultaneously. Four multiparous, 5 yr old, single pregnant Nellore cows with 488 ± 22 kg average BW, 4.7 ± 0.3 average BCS and 210 ± 10 d of gestation were assigned to a 4 × 4 Latin square design, with 4 experimental periods of 15 d each.

Cows were individually housed in an experimental area of Brachiaria decumbens divided in 4 paddocks of 0.34 ha with free access to water, mineral salt and feeders. Experiment 2 started 15 d later than Experiment 1.

The experimental treatments evaluated were: 3.0kg – cows received 3.0 kg of supplement daily; 1.5kg – cows received 1.5 kg of supplement daily; 1.0kg – cows received 1.0 kg of supplement daily; and 0.0kg – no supplement was fed. Supplement was composed of corn, sorghum and soybean meal, and formulated to contain approximately 20% CP (Table 1).

Pasture chemical composition (Table 3) was assessed by hand-plucked samples collected on the 8th d of each experimental period. On the same day, a second pasture sample was also collected to estimate forage potentially digestible dry matter (pdDM) as proposed by Paulino et al. (2008), 4 subsamples were randomly collected in each plot by cutting it close to the ground using a metal square (0.5 × 0.5 m). Samples were weighed and oven dried at 60ºC for 72 h. After that, mill grounded to pass through a 2 mm screen for indigestible neutral detergent fiber (iNDF) analysis (Casali et al., 2008). A sub portion of 20 g of each sample

was grounded to pass through a 1 mm screen for analyses of dry matter (DM), ash, crude protein (CP) and neutral detergent fiber (NDF).

After 6 days of adaptation to new treatments in each period, a 9 d intake trial was carried out in each period. To estimate fecal excretion, chromium oxide (Cr_2O_3) was used as external maker in the amount of 15 g per animal. The chromium oxide was packed in paper cartridges and delivered via esophagus with a metal probe once daily, at 10 a.m. To estimate DM intake, indigestible neutral detergent fiber (iNDF) was used as internal marker. After the start of the intake trial, six days were allowed for stabilization of marker excretion; after that, fecal samples were collected at 3 p.m. on the 7th day, at 11 a.m. on the 8th day, and at 7 a.m. on the 9th day of the intake trial (13th, 14th and 15th day of each experimental period, respectively).

Feces samples were collected directly into the rectum of animals at amounts of approximately 200 g, dried (60°C/72 h) and mill grounded to pass through a 2 mm screen for indigestible neutral detergent fiber (iNDF) analysis (Casali et al., 2008). A sub portion of 20 g of each sample was grounded to pass through a 1 mm screen for analyses of dry matter (DM), ash, crude protein (CP) and neutral detergent fiber (NDF) as described for forage samples. Grounded samples were proportionally sub-sampled to a pooled 3 d sample per animal per period.

Samples of forage, feces and supplement were analyzed following procedures described by Detmann et al. (2012) for DM (index INCT-CA G-003/1), CP (index INCT-CA N-001/1), ash (index INCT-CA M-001/1), NDF (index INCT-CA F-002/1) corrected for ash residue (index INCT-CA M-002/1) and residual nitrogen compounds (index INCT-CA N-004/1), and iNDF (index INCT-CA F-009/1) was evaluated using F57 (Ankon®) bags incubated in rumen by 288 h. Fecal samples were also analyzed for levels of chromium by

atomic absorption spectrophotometry (index INCT-CA M-005/1) and titanium dioxide by colorimetry (index INCT-CA M-007/1) as recommended by Detmann et al. (2012).

The potentially digestible dry matter (pdDM) was estimated using the second sample collected in each period as described previously using the following equation (Paulino et al., 2008):

pdDM (%; dry matter basis) = 0.98 × (100 – apNDF) + (apNDF – iNDF)

Where: 0.98 is the true digestibility coefficient of intracellular content; apNDF is forage content of neutral detergent fiber corrected for residual ash and nitrogen; iNDF is forage content of indigestible neutral detergent fiber.

Fecal excretion (FE) was estimated by the ratio of chromium oxide and its concentration in the feces. Dry matter intake (DMI) was estimated by using the iNDF as an internal marker and calculated by the following equation:

DMI (kg/d) = [((FE × iNDF feces) – iNDF supplement) ÷ iNDF forage] + SI

Where FE is the fecal excretion (kg/d); iNDF feces is the concentration of iNDF in the feces (kg/kg); iNDF supplement is the iNDF in the supplement (kg); iNDF forage is the concentration of iNDF in forage (kg/kg) and SI is supplement intake.

At the 15th d of each period 2 blood samples were collected immediately before and 4h after supplementation to estimate insulin levels pre and post-supplementation, respectively. Blood was collected in tubes with clot activator and gel for serum separation (BD Vacuntainer® SST II Plus, São Paulo, Brazil), centrifuged at 3600 × g for 20 min and serum was immediately frozen at -20°C in duplicates until further analysis. The same blood collected 4 h after supplementation was used for quantification of serum urea concentrations.

Insulin was analyzed by chemiluminescent method using Access Ultrasensitive Insulin Reagent (Ref. Number 33410, Beckman Coulter®, Brea, USA) in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA). Urea was quantified by enzymatic-colorimetric method using reagents provided by commercial kits (Ref. Number K056, Bioclin® Quibasa, Belo Horizonte, Brazil) in an automatic biochemistry analyzer (Mindray BS200E, Shenzhen, China). Serum urea N (SUN) was estimated as 46.67% of total blood urea.

Spot sampling at the 15th d of each experimental period was used to assess the excretion of urinary nitrogenous compounds (Valadares et al., 1999). Urine volume was estimated using creatinine concentration as a marker and assuming a daily creatinine excretion (mg/d) of $34.5 \times SBW^{0.9491}$ (Silva et al., 2012). Microbial N synthesis was estimated by using the technique of the purine derivatives in urine. Allantoin was estimated by colorimetric method as proposed by Chen and Gomes (1992). The urinary concentrations of creatinine and uric acid were obtained by colorimetric and enzymatic-colorimetric methods, respectively. The analyses of creatinine and uric acid were performed in an automatic biochemistry analyzer (Mindray BS200E, Shenzhen, China) using commercial kits (Ref. Number K067 for creatinine and K139 for uric acid, Bioclin® Quibasa, Belo Horizonte, Brazil).

Excretion of the purine derivatives in urine was calculated by the sum of the allantoin and uric acid excretions, which were obtained by the product between their concentrations in urine by the daily urinary volume. Absorbed purines were calculated from the excretion of purine derivatives (Barbosa et al., 2011) as follows:

$$Y = \frac{x - 0.301 \times BW^{0.75}}{0.8}$$

where Y = absorbed purines (mmol/d), x = excretion of purine derivates (mmol/d), 0.8 = recovered absorbed purines. The $0.301 \times BW^{0.75}$ value = endogenous excretion of purine derivates.

Ruminal synthesis of nitrogen compounds was calculated as a function of the absorbed purines (Barbosa et al., 2011) as follows:

$$Z = \frac{70 \times Y}{0.93 \times 0.137 \times 1000}$$

Where Z = ruminal synthesis of nitrogen compounds (g/d), Y = absorbed purines (mmol/d), 70 = purine N content (mg/mol), 0.93 = purine digestibility and 0.137 = relation of purine N:total N of microorganisms.

Linear, quadratic and cubic effects of amount of supplement fed were analyzed using GLIMMIX in SAS 9.4. Animal and period were considered random effects. Significant difference was considered at $P < 0.05$.

Results

Experiment 1

There was a linear effect of days of supplementation on cow BW at calving (Table 4; $P < 0.05$), BW at calving ranged from 508 kg for cows in 30d strategy to 531 kg for cows in 90d strategy. Birth BW of calves averaged 34.4 kg and was not different according to supplementation strategy used for their mothers in the last third of gestation ($P > 0.10$).

Thirty one days after calving cows that received supplementation pre calving tended to be heavier (480 kg, $P < 0.10$) compared to control cows (465 kg). Supplemented cows also lost less BW from parturition to 31 d post-calving ($P < 0.05$), within supplemented treatments

there was a quadratic effect of days of supplementation on post-partum BW change ($P < 0.05$), with cows from 60d strategy losing less BW (-25 kg) compared to cows in strategies 30d and 90d (-50 kg on average). Supplementation or level of supplementation did not affect BCS at any point evaluated ($P > 0.10$).

At 21 d post calving, progesterone concentration linearly reduced with days of supplementation ($P = 0.05$). Ten days later, at 31 d post calving, supplemented cows tended to have greater progesterone concentration compared to cows receiving no supplement ($P < 0.10$), within supplemented cows, no significant difference was observed ($P > 0.10$).

Besides no difference among treatments in overall pregnancy rate ($P > 0.10$), there was a tendency for a significant quadratic effect in pregnancy rate in 1st FTAI ($P < 0.10$) and a significant quadratic effect on interval from parturition to conception ($P < 0.05$). Overall pregnancy rate averaged 77.1% across strategies and calving to conception interval means were 64.1 days for control, 57.2 for 30d strategy, 63.1 days for treatment 60d, and the highest calving to conception interval was observed for cows in 90d strategy, 84.2 days.

Experiment 2

Level of supplementation linearly increased intake of DM (kg/d and g/kg of BW), DM digested (kg/d and g/kg of BW), OM (kg/d and g/kg of BW) and OM digested (kg/d) and crude protein (g/d – Table 5; $P < 0.05$), there was also a cubic effect of level on intake of crude protein (g/d; $P < 0.05$). There was no effect of level of supplementation on intake (g/d and g/kg BW) of forage DM, forage OM, forage apNDF, apNDF and iNDF.

Level of supplementation increased the digestibility of OM and CP linearly ($P < 0.05$; Table 6). There was also a cubic effect of level on digestibility of OM ($P < 0.05$).

Microbial N produced in g/d was not different among levels ($P > 0.10$; Table 7), nor was efficiency of microbial N produced in relation to N ingested and OM digested ($P > 0.10$). Level of SUN was similar among strategies ($P > 0.10$) but ureic nitrogen excreted in g/d (UUN) tended to increase linearly with level of supplementation ($P < 0.10$).

Pre supplementation levels of insulin were not different according to level of supplementation ($P > 0.10$; Table 8) but insulin levels 4h post supplementation increased linearly with amount of supplement fed ($P < 0.05$).

Discussion

Nutritional status at calving is the most important factor that influences the interval from parturition to conception in beef cows. Postpartum nutrient intake can modulate the duration of the postpartum anestrous interval; however, if thin cows gain great amounts of weight after calving, ovulation occurs later than for cows that calve in good body condition and maintain body weight (Wettemann et al., 2002).

Cabral et al. (2012), supplementing pregnant cows grazing pastures in similar conditions of the present study with different amounts of supplement/d, found a quadratic pattern on performance, with cows receiving 1.0 kg of supplement daily gaining more BW. Total amount of supplement provided for treatment 1.0 kg in Cabral's work (84 kg) was similar to the present study (90 kg).

Based on the magnitude of post-partum BW change, supplementing cows with 1.5 kg of supplement during the last 60d prior to calving was an efficient strategy to lessen the post-partum negative energy balance, since cows on that strategy lost about half (-25 kg) of the BW lost in strategies 30d and 90d (average -50 kg). These results provide evidence that adopting a strategy to deliver the same amount of supplement in 60 days prior to calving,

instead of the usually recommended 90 days, can be not only economical but also improve cows' post-partum performance.

Not satisfactory results using FTAI are associated with low BCS of cows that limits the percentage of cows cycling in the breeding season (Madureira et al., 2014). No difference in BCS at calving was observed among treatments in the present study thought. Cows from all strategies presented adequate BCS at the breeding season, 5.0 or slightly below in a 1 to 9 scale, what probably accounts for the lack of difference in overall pregnancy rates among strategies.

Previous studies in cattle during late gestation (Loerch, 1996; Radunz et al., 2010) have provided evidence that feeding systems during the last third of gestation can alter subsequent birth weight of progeny, thereby suggesting maternal dietary energy source may affect fetal growth (Radunz et al., 2011).

Although maternal intake of protein has been shown to be an important factor for fetal growth, no difference was observed for calf birth BW in the present study, probably because forage presented median quality (Table 2). Similarly Summers et al. (2015) found no difference in calves' birth BW according to supplementation strategy applied to their mothers.

In tropical pastures the use of energetic-protein supplements can impact forage intake in different ways. The most desirable is increasing forage intake, usually observed when pasture quality is low and CP content of forage is below 70 g/kg (Lazzarini et al., 2009). When forage CP is below this threshold, N provided to ruminal microorganisms via supplement increases microorganisms' growth and consequently fiber digestibility (Costa et al., 2008) and forage intake.

Not desirable in most situations, feeding high amounts of supplement can cause reduction in forage DM intake. The animal partially substitutes the forage by the supplement maintaining total DM intake. In tropical regions this can be observed when low-protein high-energy supplement is fed (Mould et al., 1983; Costa et al., 2011).

Additive effect of supplementation is a third type of pasture × supplement interaction and can be observed in the present study. Linear increase of DM, OM and CP intake with levels of supplementation is simply due supplement intake, as no difference in forage DM intake was observed in the present study.

During the intake and metabolism experiment, average forage CP content was adequate or slightly below the minimum required by ruminal microorganisms (Lazzarini et al., 2009), as presented in Table 3. In this way, level of supplementation did not affected apNDF digestibility or intake, once more, attesting the additive effect of supplementation in the present study.

Although no difference in insulin levels have been observed pre supplementation, level of supplementation significantly increased insulin levels 4h post supplementation in a linear pattern. For animals in an exclusive forage diet, relative amount of propionate, a glycogenic precursor, available for metabolism is low and supplementation significantly increases proportion of propionate produced in the rumen (Martin et al., 1999; Philippeau et al., 1999; Huntington et al., 2006).

Hawkins et al. (2000) have suggested that the increase in insulin, concomitant with decreased GH, it is an important relationship to consider when evaluating the impact of nutrition on reproduction. Insulin is an important mediator of nutritional effects on follicular dynamics in cattle and can stimulate the release of GnRH from the hypothalamus. In the

ovaries, insulin may also stimulate cell proliferation and steroidogenesis (Wettemann and Bossis, 2000) and in the liver to produce IGF-1 (Webb et al., 2004). In accordance with these findings, in the present study supplementing with higher amounts/d linearly increased progesterone concentrations 21 d after calving and supplemented animals had higher progesterone concentrations 31 d post calving.

Cows in the 60d strategy had the highest pregnancy rate at first FTAI, even though no difference was found in the overall pregnancy rates. Cows that conceive early calve early, and have better opportunity to start reproductive cycles in time to conceive in next breeding season. Calving date also affects the value of offspring, demonstrating the importance of strategies to improve conception rates at the beginning of breeding season.

Cushman et al. (2013) and Funston et al. (2012) reported that heifer calves born early tend to conceive early in their first breading season and remain in the herd. Calving date can also impact male offspring performance; steer calves born earlier in the calving season have greater weaning BW, hot carcass weight, and marbling scores (Funston et al., 2012). In this way, increasing early calving by early conception may increase progeny value at weaning, enhance carcass value of the steers and increase heifer's pregnancy rate in their first breeding season.

Early luteal activity and high progesterone concentration can delay uterine involution (Smith and Wallace, 1998) and a persistent corpus luteum causes lower reproductive efficiency (Lamming and Darwash, 1998). High incidence of persistent corpus luteum and reduced conception rate was observed in cows having early postpartum ovulation (Smith and Wallace, 1998). This may explain lower FTAI performance of cows in 30d strategy, which had numerically higher progesterone levels at 21 and 31 days post-partum.

The interval from calving to conception greatly influences profitability of beef production. Hence, in beef systems, it is preconized calving internal to be no longer than 85 days in order to assure cow is going to produce a calf per year. All treatments in the present study presented acceptable calving interval, but cows receiving higher amounts of supplement per day for reduced number of days, had lower calving interval compared to cows receiving 1 kg/d during 90 days.

We conclude that, providing 1.5 kg of supplement during the last 60 days of gestation is a nutritional management strategy that can be adopted, as it improves cow reproductive performance in the following breeding season with no negative effect on forage intake.

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Table 1. Ingredients and composition of supplements provided to cows on the last third of gestation

[A] OM – organic matter; CP – crude protein; apNDF – neutral detergent fiber corrected for ash and protein residue.

Item[A]	Supplement
Ingredients (%; as-fed basis)	
Corn	33
Sorghum	33
Soybean Meal	34
Chemical Composition (g/kg)	
OM	971
CP	208
apNDF	164

Table 2. Potentially digestible forage mass and chemical composition of forage in Experiment 1

[A] pdDM – potentially digestible forage dry matter; OM – organic matter; CP – crude protein; NDIN – neutral detergent insoluble N; apNDF – neutral detergent fiber corrected for ash and protein residue; iNDF – indigestible neutral detergent fiber.

[B] pdDM was estimated for forage sampled in the area delimited by a metal square 0.5 × 0.5; chemical composition was evaluated in the hand-plucked forage sample.

Item[A,B]	Experimental Period		
	1	2	3
pdDM (kg/ha)	4816	4054	3405
OM (g/kg)	912	913	922
CP (g/kg)	77.4	66.6	65.2
NDIN (% of total N)	6.94	7.54	9.18
apNDF (g/kg)	618	628	679
iNDF (g/kg)	225	251	272

Table 3. Potentially digestible forage mass and chemical composition of forage in Experiment 2

[A] pdDM – potentially digestible forage dry matter; OM – organic matter; CP – crude protein; NDIN – neutral detergent insoluble N; apNDF – neutral detergent fiber corrected for ash and protein residue; iNDF – indigestible neutral detergent fiber.

[B] pdDM was estimated for forage sampled in the area delimited by a metal square 0.5 × 0.5; chemical composition was evaluated in the hand-plucked forage sample.

Item[A,B]	Experimental Period			
	1	2	3	4
pdDM (kg/ha)	4320	3742	3245	1643
OM (g/kg)	925	917	922	938
CP (g/kg)	68.1	75.7	59.8	52.3
NDIN (% of total N)	10.4	10.4	12.3	10.9
apNDF (g/kg)	652	644	712	719
iNDF (g/kg)	257	239	312	345

Table 4. Cow BW and BCS, calf BW, progesterone concentrations and reproductive performance of cows according to supplement delivery strategy applied on the last third of gestation

[A] BW- body weight; BCS – body condition score; FTAI – fixed time artificial insemination.

[B] Treatments: 30d - cows received 3.0 kg of concentrate supplement beginning 30 d prior calving; 60d - cows received 1.5 kg of concentrate supplement beginning 60 d prior calving; 90d - cows received 1.0 kg of concentrate supplement beginning 90 d prior calving; and control - no concentrate supplement was fed.

[C] S – effect of supplementation, supplemented treatments compared to the control; L and Q – effects of linear and quadratic order of supplement delivery strategy (30, 60 or 90 d).

Item[A]	Treatment[B]				SEM	P-value[C]		
	30d	60d	90d	Control		S	L	Q
Supplement offered (kg/d)	3.00	1.50	1.00	-				
Cow BW (kg) and BCS and Calf BW (kg)								
Initial BW	494	517	503	503	18.0	0.95	0.74	0.43
Initial BCS	4.66	4.65	4.54	4.87	0.20	0.28	0.65	0.85
Calving BW	508	515	531	522	6.79	0.56	0.02	0.55
Calving BCS	4.74	4.83	4.83	4.82	0.15	0.95	0.70	0.82
Calf Birth BW	33.8	31.7	35.8	36.2	1.93	0.28	0.43	0.10
Cow BW 31d after Calving	468	490	482	465	7.73	0.09	0.21	0.15
BW change from parturition to d 31 post-calving	-46.7	-24.9	-53.5	-62.1	8.24	0.04	0.58	0.03
Breeding Season BCS	5.00	5.11	4.83	5.03	0.21	0.84	0.57	0.43
Progesterone Concentration (ng/dL)								
21d after Calving	0.27	0.21	0.12	0.14	0.06	0.34	0.05	0.82
31d after Calving	1.24	0.70	0.69	0.14	0.37	0.08	0.29	0.55
Cow Reproductive Performance (%)								

Pregnancy Rate 1st FTAI	37.5	66.7	22.2	44.4	0.80	0.87	0.50	0.08
Overall Pregnancy Rate	75.0	88.9	55.6	88.9	1.06	0.43	0.41	0.24
Calving to Conception, d	57.2	63.1	84.2	64.1	5.85	0.53	0.01	0.30

Table 5. Intake according to supplement delivery strategy applied to cows on the last third of gestation

[A] DM – dry matter; OM – organic matter; apNDF – neutral detergent fiber corrected for ash and protein residue; iNDF – indigestible neutral detergent fiber; CP – crude protein.

[B] Treatments: 3.0kg - cows received 3.0 kg of concentrate daily; 1.5kg - cows received 1.5 kg of concentrate daily; 1.0kg - cows received 1.0 kg of concentrate daily; and 0.0kg - no concentrate supplement was fed.

[C] L, Q and C – effects of linear, quadratic and cubic order of level of supplementation.

Item[A]	Treatment[B]				SEM	P-value[C]		
	3.0kg	1.5kg	1.0kg	0.0kg		L	Q	C
kg/d								
Forage DM	5.56	5.69	6.00	5.61	0.72	0.96	0.73	0.86
DM	8.03	6.92	6.82	5.61	0.72	0.05	0.94	0.15
DM Digested	4.00	2.97	2.76	2.08	0.45	0.01	0.67	0.08
Forage OM	5.11	5.26	5.55	5.22	0.65	0.90	0.72	0.89
OM	7.51	6.46	6.35	5.22	0.65	0.05	0.95	0.15
OM Digested	4.03	3.01	2.83	2.13	0.41	0.01	0.68	0.06
Forage apNDF	3.70	3.89	4.11	3.85	0.48	0.83	0.66	0.94
apNDF	4.11	4.09	4.25	3.85	0.48	0.72	0.71	0.68
iNDF	1.56	1.69	1.71	1.60	0.19	0.88	0.53	0.95
g/d								
CP	882	613	556	359	58.8	<0.01	0.48	<0.01
g/kg BW								
Forage DM	9.95	9.78	10.15	9.58	1.22	0.84	0.88	0.77
DM	14.35	11.92	11.53	9.58	1.23	0.03	0.85	0.12
DM Digested	7.16	5.09	4.68	3.55	0.77	0.01	0.54	0.07
Forage OM	9.14	9.05	9.39	8.92	1.12	0.89	0.87	0.81
OM	13.42	11.13	10.74	8.92	1.13	0.03	0.84	0.12
OM Digested	5.06	4.74	4.75	6.25	0.95	0.24	0.20	0.39
Forage apNDF	6.64	6.70	6.94	6.59	0.84	0.97	0.81	0.87
apNDF	7.36	7.05	7.17	6.59	0.84	0.54	0.88	0.61
iNDF	2.81	2.93	2.87	2.74	0.33	0.89	0.70	0.99

Table 6. Coefficients of digestibility (%) according to supplement delivery strategy applied to cows on the last third of gestation

[A] DM – dry matter; OM – organic matter; apNDF – neutral detergent fiber corrected for ash and protein residue; CP – crude protein.

[B] Treatments: 3.0kg - cows received 3.0 kg of concentrate daily; 1.5kg - cows received 1.5 kg of concentrate daily; 1.0kg - cows received 1.0 kg of concentrate daily; and 0.0kg - no concentrate supplement was fed.

[C] L, Q and C – effects of linear, quadratic and cubic order of level of supplementation.

Item[A]	Treatment[B]				SEM	P-value[C]		
	3.0kg	1.5kg	1.0kg	0.0kg		L	Q	C
OM	53.87	45.94	44.44	39.57	3.30	<0.01	0.42	0.01
apNDF	52.39	49.52	50.22	48.77	2.55	0.18	0.69	0.25
CP	48.12	39.14	36.54	17.70	8.98	0.03	0.53	0.11

Table 7. Nitrogen utilization according to supplement delivery strategy applied to cows on the last third of gestation

[A] Nmic – microbial N; OMD – organic matter digested; SUN – Serum urea nitrogen; UUN – Urine urea N.

[B] Treatments: 3.0kg - cows received 3.0 kg of concentrate daily; 1.5kg - cows received 1.5 kg of concentrate daily; 1.0kg - cows received 1.0 kg of concentrate daily; and 0.0kg - no concentrate supplement was fed.

[C] L, Q and C – effects of linear, quadratic and cubic order of level of supplementation.

Item[A]	Treatment[B]				SEM	P-value[C]		
	3.0kg	1.5kg	1.0kg	0.0kg		L	Q	C
Nmic (g/d)	72.87	77.99	48.02	36.14	16.4	0.17	0.58	0.82
Nmic (g/g N ingested)	0.747	0.788	0.605	0.533	0.20	0.45	0.76	0.93
Nmic (g/kg OMD)	26.71	26.21	19.99	14.87	7.19	0.25	0.72	0.67
SUN (mg/dL)	13.77	12.72	12.37	10.85	1.70	0.20	0.88	0.41
UUN (g/d)	61.36	47.59	33.35	32.39	9.85	0.09	0.53	0.48

Table 8. Insulin levels (µIU/mL) according to supplement delivery strategy applied on the last third of gestation

[A] Treatments: 3.0kg - cows received 3.0 kg of concentrate daily; 1.5kg - cows received 1.5 kg of concentrate daily; 1.0kg - cows received 1.0 kg of concentrate daily; and 0.0kg - no concentrate supplement was fed.

[B] L, Q and C – effects of linear, quadratic and cubic order of level of supplementation.

Item	Treatment[A]				SEM	P-value[B]		
	3.0kg	1.5kg	1.0kg	0.0kg		L	Q	C
Pre supplementation	1.68	1.50	1.18	1.13	0.43	0.13	0.79	0.63
Post supplementation	2.20	1.48	1.33	1.25	0.38	0.03	0.21	0.13