LEANDRO SOARES MARTINS

EFFECTS OF SUPPLEMENTATION ON PERFORMANCE AND NUTRITIONAL AND METABOLIC ASPECTS OF BEEF COWS AND CALVES AT PASTURE

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Zootecnia, para obtenção do título de *Doctor Scientiae*.

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"Se quiser ir rápido, vá sozinho. Se quiser ir longe, vá acompanhado."
(Provérbio africano)

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BIOGRAPHY

LEANDRO SOARES MARTINS, son of Francisco Márcio Portes Martins and Maria Aparecida Soares Martins, born in Piedade de Ponte Nova, Minas Gerais, on March 8th of 1989.

In March of 2007, joined the Universidade Federal de Viçosa, in an Animal Science course, concluding the course in July of 2011.

In August of 2011 started the Master's degree course in the Animal Science Department of Universidade Federal de Viçosa, concentrating his studies in Ruminants Nutrition and Production area, concluding this course in March of 2013. In this date was started the PhD course in the same area and department, concluding the thesis in February of 2017, after a period of "Doutorado Sanduíche" in the University of Queensland, Australia, between June of 2015 and March of 2016.

SUMMARY

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ABSTRACT

MARTINS, Leandro Soares, D.Sc., Universidade Federal de Viçosa, February, 2017. **Supplementation effects on performance and nutritional and metabolic characteristics of beef dams and calves at pasture**. Adviser: Mário Fonseca Paulino. Co-advisers: Edenio Detmann and Luciana Navajas Rennó.

For the thesis composition were prepared three scientific articles based on studies with dams of Nellore breed and their respective calves, submitted to different supplementation schedules in different phases of production. In the first article the objective was to evaluate the effect of *Bos indicus* dams' supplementation in the pre and/or post calving period, on the performance of their male and female calves in the suckling phase. Eighty four pluriparous Nellore cows (body weight (BW) of 522 ± 9 kg and body condition score (BCS) of 5 ± 0.08 (1-9 scale)) and their calves (44 females and 40 males) were used. The dams' treatments were: 1 kg of supplement during 90 days before and 90 days after calving (Supplemented all time), 1 kg of supplement during 90 days before calving and mineral mixture after calving (Supplemented before calving), mineral mixture before calving and 1 kg of supplement during 90 days after calving (Supplemented after calving) and a control treatment, receiving only mineral mixture *ad libitum* during entire experiment (Control). Cows supplemented before calving, calved heavier calves than supplemented after calving group. No differences were observed in BW at weaning, cows' BCS and cow's milk production ($P > 0.05$). Dams from the Control treatment had smaller milk fat in the first milk collection in the calves' supplementation period, when contrasted with the supplemented groups ($P < 0.05$). Male calves of cows from the Control treatment showed higher ($P < 0.05$) dry matter intake (DMI) than sons of supplemented cows' groups (average of DMI was: 5.5 kg and 3.4 kg to male calves of cows from Control treatment and cows which received supplement, respectively). No differences were found among the different dams' treatments to T3, T4, glucose, rib eye area (REA), subcutaneous fat thickness (SFT), *Gluteus medius* depth (GMD) and P8 thickness fat (P8TF) of male calves. Daughters of cows from the Control treatment showed lower serum glucose, but had greater serum urea compared to daughters of supplemented dams. It was concluded that supplementation of cows before calving improves calves' birth weight and the non-supplementation in the pre/post calving

increase the male calves' DMI during suckling phase. In the second article was evaluated the effect of different schedules of multiple supplementation on performance and carcass composition during the suckling phase of grazing Nellore male calves, as well as the influence of this supplementation schedule on performance of these animals in the feedlot. Forty-eight Nellore male calves (147 ± 7 kg BW and 3 months of age) in the suckling phase and their dams (512 ± 9 kg BW and 6 years of age), were used in a completely randomised design. The supplementation period was 142 days in duration and consisted of two equal 71 day periods (1 and 2), followed by a 125 days feedlot period. The treatments were: 5 and 10 g supplement dry matter (DM)/kg BW.day offered in period 1 and 2 respectively (5S/10S); 10 and 5 g supplement DM/kg BW.day offered in period 1 and 2 respectively (10S/5S); 7.5 g supplement DM/kg BW.day in both periods 1 and 2 (7.5S) and mineral mix *ad libitum* in both periods 1 and 2 (MM). Supplemented calves showed greater final BW and ADG (0.900 kg and 0.560 kg, the ADG in the suckling phase of supplemented and unsupplemented calves, respectively; $P < 0.05$), during the first and second experimental period. Calves from 5S/10S and 7.5S showed higher final BW and total ADG than calves from 10S/5S. No differences in ADG and FBW were observed in the first phase among supplemented calves. Animals from MM presented smaller ($P < 0.05$) rib eye area (REA), subcutaneous fat thickness (SFT) and *Gluteus medius* depth (GMD). In the feedlot period, supplemented animals had greater initial ($P < 0.05$) BW and tendency ($P < 0.10$) of greater final BW. It was concluded that male calves supplemented during the suckling phase, show better performance at weaning. The amount of 7.5 g supplement DM/kg BW.day is recommended to improve the performance. In the third article the aim was to evaluate different schedules of supplementation at pasture to calves in a creep feeding system, as described in the second article, on dam's performance and behaviour of calves and their dams. No differences ($P < 0.05$) in BCS, final BW and ADG were found on dams' performance. Calves from MM treatment spent more time ($P < 0.05$) grazing than supplemented calves from 5S/10S and 10S/5S treatments, in the first period. No difference in suckling time was found among the treatments ($P > 0.05$) in the first evaluated period. Calves from 10S/5S treatment spent more time suckling and less time eating supplements ($P < 0.05$) than 5S/10S treatment animals, in the second evaluated period. Dams of MM treatment's calves showed more idle time and

lower grazing time when compared with the mothers of calves from 5S/10S and 10S/5S treatments. It was concluded that, different schedules of Nellore calves' supplementation at pasture, do not affect their mothers' performance, and supplementation decreases grazing time of calves in the suckling phase.

RESUMO

MARTINS, Leandro Soares, D.Sc., Universidade Federal de Viçosa, fevereiro de 2017. **Efeitos da suplementação sobre desempenho e características nutricionais e metabólicas de vacas e bezerros de corte a pasto**. Orientador: Mário Fonseca Paulino. Coorientadores: Edenio Detmann e Luciana Navajas Rennó.

Para a composição desta tese foram elaborados três artigos científicos baseados em estudos realizados com matrizes da raça Nelore e suas respectivas crias, submetidas a diferentes estratégias de suplementação em diferentes fases de produção. No estudo descrito no primeiro artigo, objetivou-se avaliar a influência da utilização de suplementação múltipla no pré e/ou pós-parto, para matrizes Nelore em boa condição nutricional, sobre o desempenho de suas progênies durante a fase de cria. Oitenta e quatro matrizes pluríparas da raça Nelore (peso corporal de 522 ± 9 kg e escore de condição corporal (ECC) de 5 ± 0.08, numa escala de 1-9) e suas respectivas crias (44 fêmeas e 40 machos) foram utilizadas. Os tratamentos das matrizes foram: 1 kg (matéria natural) de suplemento durante 90 dias pré-parto e 90 dias pós-parto (suplementação o tempo todo), 1 kg de suplemento (matéria natural) durante 90 dias pré-parto e mistura mineral após o parto (suplementação antes do parto), mistura mineral no período pré-parto e 1 kg de suplemento (matéria natural) durante 90 dias pós-parto (suplementação pós-parto) e tratamento controle, recebendo apenas mistura mineral no período avaliado (Controle). As matrizes que foram suplementadas antes do parto, pariram bezerros mais pesados que aquelas que receberam suplemento apenas no período pós-parto. Também não foram identificadas diferenças para peso corporal (PC) dos bezerros no desmame, ECC e produção de leite das matrizes, entre os diferentes tratamentos ($P > 0.05$). Matrizes do tratamento controle apresentaram menor teor de gordura no leite na primeira coleta, em relação às suplementadas ($P < 0.05$). Os bezerros (apenas machos) filhos das matrizes do tratamento controle apresentaram maio ($P < 0.05$) consumo de matéria seca (CMS) que os filhos de matrizes suplementadas. Os tratamentos das matrizes não diferenciaram em relação à concentração de T3, T4, glicose, área de olho de lombo (AOL), espessura de gordura subcutânea (EGS), profundidade do *Gluteus medius* (PGM) e espessura de gordura na P8 (EGP8), nos seus respectivos bezerros. Bezerras, filhas de matrizes do tratamento Controle,

apresentaram menor teor de glicose e maior teor de ureia no sangue, quando comparadas aquelas filhas de matrizes suplementadas. O uso de suplementação de matrizes, antes do parto proporciona o nascimento de bezerros mais pesados, e o não uso de suplementação para as matrizes no período pré e /ou pós-parto causa aumento do consumo de matéria seca dos bezerros na fase de cria. No segundo artigo o objetivo foi avaliar o efeito de diferentes esquemas de suplementação a pasto, sobre o desempenho e composição de carcaça de bezerros na fase de cria, assim como a influência destes esquemas de suplementação sobre o desempenho destes animais no período de confinamento. 48 bezerros da raça Nelore (147 ± 7 kg e três meses de idade) na fase de cria e suas mães (512 ± 9 kg e seis anos de idade) foram utilizados em um delineamento inteiramente casualizado. O período de suplementação foi de 142 dias, subdividido em dois períodos (1 e 2) de 71 dias, seguidos de um tempo de 125 dias de confinamento. Os tratamentos dados aos bezerros foram: fornecimento diário de 5 e 10g de matéria seca (MS) de suplemento/kg de PC nos períodos 1 e 2 respectivamente (5S/10S), 10 e 5g de MS de suplemento/kg de PC nos períodos 1 e 2 respectivamente (10S/5S), 7.5g de MS de suplemento/kg de PC em ambos períodos (7.5S) e mistura mineral *ad libitum* em ambos períodos (MM). Os bezerros suplementados apresentaram maior ($P < 0.05$) PC ao desmame e GMD durante toda a fase de cria, comparados aos animais do tratamento MM. Bezerros dos tratamentos 5S/10S e 7.5S apresentaram maior ($P < 0.05$) GMD e PC ao desmame, que bezerros do tratamento 10S/5S. Não foram observadas diferenças no GMD e PC entre os bezerros suplementados, durante o primeiro período. Os bezerros do tratamento MM apresentaram menor ($P < 0.05$) AOL, EGS, PGM e também menor PC no início do período de confinamento. Bezerros suplementados na fase de cria apresentam melhor desempenho ao desmame e tendem a apresentar maior peso ao final do confinamento. A quantidade de 7.5g de MS de suplemento/kg de PC/dia é recomendado para a otimização do desempenho de bezerros Nelore na fase de cria. No terceiro artigo objetivou-se avaliar o efeito dos diferentes esquemas de suplementação para bezerros na fase de cria, como descrito no artigo 2, sobre o comportamento dos bezerros lactentes e suas respectivas mães, assim como a influência dos diferentes esquemas de suplementação no desempenho das matrizes lactantes. Não foram identificadas diferenças ($P > 0.05$) em ECC, PC final e GMD das matrizes

durante o período de avaliação. Bezerros do tratamento MM passaram mais tempo ($P < 0.05$) pastejando do que animais dos tratamentos 5S/10S e 10S/5S, no primeiro período avaliado. O tempo de mamada não diferenciou ($P > 0.05$) entre os bezerros de diferentes tratamentos, no primeiro período. Bezerros do tratamento 10S/5S passaram mais tempo ($P > 0.05$) mamando e menos tempo ($P > 0.05$) consumindo suplemento que os do tratamento 5S/10S, no segundo período. Mães de bezerros do tratamento MM apresentaram maior tempo de ócio e menor tempo de pastejo ($P > 0.05$), quando comparadas às mães de bezerros dos tratamentos 5S/10S e 10S/5S. Neste estudo foi concluído que diferentes esquemas de suplementação de bezerros a pasto, não afetam o desempenho de suas mães e que a suplementação diminui o tempo de pastejo destes bezerros.

GENERAL INTRODUCTION

To be successful in beef cattle production, attention must be paid to the cows, since they are the ones that will produce the calves, which can be used in the reproduction or production of meat, producing income and allowing the production system to be perpetuated.

According to Ferrel and Jenkins (1985), about 75% of all dietary energy consumed for meat production is destined to meet the cows' requirements of maintaining, most of them created under grazing only with mineral supplementation. This fact means that many of these animals do not fulfill their role of producing one calf per year, since pasture often does not provide nutrients in quantity or quality, especially for the category of breeding females, which are highly demanding of these nutritional resources.

To optimize forage utilization, Paulino *et al*. (2008) suggest the concept of potentially digestible dry matter (pdDM), which is represented by the fraction that has the potential to be used by the animal, encompassing the potentially digestible neutral detergent fibre fraction (pdNDF) and also the cellular content. The amount to be daily offered to the animal is 4 to 6% of the body weight. Although it is indispensable to guarantee this supply of forage to the animal, the optimization of the performance occurs with the provision of multiple supplements.

By providing multiple supplements, there is an objective of provide nutrients and also allow a greater use of the basal resource, which is the forage. In the case of cows, it is not yet clear the most appropriate period for supplementation, whether before, before and after or just after calving. If supplied immediately before calving, this will occur in the dry season of the year, a strategic phase due to the less availability of quantity and quality of the

forage. However, this is the phase in which the greatest fetal development occurs, about 75% in the final third of gestation, and the idea that the input of nutrients at this stage would be directed mainly to fetal growth. On the other hand, supplementation of these animals in the postpartum period could only guarantee an increase in milk production, since reproduction is the last priority of energy targeting in bovine female metabolism (Short & Adams, 1988).

In addition to the direct supplementation's effect on the performance of the beef cows, there is an indirect effect on their offspring, whether in the gestational period or even during lactation. When the breeding season is carried out at the appropriate time (November to February), the dams reach the final third of gestation, a period of higher nutritional requirement due to fetal growth, during the dry season with restricted quality and quantity of forage (Duarte *et al.*, 2012), and the strategic supplementation performed in this period represents an alternative to overcome this nutritional deficiency. Nutritional restriction of dams during the middle and the late gestation can lead to irreversible effects on bovine foetuses, such as decreased numbers of intramuscular adipocytes, muscle fibre size (Du *et al*., 2010), and changes in the surface area of the intestine (Duarte *et al.,* 2013). Dairy cows supplementation in the postpartum period may affect milk production (Deresz, 2001) and its composition (Stelzer *et al*., 2009), which could affect the performance of suckling calves.

A measurement that can be used as a driver of the metabolic and consequently productive condition of the bovine dams is body condition score (BCS). This measure presents a high correlation with the energetic status of the animal, since females with better BCS present higher levels of lipids composing their body. Working with beef cows with high and low BCS, Bohnert *et al*. (2013)

concluded that high BCS animals showed higher birth weight and lower mortality rates of their calves, as well as a higher pregnancy rate.

After a conception of beef cow during the mating season, attention should be directed to their offspring. In the case of calves, milk is able to meet its nutritional requirements until about three months of age (Henriques *et al*., 2011), so to optimize the performance of these animals, multiple supplementation is necessary since tropical forage do not have sufficient nutrients to meet the nutritional needs of this group to achieve high weight gains.

The decrease in the slaughter age of cattle offers several benefits, such as: greater meat tenderness (Schönfeldt & Strydom, 2011), optimization of land use and lower production of methane / unit of meat (McAllister, 2011). In view of the advantages of slaughtering young animals and knowing that what determines the slaughter point is mainly the animal's body weight, it is understood that high weight gains should be explored since the birth of the animal. Valente *et al*. (2013) working with Nellore male calves, obtained at the end of their study, lower final weight and average daily weight gain for the control treatment (mineral mix only) compared to the other treatments that received multiple supplementation in the Creep feeding system.

In addition to greater weight gain, supplementation of calves in the suckling phase also has responses in the production of hormones related to the development of the animal, such as IGF-1 (insulin-like growth factor), GH (growth hormone), T4 (thyroxine) and T3 (triiodothyronine). The hormone IGF-1 has a role in the development and differentiation of tissues, especially in the increase of protein synthesis (Lima *et al*. 2011). Studying steers receiving restricted diets (below the maintenance requirement) and for gains of 900g,

Lima *et al*. (2011) concluded that IGF-1 was the main indicator of energy status because it decreased in a sensitive and fast way during the nutritional deficiency.

Growth hormone (GH) is an anabolic hormone, secreted by acidophilic cells of the anterior lobe of the pituitary gland, which decreases fat deposition as a result of increased protein deposition. Its secretion is pulsatile and can be influenced by several feedback systems, among which are mentioned glucose, insulin, free fatty acids, somatostatin. GH is responsible for the increase of hepatic levels of insulin-like growth factor 1 (IGF-1) (Amorim *et al*., 2007). The somatotrophic axis has as its main component the GH, but the thyroid hormones (T3 and T4) are directly linked to it, regulating the synthesis and availability of GH and IGF-1 (Renaville *et al*., 2002).

The hormone T3 is produced in the thyroid gland together with the hormone T4, which is produced in greater quantity, but has less activity in the organism and when entering the cell by facilitated diffusion is converted into T3. The thyroid hormones present a great relation with the somatotrophic axis, since they act in the synthesis of receptors of somatomedins, they influence the synthesis and the secretion of the GH by the pituitary, they regulate the action of this hormone on the bones and they control the expression of the IGF-I mRNA (Moreira *et al*., 2010). Thus T3 has a great influence on animal metabolism, in the sense of accelerating it, especially in high performance conditions.

In a production system that seeks to explore the optimum performance of the animal, combining the economic and biological aspects, should be based on knowledge about nutrition and physiology. On the nutritional side, is known the importance of providing of adequate amounts of nutrients, supplying the

animal's requirements, on the physiological side it is known that when exploring the growth curve of a bovine, better responses are obtained in the relation weight gain / consumed nutrients, in the early life of these animals, where they have a higher rate of growth.

Biffani (1997) established four phases for the animal growth curve: 1) progressive phase: in this phase the growth rate is very high and positive, reaching the maximum at the inflection point of the curve, which corresponds to the puberty of the animal; 2) regressive phase: from the point of inflection the rate of growth begins to decrease because of a series of factors that progressively inhibit growth, although the animal does not stop growing. (Graphically the inflection point marks this inversion in the rate of growth); 3) stationary phase: when the animal stops growing and the growth can be considered only a renewal of the tissues; 4) Death: the tissues are not renewed and the animal dies. According to Hammond (1966), the speed of growth of each body region advances until reaching the maximum and begins to decrease as the animal approaches the adult size.

The phase-division of the growth curve of the animals occurs due to the allometric growth of the different body tissues. The first tissue to develop is the nervous, followed by the bone, muscular and finally the adipose. Further development of muscle tissue early in animal life over fatty tissue implies increased deposition of proteins, which carry water molecules in the weight ratio of 1: 3 or 1: 4 (Halas *et al.,* 2010), making younger animals more efficient at gaining weight when compared to older animals, which are already close to reaching the maximum limit of protein deposition, depositing mainly fat, which adds small amount of water in this process.

Knowing the efficiency in weight gain and knowing that the slaughter weight is the same for animals raised in different production systems, it is understood that it would be more interesting from an economic and also biological point of view, to explore higher gains in the phases of greater responsive capacity to the nutrient supply of the animals, instead of doing it later, which is generally the case in Brazil, where greater weight gains are explored in feedlots of older (heavy) animals already in the finishing phase. The objectives of these studies were to evaluate the effect of beef cows' supplementation before and/or after calving, on calves performance, milk production and composition and dams' body condition score, and also to quantify the effects of calf' supplementation in the pre-weaning on their behavior, performance and the consequences in their performance in the post-weaning feedlot.

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How does cows' supplementation before and, or after calving affect calf performance in the suckling phase?

Abstract

The objective of the present study was to evaluate the effect of beef cows' supplementation before and/or after calving, on calves performance, milk production and composition and dams' body condition score. Eighty four pluriparous Nellore cows (body weight (BW) of 522 ± 9 kg (mean ± SEM) and body condition score of 5 ± 0.08 (1-9 scale)) and their calves (44 females and 40 males) were used. It was considered day 1 and day 322 as first and last experimental day, respectively. The dams' treatments were: 1 kg of supplement (as is) during 90 days before and 90 days after calving (Supplemented all time), 1 kg of supplement (as is) during 90 days before calving and mineral mixture after calving (Supplemented before calving), mineral mixture before calving and 1 kg of supplement (as is) during 90 days after calving (Supplemented after calving) and a control treatment, receiving only mineral mixture *ad libitum* during entire experiment (Control). Cows supplemented before calving, calved heavier calves than supplemented after calving group. The average daily gain (ADG) between birth and the day 180 didn't show differences among the treatments. No differences were observed in body weight (BW) at weaning, cows' body condition score (BCS) and cow's milk production ($P > 0.05$). Dams from the Control treatment had smaller milk fat in the first milk collection in the calves' supplementation period, when contrasted with the supplemented groups ($P < 0.05$). Male calves of cows from the Control treatment showed higher ($P < 0.05$) dry matter intake (DMI) than sons of supplemented cows' groups (average of DMI was: 5.5 kg and 3.4 kg to male calves of cows from Control treatment and

cows which received supplement, respectively). No differences were found among the different dams' treatments to T3, T4, glucose, rib eye area (REA), subcutaneous fat thickness (SFT), *Gluteus medius* depth (GMD) and P8 thickness fat (P8TF) of male calves, in the weaning. Daughters of cows from the Control treatment showed lower serum glucose, but had greater serum urea compared to daughters of supplemented dams. Supplementation of cows before calving improves calves' birth weight and the non-supplementation in the pre/post calving increase the male calves' DMI during suckling phase.

Keywords: beef cattle, calves, tropical pasture

Introduction

In tropical regions of the Southern Hemisphere, the recommended breading season occurs between November and February in pasture based production systems, when there is a greater quantity and quality of the available forage. Thus, pregnant cows usually experience feeding restrictions during the middle and final gestation period which overlaps with a season of a low quantity and quality of forage (Duarte *et al*., 2012), in the dry season.

The main focus for beef cow supplementation is usually about improving their reproductive performance, but the improvement of the nutritional quality of beef cow' diets may also influence the development of the future calf (Bohnert *et al*., 2013). During the early phase of fetal development, maximal placental growth, differentiation, and vascularization occur, as well as fetal organogenesis, all of which are critical events for normal conceptus development (Funston *et al*., 2010). Therefore, an adequate nutrient supply is of extreme importance, even demanded in low quantities. Approximately 75% of

fetal growth takes place in the last trimester of the pregnancy (Robinson *et al.*, 1977), and from this point most beef cattle requirement systems start to consider the pregnancy requirement.

Restricted dam nutrition during mid- to late gestation may cause irreversible effects in the bovine fetus, like decreases in the number of intramuscular adipocytes and muscle fiber sizes (Du *et al.*, 2010), and changes the surface area of the small intestine (Duarte *et al.*, 2013). Supplementing cows in the post-partum period may affect the milk production (Deresz, 2001) and composition (Stelzer *et al.*, 2009), which could affect the suckling calves' performance.

Considering the importance of dam nutrition on future calf production and the nutrient limitations (primarily of nitrogen compounds) of tropical pastures during the beef cows' pregnancy, concentrate supplementation appears as a viable alternative to minimize such restrictions. A considerable number of studies have been performed, to evaluate the impact of feed restriction during pregnancy on the bovine fetus (Duarte *et al*., 2013; Du *et al*. 2010; Bohnert *et al*., 2013; Funston *et al*., 2010), but not many have evaluated the effect of non-feed restricted cows supplemented before and/or after calving, on performance, hormone and metabolite production of their male and female calves in the suckling phase. The objective of the present study was to evaluate the effect of beef cows' supplementation before and/or after calving, on calves performance, hormone and metabolite production, milk production and composition and dams' body condition score.

Materials and methods

Animals, experimental design and supplements

This study was approved by the Ethics Committee on Animal Use (CEUAP/UFV – Process nº 40/2014), according to ethical principles of animal experimentation established by the National Council of Animal Experimentation Control - CONCEA. The experiment was conducted in the Beef Cattle Section of Universidade Federal de Viçosa, located in Viçosa-MG, Brazil (20º45' S and 42º52' W), from July 2014 to July 2015. It was considered day 1 and day 322 as first and last experimental day, respectively. Between the days 1 and 180, Nellore cows received supplement in a factorial scheme, being this period correspondent to 90 days before calving and 90 days post-calving. Between the days 180 and 322, calves and cows kept being evaluated, being day 322 the weaning day.

Eighty four pluriparous Nellore cows (body weight (BW) of 522±9 kg and body condition score of 5±0.08 (1-9 scale)) were divided into four treatments: 1 kg of supplement (as is) during 90 days before and 90 days after calving (Supplemented all time), 1 kg of supplement (as is) during 90 days before calving and mineral mixture after calving (Supplemented before calving), mineral mixture before calving and 1 kg of supplement (as is) during 90 days after calving (Supplemented after calving) and a control treatment, receiving only mineral mixture *ad libitum* until day 180 (Control).

The dams were adapted for 14 days and then spent 180 days receiving the different scheme of supplementation. During the adaptation all pregnant cows were kept in the same paddock receiving the same treatment, which was

a 500 g/animal.day^{-1} of a 25% CP (dry matter) supplement composed of soybean meal, sorghum, corn and a mineral mixture.

Cows were kept in two separate areas - total of 105 ha – divided into eight paddocks formed by *Brachiaria decumbens* Stapf. provided with covered drinkers and feeders. The experimental design was a completely randomized design, consisting of four treatments and two replicates (groups), of 21 cows in each treatment. Supplements were offered to cows at 1000 h every day in collective feeders. The supplement used during the experiment is described in the Table 1.

After day 180, only 20 dams and their respective calves (10 male and 10 female), being 5 pairs (cow-calve) from each cow's treatment, were evaluated and kept receiving mineral mixture *ad libitum* until the weaning (day 322). The others animals were directed to another study, with different treatments.

All animals were subjected to control of endo and ectoparasites at the beginning of the experiment and throughout the experimental period whenever necessary. The calf average daily weight gain (ADG) was estimated by the difference between the final and initial body weights, both after a fasting period of 14 hours, which was then divided by the number of experimental days.

Experimental procedures and sampling

The pasture was sampled every 28 days of the experiment to quantify the availability of dry matter and potentially digestible dry matter (pdDM) (Paulino *et al.*, 2008) by cutting forage at the ground level in four randomly chosen areas, delimited by a 0.5 × 0.5 m metal square in each paddock. All the samples were oven-dried (60°C) and ground (1 and 2 mm). Qualitative

evaluations of the pasture consumed by the animals were performed by the hand-plucking method every 14 days. The collection was performed by a single sampler throughout the trial period.

To evaluate the calves' diet dry matter intake, two digestibility trials (nine days each) were conducted between experimental days 210 and 219 and between days 280 and 289, using the marker method. To estimate fecal excretion the calves received the chromic oxide (Cr_2O_3) external marker (Detmann *et al*., 2001), which was placed in paper cartridges at 10 g per calf/day in the first digestibility trial and 14 g per calf/day in the second digestibility trial, applied with the use of a metal probe directly into the esophagus, at 0900 h. To estimate the forage dry matter intake, indigestible neutral detergent fiber (iNDF) was used as internal marker (Detmann *et al*., 2001). The first six days of the trials were used for the calves' adaptation to Cr_2O_3. Feces were collected on the last three days at different times: 1500 h, 1100 h and at 0700 h. Samples of feces were collected immediately after defecation or directly from the rectum of the animals, immediately oven-dried (60 ºC), and then ground in a knife mill (1 and 2 mm).

Milk production was estimated on days 203 and 273. Calves and their dams were separated for 2 hours, reunited for 1.5 hours and allowed to suckle to satiety before separating again at 1800 h. At 0600 h the following day, 2 mL of oxytocin (10 IU/mL; Ocitovet®, Brazil) was administered into the mammary vein of cows and the udders were completely emptied, using a milking machine and total milk output was weighed immediately. Calves and their dams remained separated and at 1400 h second milking was conducted, following the same aforementioned procedures. The exact time of milking of each cow was recorded and total milk production was converted into 24 hours milk production.

On the last day of the digestibility trials, blood samples were collected via jugular venipuncture. Blood samples were also collected at the end of the experiment. Blood samples were collected using vacuum tubes containing a clot activator and separator gel (BD Vacuntainer®, SST II Advance). The blood was immediately centrifuged at 3,600 x *g* for 15 min, and the serum was stored at -20 ºC, for later evaluation of the contents of glucose, total triiodothyronine (T3), total thyroxine (T4) to male calves and urea, insulin, glucose and non-esterified fat acids (NEFA) to female calves.

In the last day of the experiment (weaning), an ultrasound evaluation of the rib eye area (REA) , from transversal section of *Longisimus dorsi* muscle (between T12 and T13 thoracic vertebrae's); subcutaneous fat thickness (SFT), from average of two measurements (the first from the same place of ribeye area and the second at pelvic region, localizing the probe between the ischium and pubis), *Gluteus medius* depth (GMD) and P8 thickness fat (P8TF) was made in the male calves, using an Aloka Ultrasound (model SSD 500V, Aloka Co., Ltd., Tokyo, Japan.) with a linear 18 cm probe. The images were analyzed in the Biosoft Toolbox® II Beef program (Biotronics Inc., Ames, Iowa, USA). Body condition score (BCS) of the cows was recorded at the beginning and end of experiment, using a scale of 1 to 9 (NRC, 1996); all evaluations were made by the same five trained evaluators.

Chemical analyses

The supplement and forage samples, obtained by the hand-plucking method were quantified with regard DM (INCT-CA G-003/1), ash (INCT-CA M-001/1), crude protein (CP; INCT-CA N-001/1), ether extract (EE; INCT-CA G-

004/1), neutral detergent fiber corrected for ash and protein (NDFap; INCT-CA F-002/1), using thermostable α-amylase, without using sodium sulfite; nitrogen insoluble in neutral detergent (NIND; INCT – CA N-004/1) according to Detmann *et al.* (2012); iNDF, according to Valente *et al.* (2011), obtained after *in situ* incubation in F57 Ankom® filter bags for 288 h.

The non-fibrous carbohydrates (NFC) were quantified according to Detmann and Valadares Filho (2010) using the following equation:

$$NFC = 100 – (\%CP + \%NDFap + \%EE + \% ash)$$

A pooled fecal sample was formed after drying, per animal, for the three collection days in each digestibility trial. Samples were analyzed for the chromium contents by atomic absorption spectrometry (INCT-CA M-005/1) according to Detmann *et al.* (2012). The level of DM was also evaluated as described previously. The fecal dry matter excretion was estimated based on the ratio between the amount of marker (chromic oxide) supplied and its concentration in the feces (Lopes *et al.*, 2014).

The voluntary dry matter intake (DMI) was estimated by using iNDF as an internal marker, according to the equation:

$$DMI\ (kg/day) = \{(FE \times CMF)/CMFO\} + MI$$

where: FE = fecal excretion (kg/day); CMF = concentration of the marker in the feces (kg/kg); CMFO = concentration of the marker in the forage (kg/kg); and MI = milk dry matter intake (kg/day).

Analyses of blood glucose and urea were performed with an automatic biochemistry analyzer (Mindray, model BS200E). The levels of NEFA were quantified using the enzymatic colorimetric method (FA115, Randox Laboratories Ltd., São Paulo, Brazil). Serum urea N (SUN) was estimated as 46.67% of the total serum urea.

Insulin, total T3 and total T4 were quantified by Beckman Coulter®, (Brea, USA) in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA). It was quantified in the milk: fat, protein, lactose and total solid.

The content of pdDM (Paulino *et al*., 2008) was calculated by the follow equation:

$$pdDM = 0{,}98 \times (100 - NDF) + (NDF - NDFi)$$

Where: pdDM = potentially digestible dry matter; NDF = neutral detergent fiber; NDFi = indigestible neutral detergent fiber

Statistical analyses

Data were submitted to analysis of variance, following completely randomized design and a 2 x 2 factorial arrangement of supplementation (supplemented or not before calving x supplemented or not after calving). The PROC MIXED procedure of SAS (Statistical Analysis System, version 9.2) software was used for all statistical analyses. All statistical procedures were performed adopting 0.05 as the critical level of probability for the type I error. Calves' sex was considered as a random effect to body weight measurements and evaluated separately to another measurements due differences in the evaluated parameters.

Results

The pdDM availability in % of BW was: 5.6; 10.2 and 4.8 to pre partum, immediately post-partum and calves suckling period, respectively. Changes were observed in the forage composition, mainly in CP and iNDF. The CP level

was 5.1; 10.3 and 7.4% of DM and 35.5; 21.7 and 26.1% of DM to iNDF, respectively from pre partum, post-partum and calf supplementation period (Table 2). The rainfall was 120.4 mm in the pre partum period (July – October), 582.0 mm in the post-partum period (November – January) and 301.6 mm in the calf supplementation period (February – June).

Cows that were supplemented before calving calved heavier calves ($P < 0.05$; Table 3). The weights of calves from cows supplemented before calving and after were respectively, 34 and 30.5 kg. The ADG between birth and the day 180 did not show differences among the different cows' treatments (Table 3). No significant differences ($P > 0.05$) were observed in calves' body weight at day 180 (BW180), final body weight (FBW) and in average daily gain between calves' 150 days of age and weaning to the different cows' treatments (Table 3).

The cows' treatments didn't show significant differences to BCS in the end of cows' supplementation period, milk production and fat milk production (Table 3).

Greater DMI ($P < 0.05$) of male calves of cows from Control treatment was observed, followed by sons of cows supplemented all time. No differences ($P > 0.05$) were observed to T4, T3, glucose, REA, SFT, GMD and P8TF in male calves among the different dams' treatments (Table 4). With regard to female calves, no differences ($P > 0.05$) were found among the different dams' treatments to DMI, insulin and NEFA, but serum urea was greater ($P < 0.05$) to female calves from cows of Control and Supplemented before calving treatments (Table 4) and glucose concentration followed the opposite way.

Discussion

During the cow life cycle, nutrient partitioning occurs in the following priority order: basal metabolism, activity, growth, basal reserves of energy, pregnancy, lactation, additional energy reserves, reproductive cyclicity and excess energy reserves, but this order may change according the current physiological functions of the moment (Short and Adams, 1988). Around 75% of fetal growth occurs in the last trimester of the pregnancy (Robinson *et al.*, 1977) and during this time, the fetus is prioritized in the energy partition in the cow metabolism. In the present study, cows which received supplementation before calving, delivered heavier calves, confirming the theory about energy partition.

Evaluating the effect of late gestation supplementation of beef cows with different BCS, Bohnert *et al.* (2013) found greater weights of calf from supplemented cows. Winterholler *et al.* (2012) concluded that cows supplemented in late-gestation had an increase in calf birth weight. Evaluating birth weight, Dillon *et al.* (2015) and Cubas *et al*. (2001) found greater values from male calves than to female calves, which was not evaluated in the present study, once females and males calves were evaluated together. Although the differences among cows' treatments to the birth weights of calves, no differences were found in their weight performance during the suckling phase.

Evaluating milk production and composition of cows from different genetic groups, Cerdótes *et al.* (2004) found greater milk production in after calving supplemented cows, which disagree with results from the present study, once no significant differences in milk production were found among different supplementation schedules to cows. Cows received a small amount of supplements (1 kg as is); consuming forage with adequate availability (Paulino

et al., 2008), and also cows presented an adequate BCS. Cows with low BCS respond more favorably to supplementation than cows in good condition (Bohnert *et al*., 2013).

Some authors have highlighted the impact of the maternal nutrition during the pregnancy (Wu *et al*., 2006; Du *et al*., 2010; Funston *et al.*, 2010), showing the negative consequences of restricted diets during this period. No statistic differences were found to calves performance in our study, however greater DMI was presented by male calves from cows of Control treatment, compared to male calves from supplemented cows. Studying the effects of maternal nutrition on the development of the gastrointestinal tract of the bovine fetus, Duarte *et al*. (2013) concluded that the weight of the small intestine per unit of body weight, the length of small intestine and its villi were respectively: 11.24%; 12.93% and 16.44% greater in fetuses from nutritional restricted dams compared to those from non-restricted dams. In that same study, restricted cows were fed at 1.2 times of maintenance and the other group was fed *ad libitum.* The cited authors attributed this response to a more efficient growth, to compensate the low supply of nutrients during the gestation, which can elucidate our DMI of male calves' results. Although not statistically significant, female calves from cows of Control treatment, showed a DMI 19% greater than female calves from supplemented cows.

Female calves from cows that received supplementation after calving (lactation period) showed greater level of serum glucose and smaller level of serum urea. These differences may be explained by the greater milk production of mothers of female calves, who received supplementation after calving (7kg and 8.9kg of milk to cows, mothers of female calves, supplemented before and after calving, respectively). Evaluating milk production and composition of cows

from different genetic groups, Cerdótes *et al*. (2004) found greater milk production to after calving supplemented cows. Studying the calf sex effect on milk production of their mothers in 1.49 million dairy cows, Hinde *et al*. (2014) concluded that mothers of female calves produce more milk. Based on this, we speculate about an improvement in nutrient (supplementation) response of female calf' mothers, when compared with male calves' mothers.

A greater milk intake, based in the greater milk production, provided bigger serum glucose concentration and also better urea utilization, doing serum urea concentration smaller to female calves of cows supplemented after calving, when compared to female calves from non-supplemented cows after calving. This fact may be explained by a better CP/energy relationship in female calves from cows which were supplemented after calving, due the greater energy consumption derivate from the greater amount of produced milk. Valadares et al., (1997) verified, that the plasma N-urea concentration range from 13.52 to 15.15 mg/dL corresponded to maximum microbial efficiency and, would represent the limit to occur protein loss for the animals. In the present study female calves from non-supplemented cows after calving, presented an average of 8.54 mg/dL of serum N-urea, indicating a protein imbalance.

Conclusion

Supplementation of cows with BCS 5 during late gestation provides birth of heavier calves, which present the same final weight and weight gain until weaning, when compared to calves from unsupplemented cows before calving. Non-supplementation in the pre/post calving increases the male calves' DMI during suckling phase.

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Table 1

Centesimal composition of supplements (as is).

Ingredient	MM[2]	SUP[2]
Mineral mixture[1]	100	5
Ground sorghum grain	---	24,65
Ground corn grain	---	24.65
Soybean meal	---	45.69
Crude Protein (%DM)	---	28,6

[1]Centesimal composition: dicalcium phosphate, 50.00; sodium chloride, 47.775; zinc sulfate, 1.4; copper sulfate, 0.7; cobalt sulfate, 0.05; potassium iodate, 0.05 and magnesium sulfate: 0.025. [2] mineral mixture (MM), supplement (SUP)

Table 2

Chemical composition of supplement and forage.

Item	Supplement	Forage[5]		
		Before calving	After calving	Suckling phase
Dry matter[2]	88.7	50.4	29.4	30.5
Organic matter[3]	91.9	92.0	92.0	91.4
Crude protein[3]	28.6	5.1	10.3	7.4
NDIN[1,4]	37.2	31.1	25.7	29.1
Ether extract[3]	2.5	0.9	1.2	1.6
NDFap[1,3]	15.6	63.4	60.6	65.9
NFC[1,3]	45.2	22.6	19.9	16.8
iNDF[1,3]	2.8	35.5	21.7	26.1

[1]/NDIN - neutral detergent insoluble nitrogen; NDFap - neutral detergent fiber corrected for ash and protein; NFC - non-fibrous carbohydrates; iNDF - indigestible neutral detergent fiber.
[2]/ In % of fresh matter (as is).
[3]/ In % of dry matter.
[4]/ In % of total nitrogen.
[5]/Mean values of the samples obtained by hand-plucking (grazing simulation) before calving, after calving and during calves' supplementation on Creep feeding system.

Table 3

Means, standard error (SE) and indicators of significance for birth weight (BW), body weight at day 180 (BW180, end of cows' supplementation), final body weight (FBW), average daily gain (ADG) of Nellore calves and body condition score at weaning (BCS) in the end of cows' supplementation period, milk production during (MP1) and after (MP2) cows' supplementation period.

Item	Cows' treatments				SE	*P*-value[5]		
	Control	Supplemented before calving	Supplemented after calving	Supplemented all time		SUB	SUA	B x A
	Calves							
BW (kg)	31	33	30	35	0.62	0.014	---	---
ADG (kg)[1]	0.848	0.758	0.876	0.864	0.02	0.266	0.186	0.399
BW180 (kg)	164	135	144	155	3.41	0.414	0.975	0.113
FBW (kg)[2]	252	214	222	234	4.67	0.426	0.744	0.169
ADG (kg)[3]	0.672	0.605	0.595	0.596	0.02	0.564	0.461	0.544
	Cows							
BCS[4]	4.8	5.1	5.0	4.9	0.07	0.549	0.643	0.364
MP1 (kg)	8.17	7.47	8.38	8.06	0.32	0.383	0.537	0.731
MP2 (kg)	5.55	5.99	5.12	5.51	0.16	0.202	0.167	0.947

[1/]Between birth and the 150 days of calves' age

[2/]Body weight in the weaning

[3/]Between 150 days of age and calves' weaning

[4/]End of cows' supplementation (1-9 scale)

[5/] SUB: supplementation before calving; SUA: supplementation after calving; B x A: interaction between supplementation before and after calving. Indicators of difference according to period of supplementation ($P<0.05$).

Table 4

Means, standard error (SE) and indicators of significance for dry matter intake (DMI), T4 concentration (T4), T3 concentration (T3), glucose concentration (Glu), rib eye area (REA), subcutaneous fat thickness (SFT), *Gluteus medius* depth (GMD) and P8 thickness fat (P8TF), insulin concentration (Ins), non-esterified fatty acids (NEFA) and N-urea concentration (N-Ure) of Nellore calves.

Item	Cows' treatments				SE	*P*-value[1]		
	Control	Supplemented before calving	Supplemented after calving	Supplemented all time		SUB	SUA	B x A
	Male calves							
DMI (kg)	5.50	3.10	3.37	3.80	0.20	0.028	0.092	0.003
T4 (ng/dL)	68.3	118.5	91.2	87.6	4.57	0.178	0.809	0.123
T3 (ng/mL)	2.18	2.82	2.58	1.56	0.11	0.748	0.465	0.175
Glu (mg/mL)	68.3	75.0	64.3	67.0	1.65	0.389	0.276	0.704
REA (cm^2)	45.1	35.3	37.0	42.4	1.35	0.668	0.928	0.177
SFT (mm)	1.6	1.9	2.5	1.6	0.10	0.242	0.235	0.078
GMD (cm)	55.6	51.1	54.3	55.2	1.03	0.379	0.473	0.205
P8TF (mm)	2.2	1.9	2.1	1.6	0.14	0.251	0.666	0.783
	Female calves							
DMI (kg)	5.41	4.69	4.36	4.57	0.11	0.548	0.190	0.284
Ins (µ IU/mL)	5.3	2.0	4.3	3.6	3.64	0.351	0.865	0.541
Glu (mg/mL)	56.3	66.6	90.5	75.3	1.80	0.720	0.017	0.099
NEFA (mmol/L)	0.21	0.23	0.27	0.15	0.01	0.168	0.619	0.067
N-Ure (mg/dL)	8.8	8.3	7.7	7.9	0.59	0.377	0.009	0.129

[1/] SUB: supplementation before calving; SUA: supplementation after calving; B x A: interaction between supplementation before and after calving. Indicators of difference according to period of supplementation ($P<0.05$).

Is it feasible to use different supplementation scheme before weaning of Nellore male calves?

Abstract

It was evaluated the effect of different schedules of supplementation on performance and carcass composition during the suckling phase of grazing Nellore male calves, as well as the influence of this supplementation schedule on performance of these animals in the feedlot. Forty-eight Nellore male calves (147 ± 7 kg body weight (BW) and 3 months of age) in the suckling phase and their dams (512 ± 9 kg live weight and 6 years of age), were used in a completely randomised design. The supplementation period was 142 days in duration and consisted of two equal 71 day periods (1 and 2), followed by a 125 days feedlot period. The treatments were: 5 and 10 g supplement dry matter (DM)/kg BW.day offered in period 1 and 2 respectively (5S/10S); 10 and 5 g supplement DM/kg BW.day offered in period 1 and 2 respectively (10S/5S); 7.5 g supplement DM/kg BW.day in both periods 1 and 2 (7.5S) and mineral mix *ad libitum* in both periods 1 and 2 (MM). Supplemented calves showed greater final body weight (FBW) and average daily gain (ADG) (0.900 kg and 0.560 kg, the ADG in the suckling phase of supplemented and unsupplemented calves, respectively; $P < 0.05$), during the first and second experimental period. Calves from 5S/10S and 7.5S showed higher FBW and total ADG than calves from 10S/5S. No differences in ADG and FBW were observed in the first phase among supplemented calves. Supplemented animals had smaller intake of forage dry matter (FDM) and grater values for crude protein (CP) and total digestible nutrients (TDN), greater digestibility for all evaluated diet' components

and higher values ($P < 0.05$) of microbial production (MicN) when compared with MM treatment calves, in the first period (44.8 g/day vs. 58.1 g/day for MM treatment and supplemented animals, respectively). Animals from MM presented smaller ($P < 0.05$) rib eye area (REA), subcutaneous fat thickness (SFT) and *Gluteus medius* depth (GMD). In the feedlot period, supplemented animals had greater initial ($P < 0.05$) BW and tendency ($P < 0.10$) of greater final BW. It was concluded that *Bos indicus* male calves supplemented during the suckling phase, show better performance at weaning and have smaller finishing phase. The amount of 5 and 10 g supplement DM/kg BW.day offered in periods 1 and 2 respectively (5S/10S) is recommended to improve the performance.

Keywords: creep feeding, feedlot, tropical pasture.

Introduction

Traditional beef cattle management strategies have been developed to increase body weight gain during the finishing phase of both forage-fed and grain-based production systems. Animal tissue growth is allometric and the body weight increase is described by a sigmoidal curve, demonstrating periods of fast and slow growth of the carcass and tissues, depending on the growth phase and physiological maturity of the animal (Berg & Butterfield, 1976). During normal body development, the bone tissue is first formed, followed by the accretion of protein and then fat, with the proportion of fat in the carcass increasing as the animal approaches maturity (Berg & Butterfield, 1968).

Muscle tissue is the primary site for protein deposition in cattle, with each g of protein associated with four g of water (Halas *et al*., 2010). Young cattle deposit more protein and display more efficient weight gain than mature cattle, and also animals approaching to the maturity, have fat deposition increases, and weight gain becomes less efficient, as there is no associated water deposition, resulting in an inflection point in the growth curve. Therefore, as weight gain of young animals is more efficient, it would be of interest to investigate strategies to increase weight gain during the early phases of growth, and the impacts these have on weight gain during the finishing phase and carcass yield.

In addition to the mentioned benefits, increasing body weight gain in younger cattle may decrease slaughter age, resulting in increased meat tenderness (Schönfeldt & Strydom, 2011), optimization of land use and lower production of methane / meat produced (McAllister, 2011). The dam's milk supply is able to meet the nutritional requirements of calves to approximately three months of age (Henriques *et al*., 2011); when the milk from the dam is unable to meet the nutritional demands of the calf, supplementation may be required to maximize growth in the early part of the growth phase.

Nutrients can make changes in metabolic levels, changing the production and/or activity of some hormones (Lima *et al*. 2011) and metabolites. The thyroid hormones (triiodothyronine (T3) and thyroxine (T4)), are strictly correlated with performance and their concentrations vary according to the nutritional status of the animal (Yambayamba *et al*. 1996).

Previous studies demonstrated increased growth, digestibility and microbial protein production of suckling male calves supplemented with low or medium levels of supplementation (Valente et al., 2013; Lopes et al., 2014).

However, little is known about the effects of supplementation of calves in the suckling phase on post-weaning feedlot growth and carcass characteristics at weaning. The objective of this study was to quantify the effects of calf' supplementation in the pre-weaning on their performance, as well the consequences in their performance in the post-weaning feedlot.

Materials and methods

Animals and experimental design

This study was approved by the Ethics Committee on Animal Use (CEUAP/UFV – Process nº 40/2014), according to ethical principles of animal experimentation established by the National Council of Animal Experimentation Control - CONCEA. The experiment was conducted in the Beef Cattle Section of Universidade Federal de Viçosa, located in Viçosa-MG, Brazil (20º45' S and 42º52' W), during the rainy-to-dry transition season and dry season. The average annual rainfall in the experimental area is 1300 mm. The experiment consisted of a 142 days supplementation period followed by a 125 days feedlot period.

Forty eight Nellore male calves (147 ± 7 kg body weight and 3 months of age) and their dams (512 ± 9 kg body weight and 6 years of age) were used in the experiment. Calves were allowed to suckle dams throughout the supplementation period. Calves were weaned at 8 months of age and then grown out in a feedlot until reach 13 months of age.

The supplementation period was 142 days in duration and consisted of two equal 71 day phases (1 and 2). The experimental design was a completely randomized design, consisting of four treatments and 12 replicates per

treatment. The four treatments were based upon the provision of different amounts of the same supplement in periods 1 and 2 of the experiment; 5 and 10 g supplement DM/kg BW.day offered in periods 1 and 2 respectively (5S/10S) and 10 and 5 g supplement DM/kg BW.day offered in periods 1 and 2 respectively (10S/5S), 7.5 g supplement DM/kg BW.day in both periods 1 and 2 (7.5S) and mineral mix *ad libitum* in both periods 1 and 2 (MM).

Cows and calves were then adapted to experimental feeding and management over 14 days prior the evaluation period. During the adaptation period all animals were kept in the same paddock and calves received 0.5 kg DM/animal.day of a supplement of a 25% CP (dry matter) containing soybean meal, corn and a mineral mixture (50% of dicalcium phosphate; 47.8% of sodium chloride; 1.4% of zinc sulfate; 0.7% of copper sulfate; 0.05% of cobalt sulfate; 0.05% of potassium iodate and 0.025% of magnesium sulfate).

Supplements and grazing management

Animals were kept in five 10-ha paddocks and grazed a *Brachiaria decumbens* Stapf. pasture. Animals and their respective treatments were rotated between paddocks every 7 days to prevent possible confounding effect of paddock on results. This resulted in each paddock grazed for 28 days, followed by a 7 day grazing-free period. Each paddock contained covered water and feed troughs. A calf creep-feeding system was established in each paddock.

Cows and calves in all treatments had *ad libitum* access to a mineral mix (50% of dicalcium phosphate; 47.8% of sodium chloride; 1.4% of zinc sulfate; 0.7% of copper sulfate; 0.05% of cobalt sulfate; 0.05% of potassium iodate and

0.025% of magnesium sulfate) throughout the supplementation period. Calves were offered their amount of the same supplement at 1000 h each day during the supplementation period. The supplement (Table 1) was provided in the creep-feeder in each paddock and each group of calves had access to the feeders at all times during the day. The amount of supplement provided was based on the most recent body weight measurement. During the feedlot period of the experiment the weaned calves were equally divided into two different offered diets, that contained either corn silage (60% DM) plus a soybean hulls and maize grain concentrate mixture (40%) or sugar-cane plus 60% of concentrate (soybean hulls and corn). All animals were subjected to control of endo and ectoparasites at the beginning of the experiment and throughout the experimental period whenever necessary.

Experimental procedures and sampling

Cows and calves were weighed after a 14 hour fasting period at 0930 h on the first and last day of each period (1 and 2) of the experiment. Weaned calves were weighed at the start and end of the feedlot phase after a similar fasting period described above.

The pasture was sampled every 28 days during the experiment to quantify the availability of DM and potentially digestible DM (pdDM) (Paulino *et al.*, 2008) by cutting biomass at ground level in four randomly selected 0.5 × 0.5 m quadrats in each paddock. All pasture samples were oven-dried (60°C) and ground through 1 and 2 mm screens prior to analysis. Qualitative evaluations of the pasture consumed by the animals were performed by the hand-plucking

method every 14 days. The collection was performed by a single sampler throughout the trial period.

To evaluate the nutritional characteristics of the calves' diet, two digestibility periods (nine days each) were conducted between days 30 and 39 (period 1) and days 100 and 109 (period 2) of the experiment, using the three-marker method. To estimate faecal excretion, calves received 10 and 14g chromic oxide (Cr_2O_3)/calf each day in period 1 and 2 respectively (Detmann *et al*., 2001). The Cr_2O_3 was weighed into paper cartridges which were inserted directly into the oesophagus of each calf at 1000 h each day during the digestibility period. Titanium dioxide (TiO_2) was included in the supplement (Titgemeyer *et al*., 2001) at 10 g/kg of supplement offered to estimate supplement intake by individual calves. The indigestible neutral detergent fibre (iNDF) content was used as an internal marker to estimate forage DM intake (Detmann *et al*., 2001). The first six days of each digestibility period were used to adapt the calves to Cr_2O_3 dosing and TiO_2 in the supplement. A single faecal sample was collected each day on the final three days of digestibility period at 1500, 1100 and 0700 h respectively. Faecal samples were collected immediately after defecation or directly from the rectum of the animals, immediately oven-dried (60ºC) and then ground in a knife mill (1 and 2 mm).

Milk production was estimated on days 15, 55 and 91. Calves and their dams were separated for 2 hours, reunited for 1.5 hours and allowed to suckle to satiety before separating again at 1800 h. At 0600 h the following day 2 mL of oxytocin (10 IU/mL; Ocitovet®, Brazil) was administered into the mammary vein of cows and the udders were completely emptied using a milking machine and total milk output was weighed immediately. Calves and their dams remained separated and at 1400 h second milking was conducted, following the same

aforementioned procedures. The exact time of milking of each cow was recorded and total milk output was converted into 24 hour milk production.

Spot urine samples were obtained four hours after supplementation, from calves on the final day of each digestibility period after spontaneous urination. Urine samples were diluted with 0.036 N H_2SO_4 and stored at -20^{o}C prior to analysis. Blood samples were collected from calves approximately four hours after supplementation at the end of each digestibility period, and at the end of periods 1 and 2 and final of the feedlot phase. Blood samples were collected from the jugular vein into containing a clot activator and separator gel (BD Vacuntainer®, SST II Advance) and were immediately centrifuged at 3,600 x *g* for 15 min. Serum was collected and stored until subsequent analysis.

On the final day of the experiment, rib eye area (REA), subcutaneous fat thickness (SFT), *Gluteus medius* depth (GMD) and fat thickness at the P8 site (P8TF) was measured, using an Aloka Ultrasound (model SSD 500V, Aloka Co., Ltd., Tokyo, Japan.) with a 18 cm linear probe. The images were analyzed in the Biosoft Toolbox® II Beef program (Biotronics Inc., Ames, Iowa, USA).

Chemical analyses

The supplement and forage samples obtained by the hand-plucking method were quantified with regard DM (INCT-CA G-003/1), ash (INCT-CA M-001/1), crude protein (CP; INCT-CA N-001/1), ether extract (EE; INCT-CA G-004/1), neutral detergent fiber corrected for ash and protein (NDFap; INCT-CA F-002/1), using thermostable α-amylase, without using sodium sulfite; nitrogen insoluble in neutral detergent (NIND; INCT – CA N-004/1) according to

Detmann *et al.* (2012); iNDF, according to Valente *et al.* (2011), obtained after *in situ* incubation in F57 Ankom® filter bags for 288 h.

The non-fibrous carbohydrates (NFC) were quantified according to Detmann and Valadares Filho (2010) using the following equation:

$$NFC = 100 – (\%CP + \%NDFap + \%EE + \% ash)$$

Dried daily faecal samples were pooled within collection periods for individual animals. The concentration of Cr and TiO_2 in faecal samples were estimated by atomic absorption spectrometry (INCT-CA M-005/1) according to Detmann *et al.* (2012), and colorimetry (Titgemeyer *et al*., 2001) respectively. The DM, CP, EE, NDFap, iNDF and ash content of faeces were determined as described previously. The faecal DM excretion was estimated based on the ratio between the amount of marker (chromic oxide) supplied and its concentration in the faeces (Lopes *et al*., 2014). The individual intake of supplements by calves was obtained by the following equation:

$$ISI = ((FE \times CMF)/MSG) \times SSG$$

where: ISI = individual supplement intake (kg/day); FE = faecal excretion, in kg/day; CMF = concentration of the marker in the faeces (kg/kg); MSG = marker present in the supplement supplied to the group (kg/day); SSG = amount of supplement supplied to the group of animals (kg/day).

The voluntary intake of DM from forage was estimated by employing iNDF as an internal marker, according to the equation:

$$DMI\ (kg/day) = \{[(FE \times CMF) – MS]/CMFO\} + SDMI + MDMI$$

where: FE = faecal excretion (kg/day); CMF = concentration of the marker in the faeces (kg/kg); MS = intake of marker from supplement (kg); CMFO = concentration of the marker in the forage (kg/kg); SDMI = supplement dry matter intake (kg/day); and MDMI = milk dry matter intake.

Analyses of creatinine, uric acid, glucose and urea were performed on automatic biochemistry analyzer (Mindray, model BS200E). The enzymatic colorimetric method was used to determine the amount of uric acid. The fixed-time kinetic method was used to quantify urea. Total T3, total T4 and growth hormone (GH) were quantified using a chemiluminescent enzyme immunoassay (Beckman Coulter, model Access 2). Creatinine was quantified by using the kinetic colorimetric technique. The daily urine volume was calculated by employing the relationship between the daily excretion of creatinine (EC), adopting the equation proposed by Silva *et al*. (2012) and its concentration in the spot samples:

$$EC\ (g/day) = 0.0345 \times BW^{0.9491}$$

where: BW = body weight.

Analyses of allantoin were performed by the colorimetric method (Chen & Gomes, 1992). The total excretion of purine derivatives was calculated as the sum of the quantities of allantoin and uric acid excreted in the urine. The absorbed purines (Y, mmol/day) were calculated based on the excretion of purine derivatives (X, mmol/day), by the following equation:

$$AP = \frac{PD - 0.301 \times BW^{0.75}}{0.80}$$

where: 0.80 = recovery of absorbed purines as purine derivatives; 0.301 × $BW^{0.75}$ = the endogenous contribution for the excretion of purines (Barbosa *et al*., 2011).

The ruminal synthesis of nitrogen compounds (Y, g micN/day) was calculated as a function of the absorbed purines (X, mmol/day), using the equation proposed by Barbosa *et al*. (2011):

$$_{mic}N = \frac{70 \times AP}{0.93 \times 0.137 \times 1{,}000}$$

where: 70 = purine N content (mg N/mol); 0.137 = purine N:total N in-the-bacteria ratio; and 0.93 = digestibility of the bacterial purines.

Statistical analyses

The PROC MIXED procedure of the SAS (Statistical Analysis System, version 9.2) software was used for all statistical analyses. Means were compared by use of Fisher's Least Significant Difference (LSD). Initial weight was used as a co-variable when significant, except to final body weight in the feedlot period. All statistical procedures were performed adopting 0.05 as the critical level of probability for the type I error.

Effect of diet type (sugar cane plus concentrate and corn silage plus concentrate) and interaction between diet and treatment were tested for the parameters measured in the feedlot period.

Results

The pdDM available during the experiment was 1621 kg/day; equivalent to 4.8% of animal live weight, which is between the 4 and 6% recommended by Paulino *et al*. (2008). The average CP content of the forage was 7.3% and the NDFap content was 66.5% (Table 2). The CP content of forage decreased 1% between the first and second experimental period.

Supplemented calves had greater ADG (0.86 kg), than unsupplemented (0.65 kg) calves (Table 3; $P < 0.05$), during the period 1, with a similar result observed in the period 2 and in the total period before weaning. Supplemented calves were heavier than unsupplemented calves at the end of both periods of

the supplementation period ($P < 0.05$). Calves that were offered 7.5S and 5S/10S treatments had a greater ADG than calves that were offered the 10S/5S treatment during period 2 and over the entire experiment ($P < 0.05$).

During the first 45 days of the first experimental period, no difference in supplement intake was found among supplemented calves. Calves from 7.5S treatment consumed less forage dry matter (FDM) than calves from MM treatment. Calves from 5S/10S and 10S/5S treatment consumed greater amount of total digestible nutrients (TDN) in the first period, and also more CP in both periods of the supplementation phase, when compared with calves offered the MM treatment (Table 4). There was also observed greater FDM intake in g/kg of body weight, for calves from MM treatment when compared with supplemented calves, in the second period ($P < 0.05$).

All evaluated digestibility, except the NDF in the second experimental period, were higher for supplemented calves compared with calves that were offered the MM treatment, in both the first and second periods of the supplementation phase (Table 5). The digestibility of all evaluated diet components, except for OM and CP in period 2, was smaller ($P < 0.05$) for calves that received the 7.5S treatment, among the supplemented calves.

Supplemented calves showed greater ($P < 0.05$) MicN production (58.1 g/day) during first period compared with unsupplemented calves (44.8 g/day) (Table 6). MicN was unaffected ($P > 0.05$) by supplementation strategy in both periods of the supplementation phase, the efficiency of microbial protein production (MicE) did not differ ($P > 0.05$) between treatments in the two periods. Unsupplemented calves had a lower ($P < 0.05$) concentration of serum urea nitrogen (SUN) in the first period and smaller ($P < 0.05$) ratio of urea nitrogen to creatinine nitrogen (UUN/CRN) in the urine in both the first and

second periods, compared to supplemented calves (Table 6). Calves offered the 5S/10S treatment had a greater ($P < 0.05$) SUN concentration and a UUN/CRN ratio among the supplemented calves in the second period. Calves from 5S/10S showed greater levels of glucose in their blood than calves from the control group ($P < 0.05$; Table 6). No differences were observed in T3 and T4 concentration, neither the first nor second evaluated period, during the suckling phase.

Evaluating the ultrasound images, supplemented calves presented 49.5 cm^2 of REA, 2.7 mm of SFT and 59.9 cm of GMD, being all these values significantly higher ($P < 0.05$), when compared with unsupplemented calves (Table 7). Calves from 5S/10S treatment had greater ($P < 0.05$) REA than animals from 10S/5S treatment, also no differences were found for ultrasound measurements between calves from 5S/10S and 7.5S (Table 7).

Unsupplemented calves were lighter than supplemented calves at the start ($P < 0.05$) and tended ($P < 0.07$) to be lighter in the end of the feedlot phase, although there were no differences in ADG between the treatment groups during the finishing period (Table 8). In addition, DM intake and the concentration of T3 and T4 in the circulation were similar between all treatment groups during the feedlot period.

Discussion

There are many possibilities of interaction between pasture and supplements consumed by ruminants, and it is basically dependent of the supplement and forage compositions. According to Hodgson & Brookes (1999), there are three main factors affecting the DMI of grazing animals: (1) nutritional

requirements, determined by the genetics and physiologic state, (2) physic limitation and (3) behavior restrictions resulting from the combination of pasture and animal factors, affecting grazing behavior. No differences were found in calves' DMI, which can be attributed to the high energy level of the diet, due the milk consumption, even for animals from the MM treatment.

During the first 45 days of the first experimental period, no difference in supplement intake was found among supplemented animals, and this can be explained by the strong relationship between *Bos indicus* calves and dams, especially during the 120 days post-partum (Pérez-Torres *et al*., 2014), when was observed that calves consumed supplement only when their dams were around the supplement trough. Another factor that could affect the supplement intake in this phase is the high relation milk intake/kg of calves BW. Lower pasture intake was observed for supplemented calves when compared with the MM treatment animals in the first period, which was reflected in a greater digestibility of all evaluated dietary components and consequentially higher values of TDN. Total digestibility increase can be expected with inclusion of concentrates in the diet, because these usually show higher digestibility values than pasture (Paulino *et al*., 2008), and also because of the greater nutrients concentration in the supplements, which increases their participation in the total diet, thereby reducing the relative participation of the fecal metabolic fraction (Van Soest, 1994). Greater NDF digestibility was observed in the first period for animals from 10S/5S treatment, when supplemented animals were compared, being consequence of amount of supplement intake. Detmann *et al*. (2003) found inverse relation between non fibrous carbohydrates intake and NDF digestibility in tropical conditions.

Comparing supplemented with unsupplemented animals was observed difference in the MicN and supplemented calves showed greater values in the first period. With the increase of starch intake, greater MicN is expected because of microbial population change, favoring amylolytic microorganism proliferation, which has faster development than fibrolytic microorganisms. Amylolytic microorganisms have a greater ability in nitrogen uses when compared with the fibrolytic ones (Olson *et al*., 1999). On the other hand, the MicE had no difference among different calves' treatment in the first and second experiment phase (Table 6). Working with Nellore male calves, Lopes *et al*. (2014) showed MicN of 65.3 and 46.1 g/day for supplemented and unsupplemented animals, respectively, these values are smaller than those found in the present study (67.2 and 58.6 g/day of MicN for supplemented and unsupplemented calves, respectively). The cited authors did not find difference for MicE between supplemented and unsupplemented groups, agreeing with the results in this study. Supplemented calves had greater MicN, but in the same time they consumed more digestible organic matter (DOM) than unsupplemented calves, causing a similar MicN/DOM intake relation between these two groups. Detmann *et al*. (2014) found negative relationship between MicE and DOM intake, evaluating data of animals in tropical pastures.

Supplemented calves showed higher levels of SUN (in the second period) and UUN/CRN (in both periods) than calves from MM group, and animals supplemented with 10 g supplement DM/kg LW.day had greater values of SUN and UUN/CRN when compared with those receiving 5 g supplement DM/kg LW.day, in the second evaluated period. Evaluating these parameters in Nellore heifers supplemented with different amounts of CP, Martins *et al*. (2015)

found highest levels of SUN and UUN/CRN for supplemented animals, justifying this event by the higher protein intake.

Calves from 5S/10S treatment and non-supplemented calves showed a difference in glucose content in the first evaluated period, which can be explained by the greater offered amount of concentrate to supplemented animals. No difference was found in calf serum glucose concentration between different supplemented animals or between supplemented and unsupplemented calves, in the second period. Studying the effect of sources of energy on performance of early-weaned steers, Schoonmaker et al. (2003) found no difference in serum glucose between steers consuming different amounts of concentrate. Murphy et al. (1994) did not find a difference in serum glucose, but observed a lower insulin concentration for ad libitum-fed steers compared with steers, whose intake was restricted. No insulin levels were measured in the present study. However, no difference was found for calf total T3 and T4 concentration between the supplemented and unsupplemented calves, neither the first nor second evaluated period. Thyroid hormones are associated with basal metabolic rate (Murphy and Loerch, 1994) and the nutritional conditions established here were not different enough to provide differences in the thyroid hormone concentration. The calves from the MM treatment had good nutritional conditions, consuming enough energy and protein to have an average daily gain of 0.69 kg/day and 0.5 kg/day in the first and second period, respectively (BrCorte, 2010), thus no difference resulted. Studying different growth rates in beef cattle, Ellenberger et al. (1989), did not find difference in T3 concentration between animals feed ad libitum and restricted diets.

Supplemented calves had greater ADG and FBW due to supplement intake and digestibility of nutrients. Greater supplement intake can explain the

better performance of calves from the 5S/10S and 7.5S treatments when compared with 10S/5S treatment calves, being the second period determining to obtain this result, since supplemented animals showed no difference in supplement intake in the first phase. The difference found in the calves' carcass at the weaning day, reflect the results in weight gain, where supplemented animals had higher values of REA, SFT, GMD and P8TF, and animals from the 5S/10S and 7.5S treatments, showed greater REA when compared with the 10S/5S treatment calves. Increasing the level of offered concentrate, tended increase the carcass fat concentration and increased the carcass weight in beef steers during the finishing phase in a Keady *et al*. (2013) study.

At the end of the feedlot period, the animals supplemented during the suckling period, tended to show greater FBW, being consequence of the greater IBW. The average of carcass weight in the first 2015 quarter in Brazil was 237.5 kg (IBGE, 2015), considering 55% of carcass yield, the supplemented group had 237.3 kg of carcass equivalent, while the MM group showed 214 kg of carcass equivalent. Considering the ADG of 1.2 kg/day (ADG during feedlot period), the animals from the MM treatment needed approximately 36 more days to reach the required weight for slaughter, which could economically unfeasible the feedlot system.

Conclusion

Bos indicus male calves supplemented during the suckling phase, show better performance at weaning and have smaller finishing phase. The amount of 5 and 10 g supplement DM/kg BW.day offered in periods 1 and 2 respectively (5S/10S) is recommended to improve the performance.

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Table 1

Centesimal composition of supplements (as is).

Ingredient	MM[2]	SUP[2]
Mineral mixture[1]	100	5.0
Corn meal	---	46.0
Soybean meal	---	46.0
Molasses	---	3.0
Crude Protein (%DM)	---	28.8

[1]Centesimal composition: dicalcium phosphate, 50.00; sodium chloride, 47.775; zinc sulfate, 1.4; copper sulfate, 0.7; cobalt sulfate, 0.05; potassium iodate, 0.05 and magnesium sulfate: 0.025.

[2] mineral mixture (MM), supplement (SUP)

Table 2

Chemical composition of supplement and forage.

		Forage[5]		
Item	Supplement	All experiment	Digestibility trial 1	Digestibility trial 2
Dry matter[2]	88.9	30.8	27.5	31.3
Organic matter[3]	91.5	91.7	92.0	90.4
Crude protein[3]	28.8	7.3	8.6	7.6
NDIN[1,4]	35.7	29.1	26.1	22.6
Ether extract[3]	2.4	1.2	1.0	1.4
NDFap[1,3]	15.2	66.5	69.1	62.1
NFC[1,3]	45.1	16.7	13.3	19.3
iNDF[1,3]	2.7	27.7	28.6	27.2

[1]/NDIN - neutral detergent insoluble nitrogen; NDFap - neutral detergent fiber corrected for ash and protein; NFC - non-fibrous carbohydrates; iNDF - indigestible neutral detergent fiber.
[2]/ In % of fresh matter (as is).
[3]/ In % of dry matter.
[4]/ In % of total nitrogen.
[5]/Mean values of the samples obtained by hand-plucking (grazing simulation).

Table 3

Means, standard error (SE) and indicators of significance for initial body weight (IBW), final body weight (FBW), average daily gain (ADG) of Nellore male calves

Item	Treatments[1]				SE	(*P*-value)
	MM	5S/10S	7.5S	10S/5S		
	First period					
IBW (kg)	148	147	148	147	---	---
FBW (kg)	191[b]	203[a]	207[a]	205[a]	3.95	<.001
ADG (kg)	0.65[b]	0.83[a]	0.89[a]	0.86[a]	0.02	<.001
	Total period					
FBW (kg)	227[c]	278[a]	277[a]	262[b]	5.22	<.001
ADG (kg)	0.56[c]	0.94[a]	0.93[a]	0.82[b]	0.03	<.001

[1]/ MM - mineral mixture; 5S/10S – 5 g supplement DM/kg LW.day in the first phase and 1 g supplement DM/kg LW.day in the second phase, 7.5S - 7.5 g supplement DM/kg LW.day during entire experiment; 10S/5S – 10 g supplement DM/kg LW.day in the first phase and 5 g supplement DM/kg LW.day in the second period.

Table 4
Means, standard error (SE) and indicators of significance for estimated voluntary intake (kg/animal.day) of Nellore male calves.

	Treatment[1]					
Intake	MM	5S/10S	7.5S	10S/5S	SE	(*P*-value)
	First Period					
Supplement dry matter	---	0.90	0.91	1.12	0.09	---
Forage dry matter	2.96[a]	2.25[ab]	1.75[b]	2.32[ab]	0.16	0.044
Milk dry matter	0.67	0.79	0.73	0.61	0.03	0.077
Total dry matter	3.63	4.05	3.40	4.04	0.20	0.598
Organic matter	3.37	3.78	3.16	3.76	0.19	0.589
Ether extract	0.27[a]	0.29[a]	0.23[b]	0.22[b]	0.25	0.006
Crude protein	0.44[b]	0.66[a]	0.58[ab]	0.70[a]	0.03	0.017
NDFap[2]	1.98	1.68	1.34	1.87	0.11	0.179
Total digestible nutrients	1.86[c]	3.09[a]	2.37[bc]	2.96[ab]	0.14	0.003
TSDM[3]	---	54.4	77.3	102.6	---	---
			g/kg BW			
Dry matter	17.88	19.13	15.64	19.68	1.06	0.549
Forage dry matter	14.51[a]	10.57[ab]	8.07[b]	11.35[ab]	0.79	0.028
NDFap[2]	9.73	7.88	6.18	9.15	0.60	0.126
	Second Period					
Supplement dry matter	---	1.85	1.58	1.05	0.15	---
Forage dry matter	3.18	2.50	2.20	2.48	0.15	0.122
Milk dry matter	0.63	0.76	0.70	0.63	0.03	0.241
Total dry matter	3.81	5.11	4.69	4.19	0.24	0.249
Organic matter	3.57	4.68	4.29	3.82	0.22	0.300
Ether extract	0.34	0.33	0.30	0.32	0.27	0.464
Crude protein	0.44[b]	0.90[a]	0.85[a]	0.68[a]	0.05	0.001
NDFap[2]	1.98	1.85	1.63	1.71	0.11	0.850
Total digestible nutrients	2.37	3.50	3.16	2.96	0.16	0.083
TSDM[3]	---	150.3	114.1	74.1	---	---
TSDM[4]	---	204.7	191.4	176.7	---	---
			g/kg BW			
Dry matter	17.13	20.18	17.44	16.50	0.98	0.592
Forage dry matter	14.31[a]	9.86[b]	8.20[b]	9.74[b]	0.69	0.008
NDFap[2]	8.90	7.29	6.06	6.71	0.47	0.345

[1]/ MM - mineral mixture; 5S/10S – 5 g supplement DM/kg LW.day in the first phase and 1 g supplement DM/kg LW.day in the second phase, 7.5S - 7.5 g supplement DM/kg LW.day during entire experiment; 10S/5S – 10 g supplement DM/kg LW.day in the first phase and 5 g supplement DM/kg LW.day in the second period.
[2]/ Neutral detergent fiber corrected for ash and protein.
[3]/ Total supplement dry matter in the period (kg/animal).
[4]/ Total supplement dry matter during all experiment (kg/animal).

Table 5

Means, standard error (SE) and indicators of significance for apparent digestibility (%) of Nellore male calves diets.

	Treatment[1]					
Digestibility	MM	5S/10S	7.5S	10S/5S	SE	(*P*-value)
	First period					
Dry matter	45.0^{c}	70.5^{a}	63.6^{b}	70.1^{a}	1.65	<.001
Organic matter	50.4^{c}	74.0^{a}	67.7^{b}	73.6^{ab}	1.52	<.001
Crude protein	42.2^{c}	71.4^{ab}	66.7^{b}	75.4^{a}	2.22	<.001
NDFap[2]	45.9^{d}	65.6^{b}	56.1^{c}	69.5^{a}	1.56	<.001
Total digestible nutrients	54.4^{c}	76.6^{a}	69.8^{b}	74.2^{ab}	1.55	<.001
	Second period					
Dry matter	53.4^{c}	64.5^{ab}	61.0^{b}	66.5^{a}	1.05	<.001
Organic matter	58.4^{b}	67.7^{a}	65.5^{a}	68.9^{a}	0.95	<.001
Crude protein	56.5^{b}	71.5^{a}	70.4^{a}	73.1^{a}	1.41	<.001
NDFap[2]	53.7^{c}	54.5^{bc}	50.9^{c}	60.6^{ab}	1.16	0.025
Total digestible nutrients	63.7^{c}	69.7^{ab}	66.6^{bc}	71.1^{a}	0.93	0.017

[1]/ MM - mineral mixture; 5S/10S – 5 g supplement DM/kg LW.day in the first phase and 1 g supplement DM/kg LW.day in the second phase, 7.5S - 7.5 g supplement DM/kg LW.day during entire experiment; 10S/5S – 10 g supplement DM/kg LW.day in the first phase and 5 g supplement DM/kg LW.day in the second period.
[2]/ NDFap: Neutral detergent fiber corrected for ash and protein.

Table 6

Means and standard error (SE) for production of microbial nitrogen (micN), microbial efficiency (micE), serum urea nitrogen (SUN), urine urea nitrogen/creatinine nitrogen ratio (UUN/CRN), Glucose, T3 and T4 of Nellore male calves.

	Treatment[1]					
Item	MM	5S/10S	7.5S	10S/5S	SE	(*P*-value)
	First period					
micN (g/day)	44.8[b]	54.2[a]	58.4[a]	61.6[a]	1.79	0.003
micE[2]	178.9	132.4	177.6	165.0	7.29	0.195
SUN (mg/dL)	13.0	13.5	14.9	15.6	0.43	0.119
UUN/CRN	14.0[b]	18.2[a]	19.7[a]	19.6[a]	0.62	0.001
Glucose (mg/mL)	64.1[b]	84.2[a]	72.3[ab]	74.3[ab]	2.56	0.049
T3 (ng/mL)	3.0	2.8	2.5	2.8	0.18	0.806
T4 (ng/dL)	106.4	140.9	102.9	124.4	6.08	0.103
GH (ng/mL)	6.4	2.8	10.2	5.6	1.81	0.271
	Second period					
micN (g/day)	72.5	74.7	74.1	79.7	4.72	0.958
micE[2]	228.9	177.7	186.6	203.9	15.29	0.760
SUN (mg/dL)	10.0[c]	16.8[a]	13.6[b]	14.0[b]	0.51	<.001
UUN/CRN	10.3[c]	21.7[a]	19.2[ab]	16.9[b]	0.91	<.001
Glucose (mg/mL)	69.0	75.8	66.8	69.8	1.62	0.263
T3 (ng/mL)	1.7	2.0	2.0	1.9	0.07	0.433
T4 (ng/dL)	84.5	94.3	88.4	89.1	3.73	0.848
GH (ng/mL)	10.9	6.2	13.0	7.4	1.80	0.557

[1]/ MM - mineral mixture; 5S/10S – 5 g supplement DM/kg LW.day in the first phase and 1 g supplement DM/kg LW.day in the second phase, 7.5S - 7.5 g supplement DM/kg LW.day during entire experiment; 10S/5S – 10 g supplement DM/kg LW.day in the first phase and 5 g supplement DM/kg LW.day in the second period.
[2]/ In g of microbial crude protein/kg digestible organic matter intake.

Table 7

Means and standard error (SE) for rib eye area (REA), subcutaneous fat thickness (SFT), Gluteus medius depth (GMD) and P8 thickness fat (P8TF) of Nellore male calves in the weaning time

	Treatments[1]					
Item	MM	5S/10S	7.5S	10S/5S	SE	(*P*-value)
REA (cm^2)	40.0^{c}	53.4^{a}	49.2^{ab}	45.8^{b}	1.11	<.001
SFT (mm)	2.0^{b}	3.0^{a}	2.5^{a}	2.6^{a}	0.10	0.006
GMD (cm)	54.0^{b}	59.9^{a}	61.7^{a}	58.0^{ab}	0.86	0.007
P8TF (mm)	2.2	3.1	2.9	2.4	0.13	0.060

[1]/ MM - mineral mixture; 5S/10S – 5 g supplement DM/kg LW.day in the first phase and 1 g supplement DM/kg LW.day in the second phase, 7.5S - 7.5 g supplement DM/kg LW.day during entire experiment; 10S/5S – 10 g supplement DM/kg LW.day in the first phase and 5 g supplement DM/kg LW.day in the second period.

Table 8

Means and standard error (SE) for hormones, initial body weight (IBW), final body weight (FBW), average daily gain (ADG) and total dry matter intake (TDMI) of Nellore steers in feedlot

	Treatments[1]					(*P*-value)		
Item	MM	5S/10S	7.5S	10S/5S	SE	Trat	Diet	Trat x Diet
T3 (ng/mL)	2.7	2.5	2.2	2.8	0.11	0.244	0.920	0.470
T4 (ng/dL)	129.4	135.9	132.8	145.9	3.97	0.394	0.739	0.723
IBW (kg)	227[c]	278[a]	277[a]	262[b]	5.22	0.001	---	---
FBW (kg)	389	447	424	423	7.01	0.065	0.889	0.636
ADG (kg)	1.2	1.1	1.2	1.2	0.03	0.970	0.317	0.769
TDMI (kg)	946.3	1011.1	972.5	1006.4	17.90	0.533	0.057	0.431

[1]/ MM - mineral mixture; 5S/10S – 5 g supplement DM/kg LW.day in the first phase and 1 g supplement DM/kg LW.day in the second phase, 7.5S - 7.5 g supplement DM/kg LW.day during entire experiment; 10S/5S – 10 g supplement DM/kg LW.day in the first phase and 5 g supplement DM/kg LW.day in the second period.

Varied supplementation scheme of male Nellore calves influencing behaviour and performance of their dams

Abstract

The objective of the present study was to evaluate the effect of different schemes of calves' supplementation in a Creep feeding system, on the behaviour of *Bos indicus* calves and dams, and also the influence of the calves' supplementation on dams' performance. Forty eight Nellore male calves (147 ± 7 kg body weight and 3 months of age) in the suckling phase and their dams (476 ± 9 kg and 6 years of age), were studied in a completely randomised design. The experiment was divided into 2 periods of 71 days. The treatments were: 5 and 10 g supplement dry matter (DM)/kg BW.day offered in period 1 and 2 respectively (5S/10S); 10 and 5 g supplement DM/kg BW.day offered in period 1 and 2 respectively (10S/5S); 7.5 g supplement DM/kg BW.day in both periods 1 and 2 (7.5S) and mineral mix ad libitum in both periods 1 and 2 (MM). No differences ($P < 0.05$) in body condition score (BCS), final body weight (FBW) and average daily gain (ADG) were found on dams' performance. Calves from MM treatment spent more time ($P < 0.05$) grazing than supplemented calves from 5S/10S and 10S/5S treatments, in the first period. No difference in suckling time was found among the treatments ($P > 0.05$) in the first evaluated period. Calves from 10S/5S treatment spent more time suckling and less time eating supplements ($P < 0.05$) than 5S/10S treatment animals, in the second evaluated period. Dams of MM treatment's calves had more idle time and lower grazing time when compared with the mothers of calves from 5S/10S and 10S/5S treatments. It was concluded that, different schedules of Nellore calves'

supplementation at pasture, do not affect their mothers' performance, and supplementation decreases grazing time of calves in the suckling phase.

Keywords: beef cattle, *Bos indicus*, creep-feeding, tropical pasture

Introduction

When forages cannot supply the nutrient requirements for a desired performance, use of supplementation becomes an alternative, especially in tropical regions. The most important variable influencing animal performance is dry matter intake (Waldo and Jorgensen, 1981). Protein and energy supplementation can change feeding behaviour of grazing animals, however, this has not been well evaluated (Manzano *et al*. 2007). Understanding the behaviour of grazing cattle is important to understand and alter cattle management and improve animal production, especially of *Bos indicus* calves and dams, which have a strong relationship (Pérez-Torres *et al*., 2014) thereby influencing each other's behaviour.

Multiple factors can affect the animal feed intake, but rumen fill and energy content are mainly correlated with feed characteristics (Mertens, 1987), consequently affecting the consumption. Therefore, calves receiving high levels of concentrate supplementation could have a large part of their energy requirement supplied and decrease milk suckling amount and, or frequency. Evaluating the effect of suckling on performance of dairy cows, Bar-Pelled *et al*. (1995) concluded that milk production was higher and body weight loss was greater for suckled cows. This could cause a body condition score loss and compromise the reproductive performance in the subsequent breeding season (Bohnert *et al*. 2013). The objective of the present study was to evaluate the effect of supplementation in a Creep feeding system on the behaviour of *Bos*

indicus calves and dams, and also the influence of the calves' supplementation on dams' performance.

Material and methods

Animals, experimental design and supplements

This study was approved by the Ethics Committee on Animal Use (CEUAP/UFV – Process nº 40/2014), according to ethical principles of animal experimentation established by the National Council of Animal Experimentation Control - CONCEA. The experiment was conducted in the Beef Cattle Section of Universidade Federal de Viçosa, Viçosa-MG, Brazil (20º45' S and 42º52' W) in the period of transition between the rainy-dry season and the dry season. The average annual rainfall in the experimental area is 1300 mm.

Forty eight Nellore male calves (147 ± 7 kg body weight and 3 months of age) in the suckling phase and their dams (476 ± 9 kg and 6 years of age), were used. Animals were acclimated for 14 days and then spent 142 days in the experimental trial, which were divided into 71-day periods. During the acclimation all animals were kept in the same paddock and received the same treatment, which was a 500 g/animal.day^{-1} of a 25% crude protein (CP) (dry matter) supplement, composed of soybean meal, corn and a mineral mixture. The supplement used during the experiment is described in the Table 1. The animals were kept in five 10-ha paddocks formed by *Brachiaria decumbens* Stapf. and provided with drinkers and covered feeders.

The experimental design was a completely randomized design, consisting of four treatments and twelve replicates. The calves' treatments were: 5 and 10 g supplement dry matter (DM)/kg BW.day offered in period 1 and 2 respectively (5S/10S); 10 and 5 g supplement DM/kg BW.day offered in

period 1 and 2 respectively (10S/5S); 7.5 g supplement DM/kg BW.day in both periods 1 and 2 (7.5S) and mineral mix *ad libitum* in both periods 1 and 2 (MM). Animals were weighed every first day of the month at 0930 h, for adjustment the level of supplement offered. Supplements were offered to calves at 1000 h every day in a collective feeder (Creep-feeding system) in each paddock. All cows and calves received the mineral mixture (Table 1) *ad libitum* during the entire experimental period. Animals (and their respective treatments) were rotated among the paddocks every seven days, to prevent possible paddock effects on the treatments.

Experimental procedures and sampling

The pasture was sampled every 28 days during the experiment to quantify the availability of DM and potentially digestible DM (pdDM) (Paulino *et al.*, 2008) by cutting biomass at ground level in four randomly selected 0.5 × 0.5 m quadrats in each paddock. All pasture samples were oven-dried (60°C) and ground through 1 and 2 mm screens prior to analysis. Qualitative evaluations of the pasture consumed by the animals were performed by the hand-plucking method every 14 days. The collection was performed by a single sampler throughout the trial period.

To evaluate behaviour, four days were selected - day 57, 58, 112 and 113 of the experimental period. The evaluation was made between 0600h to 1800h by 12 evaluators, where each was responsible for one treatment for four consecutive hours. The observers stayed at a minimum distance of 50 m from the animals. The behaviours were classified as - grazing, idle, suckling and supplement consumption for the calves, and grazing, idle and mineral mixture consumption for the cows. The time was noted when the animals changed their

activity. The animals were individually identified with numbers painted on the rib area two days before the evaluation, and each group was kept in the same paddock during the two evaluated days in each period. The average of the two days of each period was used to evaluate the behaviours.

To estimate milk production (and calves milk intake) two collections were made in experimental days 15 and 91. Calves and their dams were separated for 2 hours, after together for 1.5 hours and separated again at 1800h, spent all night separated. At 0600h the next day the first milk production was measured by a mechanical milking device, immediately after an injection of 2 mL of oxytocin (10 IU/mL; Ocitovet®, Brazil) in the mammary artery. Milk was weighed and a sample was sent for composition evaluation, immediately after milking. Calves and their dams were then kept separate and at 1400h the second milk production was measured by the same procedure. The exact time when each cow was milked was recorded, and the milk produced was converted into a 24-h production. The milk produced was corrected to 4 % of fat (NRC, 2001) calculated by the following equation:

$$FCM = 0.4 \times MP + 15 \times (MF/100) \times MP$$

Where: FCM = 4% fat corrected milk in kg/day; MP = milk production in kg/day; MF= milk fat in %

BCS of the cows was recorded using a scale of 1 to 9 (NRC, 1996); all evaluations were made by the same five trained evaluators. The ADG by cows was estimated by the difference between the final and initial body weights, both after a feed-deprivation period of 14 hours (water available), which was then divided by the number of experimental days.

Chemical analyses

The supplement and forage samples obtained by the hand-plucking method were quantified with regard dry matter (DM; INCT-CA G-003/1), ash (INCT-CA M-001/1), CP (INCT-CA N-001/1), ether extract (EE; INCT-CA G-004/1), neutral detergent fiber corrected for ash and protein (NDFap; INCT-CA F-002/1), using thermostable α-amylase, without using sodium sulfite; nitrogen insoluble in neutral detergent (NIND; INCT–CA N-004/1) according to Detmann *et al.* (2012); iNDF, according to Valente *et al.* (2011), obtained after *in situ* incubation in (F57 Ankom®) bags for 288 h.

The pdDM (Paulino *et al.*, 2008) was calculated by the follow equation:

$$pdDM\ (\%) = 0{,}98 \times (100 - NDF) + (NDF - NDFi)$$

Where: pdDM = potentially digestible dry matter; NDF = neutral detergent fibre; NDFi = indigestible neutral detergent fibre

Statistical analyses

The PROC MIXED procedure of the SAS (Statistical Analysis System, version 9.2) software was used for all statistical analyses. Means were compared by use of Fisher's Least Significant Difference (LSD) Test. Cows' initial weight was used as a co-variable when significant, in their performance evaluation. All statistical procedures were performed adopting 0.05 as the critical level of probability for the type I error, excluding the behaviour measurements, which were evaluated adopting 0.10 as the critical level of probability for the type I error, because the high incidence of type II error in this parameters.

Results

The climate data during the experiment were respectively for the days 57, 58, 112 and 113: average temperature (°C): 22.2; 21.9; 14.4 and 15.1; maximum temperature (°C): 28.6; 28.2; 22.8 and 24.8; minimum temperature (°C): 19.5; 19.0; 8.6 and 8.5; humidity (%): 85; 83; 74 and 78; rain (mm): 1.2; 0.4; 0.0 and 0.0; wind speed (km/day): 90; 61; 45 and 46. The average of herbage mass DM and pdDM availability were respectively, 5894 kg/ha and 3900 kg/ha in the first behaviour evaluation (days 57 and 58); and 5886 kg/ha and 3623 kg/ha in the second behaviour evaluation period (days 112 and 113). The average of pdDM during the experiment was 4.8% of animal live weight (kg). The average of forage CP was 7.3% during experimental period (Table 1).

No differences ($P > 0.05$) in BCS, FBW and ADG were found among cows (Table 2). The dams of calves from 5S/10S treatment showed higher ($P < 0.05$) milk production than mothers of calves from 10S/5S in the first evaluated period (Table 2).

Calves from the MM treatment spent 10% more time grazing than supplemented calves in the first period, presenting greater ($P < 0.05$) time grazing than calves from the treatments 5S/10S and 10S/5S in this same evaluated period (Table 3). Calves from the 5S/10S treatment spent 7% less time grazing and 14% more idle time than calves from the 10S/5S treatment in the first period. Dams showed the same behaviour in different proportions. No difference in suckling time was found among the treatments ($P > 0.05$), and calves that received greater amounts of concentrate spent more time eating the supplement in the first evaluated period (Table 3).

In the second period, unsupplemented calves spend more ($P < 0.05$) grazing time than calves from 5S/10S and 7.5S treatments. Calves from the 10S/5S treatment spent more time suckling (19 minutes vs. 10 minutes) and less time eating supplements (33 minutes vs. 58 minutes) when contrasted with the calves from 5S/10S treatment, in the second evaluated period. Calves from the 10S/5S treatment suckled 2.3 times day^{-1} and calves from the 5S/10S treatment, suckled 1.3 times/day in the second period (Table 3). The dams of the MM treatment' calves showed more idle time and lower grazing time when compared with the mothers of calves from the 5S/10 and 10S/5S treatments ($P < 0.05$). The calves' average suckling was 2.3 and 1.8 times in the first and second period, respectively.

The activities followed a cyclic rhythm, where the grazing was concentrated during the morning and in the end of afternoon, idle time was concentrated during the beginning of the morning and early afternoon, for both calves (Figure 1) and cows (Figure 2). Suckling activities were distributed throughout the day and supplement intake concentrated after the times that the supplement was provided (between 1000h and 1140h) (Figure 1). Supplemented calves consumed the supplement 3.2 times day^{-1} in the first and 2.5 times day^{-1} in the second period.

Discussion

The cows' performance was not affected by calves' treatments, except the milk production when the treatments 5S/10S and 10S/5S were compared, in the first period. Independently of the period (amount of offered supplement) the dams of calves from the 5S/10S treatment always produced more milk (in absolute values), indicating that this effect was not correlated with the calves'

treatment but with the cows. Fordyce *et al*. (1996) suggested that the supplementation of calves may decrease milk production of cows due to a reduction in suckling stimulation, but in the present study no difference was found in this parameter. The results in this study agree with those of Valente *et al.* (2013) and Lopes *et al.* (2016) where the supplementation of calves did not affect their dams' performance, when cows had adequate body condition score in tropical pastures conditions. Comparing different schedules of nursing, Alvarez-Rodriguez *et al*. (2009) concluded that cow live weight losses, fat-corrected milk yield and calf average daily gain during lactation were higher in the *ad libitum* nursing treatment, when compared with the restricted nursing groups. In the present study all animals were kept with their dams during the entire experiment.

Multiple associative effects can be observed when grazing animals are supplemented (Paulino *et al.* 2004). There is an associative-addictive effect, where the pasture intake is increased by the supplement on offer and there is a substitutive effect, where the pasture intake is decreased by animal supplementation, which is common when energy based or concentrate based supplements are used. In the present study, calves from the MM treatment spent more time grazing in the first period than supplemented groups, and the same behaviour tended to happen in the second period. This suggests that supplemented calves had a lower pasture intake through the substitutive effect. The herbage mass on offer and the pdDM on offer were high and not limiting (Paulino *et al*. 2008).

Bodine and Purvis (2003) found that grazing time, intensity, and harvesting efficiency were reduced by corn supplementation. Calves from the 5S/10S treatment spent more time consuming supplements than animals from

the 10S/5S treatment in the second period, which was expected, since the calves from 5S/10S treatment received twice of body weight in supplement. The greater supplement intake made the calves from the 5S/10S treatment suckling for less time (and did this 1.3 times during the day) when compared with the 10S/5S treatment's calves (2.3 sucklings/day), even the milk production was greater for the mothers of calves from 5S/10S treatment.

Young calves have the following descending preference order: milk, concentrate and pasture (Webb *et al.* 2013), but this order can be changed according to the age of the animal. Webb *et al.* (2013), found that calves of 3 months of age selected the following proportion (average of individual proportions) of milk replacer, concentrate and roughage in relation to total g dry matter intake: 51.6 ± 5.0%, 25.0 ± 4.7% and 23.4 ± 2.8%. At 6 months, the calves conserved the roughage proportion (23.3 ± 1.6%), but increased concentrate intake (47.1 ± 2.1%) at the expense of milk replacer treatment (29.6 ± 1.9%). In the present study was observed that cows started grazing earlier, and were followed by grazing by the calves.

Conclusion

Supplementing calves at pasture by a different sequence over time do not affect their dams' performance. Supplementation decreases the grazing time of the calves. There was no advantage of a sequence low-high or high-low level of supplement compared to offering a constant level of supplement throughout lactation.

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Table 1

Chemical composition of supplement and forage.

Item	Supplement	Forage
Dry matter[2]	88.9	30.8
Organic matter[3]	91.5	91.7
Crude protein[3]	28.8	7.3
NDIN[1,4]	35.7	29.1
Ether extract[3]	2.4	1.2
NDFap[1,3]	15.2	66.5
NFC[1,3]	45.1	16.7
iNDF[1,3]	2.7	27.7

[1]/NDIN - neutral detergent insoluble nitrogen; NDFap - neutral detergent fiber corrected for ash and protein; NFC - non-fibrous carbohydrates; iNDF - indigestible neutral detergent fiber.
[2]/ In % of fresh matter (as is).
[3]/ In % of dry matter.
[4]/ In % of total nitrogen.

Table 2

Means, standard error (SE) and indicators of significance for initial body weight (IBW), final body weight (FBW), average daily gain (ADG), initial body condition score (IBCS), final body condition score (FBCS), milk production in the first (MP1) and second (MP2) experiment period of lactating Nellore cows.

	Treatments[1]					
Item	MM	5S/10S	7.5S	10S/5S	SE	*P*-value
IBW (kg)	479	478	478	468	8.26	0.955
FBW (kg)	483	481	477	465	8.34	0.888
ADG (kg)	0.03	0.02	-0.02	-0.02	0.02	0.679
IBCS[2]	5.0	4.7	4.9	4.7	0.10	0.711
FBCS[2]	5.0	4.9	4.8	4.7	0.09	0.686
CMP1 (kg)[3]	5.5[ab]	6.5[a]	5.6[ab]	4.5[b]	0.22	0.012
CMP2 (kg)[3]	5.3	6.1	5.4	5.0	0.21	0.379

[1]/ MM - mineral mixture; 5S/10S – 5 g supplement DM/kg LW.day in the first phase and 1 g supplement DM/kg LW.day in the second phase, 7.5S - 7.5 g supplement DM/kg LW.day during entire experiment; 10S/5S – 10 g supplement DM/kg LW.day in the first phase and 5 g supplement DM/kg LW.day in the second period.
[2/] 1-9 scale
[3/] Milk production corrected to 4% of fat

Table 3

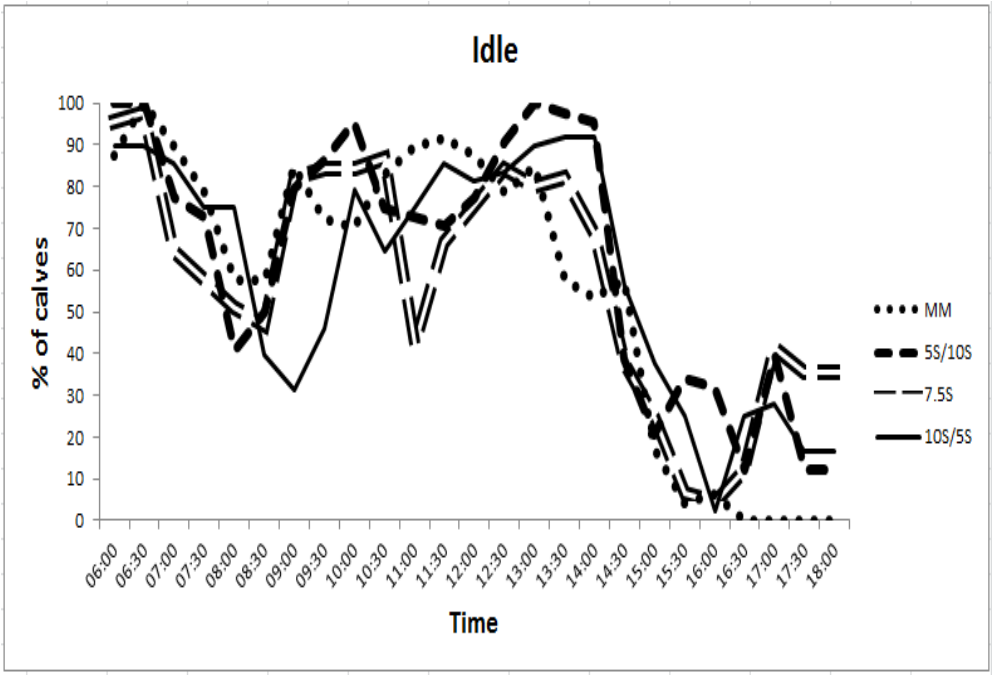
Means and standard error (SE) for behaviour (% of the evaluated period) at pasture of Nellore male calves and their dams

	Treatments[1]					
Behaviour	MM	5S/10S	7.5S	10S/5S	SE	*P*-valor
	First period - Calves					
Grazing	39.6^{a}	22.9^{c}	35.2^{ab}	30.5^{b}	1.34	<.001
Idle	54.4^{b}	67.6^{a}	51.8^{b}	53.6^{b}	1.49	<.001
Suckling	4.9	3.2	4.4	7.2	0.94	0.468
Supplement eating[2]	1.1^{c}	6.3^{b}	8.6^{a}	8.7^{a}	0.64	<.001
Number of suckling/day	2.4	2.6	2.0	2.3	---	---
Number of supplement eating/day	---	3.3	2.7	3.7	---	---
	First period - Cows					
Grazing	53.0^{b}	45.2^{c}	58.5^{a}	58.3^{ab}	1.46	0.001
Idle	46.3^{b}	53.4^{a}	41.5^{c}	39.7^{c}	1.48	0.001
Mineral mix eating	0.8	1.4	0.4	2.0	0.22	0.220
	Second period - Calves					
Grazing	45.4^{a}	41.4^{cb}	40.3^{c}	45.2^{ab}	0.88	0.091
Idle	52.2	48.8	50.7	47.6	0.92	0.276
Suckling	1.6^{b}	1.4^{b}	1.6^{b}	2.6^{a}	0.18	0.064
Supplement eating[2]	0.8^{c}	8.4^{a}	7.4^{a}	4.9^{b}	0.58	<.001
Number of suckling/day	1.7	1.3	1.7	2.3	---	---
Number of supplement eating/day	---	2.8	2.2	2.6	---	---
	Second period - Cows					
Grazing	53.4^{c}	60.5^{ab}	57.2^{bc}	64.6^{a}	1.15	0.001
Idle	46.4^{a}	39.1^{bc}	42.2^{ab}	34.9^{c}	1.16	0.001
Mineral mix eating	0.2	0.4	0.5	0.4	0.08	0.840

[1]/ MM - mineral mixture; 5S/10S – 5 g supplement DM/kg LW.day in the first phase and 1 g supplement DM/kg LW.day in the second phase, 7.5S - 7.5 g supplement DM/kg LW.day during entire experiment; 10S/5S – 10 g supplement DM/kg LW.day in the first phase and 5 g supplement DM/kg LW.day in the second period.

[2/] Multiple or mineral mixture eating

Figure 1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% of calves
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90
80
70
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50
40
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Time
MM
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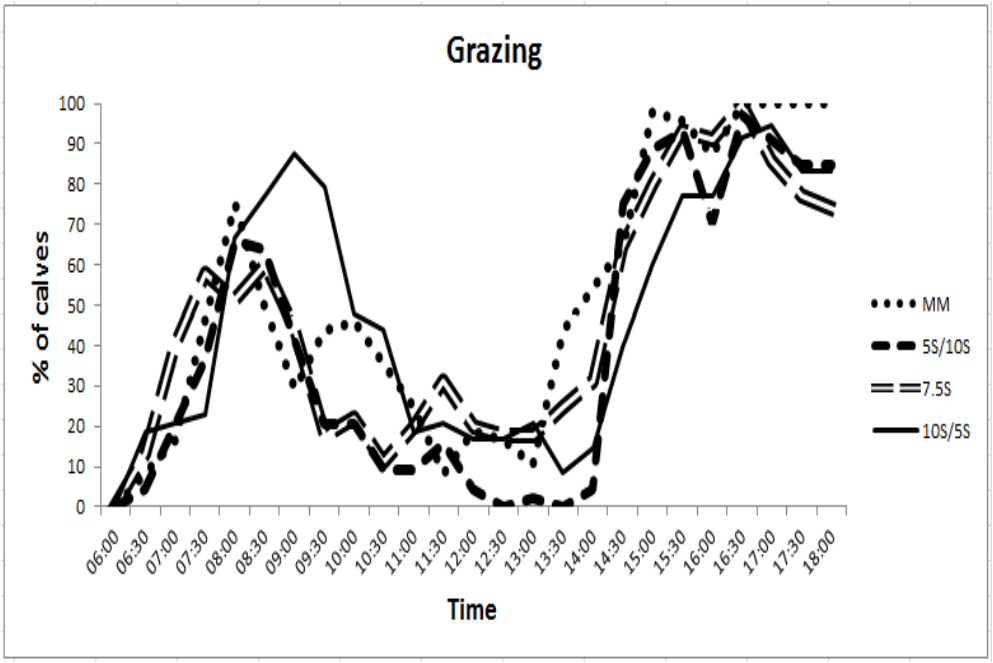
10S/5S


Grazing
% of calves
100
90
80
70
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Time
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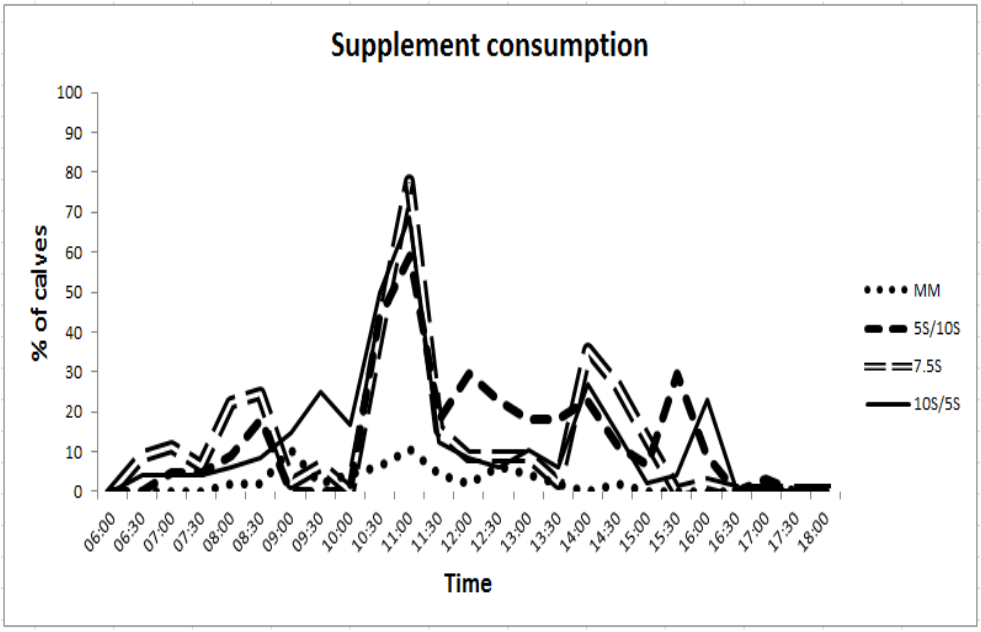
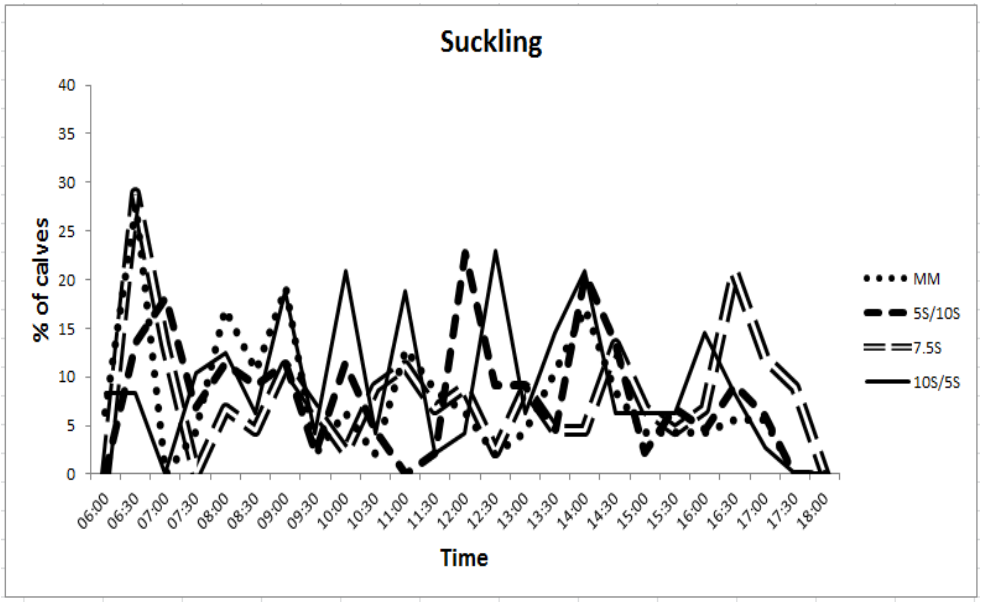
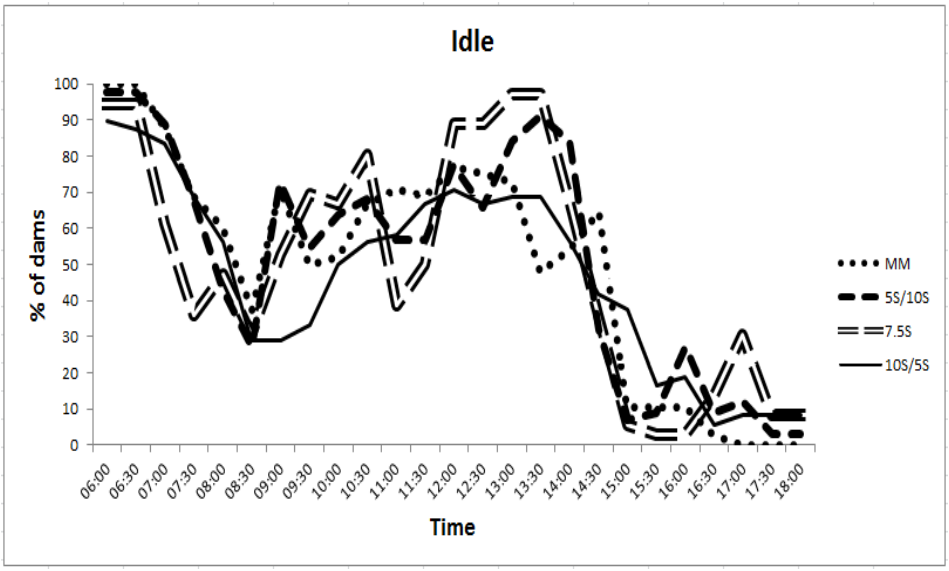
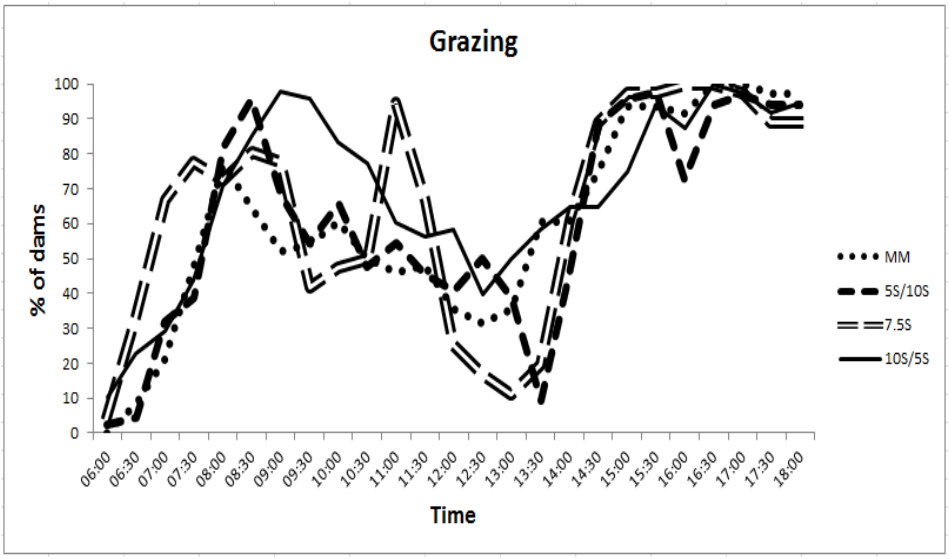


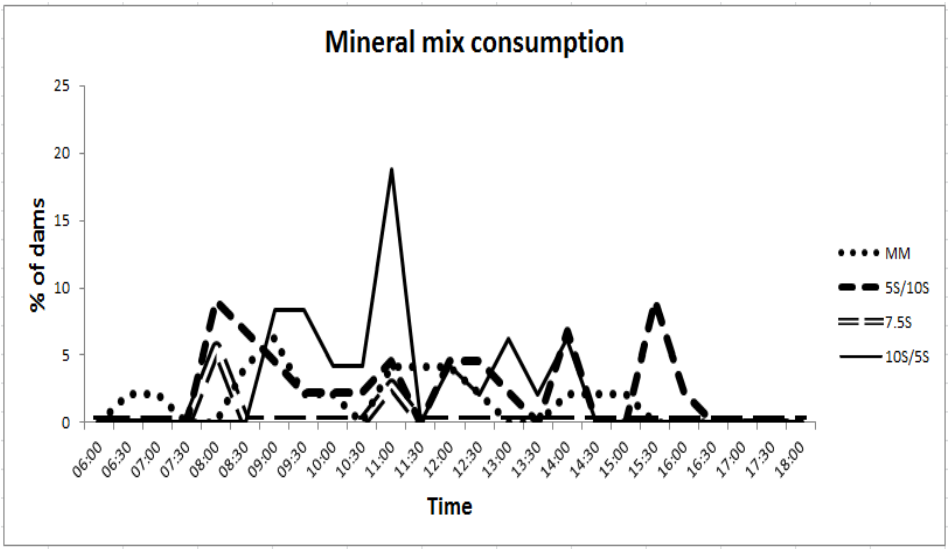


Male calves' behaviour during suckling phase at pasture receiving different supplementation schedule

Figure 2







Lactating dams behaviour with their calves receiving different supplementation schedules