

DANIEL MAGESTE DE ALMEIDA

**EFFECTS OF SUPPLEMENTATION LEVELS ON PERFORMANCE AND
METABOLIC AND NUTRITIONAL CHARACTERISTICS OF COWS,
SUCKLING FEMALE CALVES AND HEIFERS ON GRAZING**

Doctoral thesis submitted to the
Animal Science Graduate Program
of the Universidade Federal de
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requirements for the degree of
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ABSTRACT

ALMEIDA, Daniel Mageste de, D.Sc., Universidade Federal de Viçosa, February, 2017. **Effects of supplementation levels on performance and metabolic and nutritional characteristics of cows, suckling female calves and heifers on grazing.** Adviser: Mário Fonseca Paulino. Co-advisers: Marcos Inácio Marcondes and Luciana Navajas Rennó.

Reproduction is the main limiting factor in meat production efficiency and the low offtake rate observed in the Brazilian herd reflects the low fertility rate of cows and the late age at puberty of replacement heifers. In order to provide more information about nutritional strategies to better develop Nellore heifers and to improve dams' performance, three studies were conducted and the results are here presented in four papers. In the first paper, were used 80 pregnant Nellore cows (6 months gestation) with initial age of six years and mean initial weight and initial body condition score of 515.5 ± 1.34 kg and 4.68 ± 0.15 , respectively. The experimental design was completely randomized, with four treatments and twenty repetitions. The strategies evaluated were supplementation with 1 kg/cow/day of supplementation only in the pre-partum, only in the postpartum, in the pre and postpartum, and only ad libitum mineral mixture during the pre and postpartum. The effect of supplementation on the body weight (BW) of cows at calving and calf weight at birth ($P < 0.05$) was observed. There was also effect of pre and postpartum supplementation ($P < 0.05$) on the final BW, mean daily gain and final body condition score of the animals. There was a trend of postpartum supplementation on milk production from the matrices ($P = 0.065$) and, for its components, there was post-partum supplementation effect only on milk protein (MP) ($P = 0.003$). There was an effect of postpartum supplementation on serum urea nitrogen, glucose, non-esterified fatty acids, β -hydroxybutyrate and progesterone ($P < 0.05$). All metabolites were influenced ($P < 0.05$) by the postpartum day. Cows supplemented postpartum had a higher conception rate than that observed in non-supplemented animals ($P = 0,005$). Supplementation with 1 kg/day supplementation with 28.6% of CP in pre-partum improved some of the productive characteristics, however, postpartum supplementation allows more expressive effects on the productive, metabolic and reproductive efficiency of cows. Therefore, postpartum supplementation is recommended when the animals have adequate body condition score (BCS) at calving. The heifer calves of cows used in the first experiment were used in the

second paper to evaluate supplementary strategies in the creep feeding system. Were used fourty-four Nellore heifer calves, with age and initial mean weight, of four months and 147.6 ± 1.34 kg, respectively. A single supplement with approximately 20% of protein was provided in different amounts depending on body weight (BW). Treatments consisted in the supply of 0.0%, 0.2%, 0.4% or 0.6% of BW from this supplement. Increasing linear effect ($P < 0.05$) of final body weight and average daily gain of heifer calves were observed with increased supplementation. Multiple supplementation increased consumption, in kg/day, of dry matter (DM), organic matter (OM), crude protein (CP), digested dry matter (dDM) and total digestible nutrients (TDN). There was no effect of supplementation ($P > 0.05$) on NEFA concentrations. There was an increasing linear effect ($P < 0.05$) of supplementation on insulin concentrations. It is concluded that supplementation improves the performance, nutritional and metabolic characteristics of the animals, being the supply of 0.6% of BW of supplement with 20% of CP the most effective treatment. The same heifers were used in the third experiment after weaning to evaluate the effects of strategic supplementation for Nellore heifers after weaning to conception. The evaluated strategies were: BAAL- supplementation with 0.2% of BW/animal/day of supplement in the first 90 days and supplementation with 0.6% of BW/animal/day in the subsequent 90 days; MEME - supplementation with 0.4% of BW/animal/day for 180 days; ALBA - supplementation with 0.6% of BW/animal/day of supplement in the first 90 days and supplementation with 0.2% of BW/animal/day in the subsequent 90 days; and MM - only mineral mix ad libitum during the 180 days. It was observed that supplementation improved the performance of the animals during the first 90 days of experiment, and this fact can be verified by the heifers' average daily gain (ADG) ($P = 0.001$). The same fact was observed in the dry/water transition phase, where supplementation improved final body weight (fBW) ($P = 0.002$) and ADG ($P = 0.001$). It was also verified that multiple supplementation increased dry matter (DM), organic matter (OM), crude protein (CP), digestible dry matter (dDM) and total digestible nutrients (TDN) during the whole experiment, and digested neutral detergent fiber (dNDF), and neutral detergent fiber corrected for ash and protein (apNDF) only in the dry/water transition. Supplementation increased the total apparent digestibility coefficient of DM, OM, CP, apNDF and TDN ($P < 0.05$), that is, of all analyzed parameters. Serum urea nitrogen (SUN), glucose (GLUC), insulin (INS) and progesterone (PROG)

levels were higher in supplemented heifers than in non-supplemented heifers ($P < 0.05$). On the other hand, supplementation reduced the concentrations of non-esterified fatty acids (NEFA) ($P = 0.001$). Finally, it was found that the conception rate was higher for supplemented heifers ($P = 0.020$). It was concluded that the best levels of SUN, INS, GLUC, NEFA and PROG of the supplemented heifers associated to the higher fBW, digestibility and consumption of the diet components provided better reproductive performance for the supplemented heifers, independently of the supplementation.

RESUMO

ALMEIDA, Daniel Mageste de, D.Sc., Universidade Federal de Viçosa, fevereiro de 2017. **Efeitos dos níveis de suplementação sobre o desempenho e características metabólicas e nutricionais de vacas, bezerras lactentes e novilhas em pastejo.** Orientador: Mário Fonseca Paulino. Coorientadores: Marcos Inácio Marcondes e Luciana Navajas Rennó.

A reprodução é o principal fator limitante na eficiência da produção de carne, e a baixa taxa de concepção observada no rebanho brasileiro reflete a baixa taxa de fertilidade das vacas e a idade tardia à puberdade das novilhas de substituição. A fim de fornecer mais informações sobre as estratégias nutricionais para o melhor desenvolvimento das novilhas Nelore e melhorar o desempenho das vacas, foram realizados três estudos e os resultados são apresentados em quatro artigos. No primeiro trabalho, foram utilizadas 80 vacas Nelore grávidas (6 meses de gestação) com idade inicial de seis anos e peso inicial médio e escore de condição corporal inicial de $515,5 \pm 1,34$ kg e $4,68 \pm 0,15$, respectivamente. O delineamento experimental foi inteiramente casualizado, com quatro tratamentos e vinte repetições. As estratégias avaliadas foram suplementação com 1 kg/vaca/dia de suplementação somente no pré-parto, somente no pós-parto, no pré e pós-parto, e somente mistura mineral ad libitum durante o pré e pós-parto. Observou-se o efeito da suplementação sobre o peso corporal (PC) das vacas no parto e sobre o peso dos bezerras ao nascimento ($P < 0,05$). Houve também efeito da suplementação no pré e pós-parto ($P < 0,05$) no PC final, ganho médio diário e escore de condição corporal final dos animais. Houve uma tendência, com a suplementação no pós-parto, de maior produção de leite das vacas ($P = 0,065$) e, para os seus componentes, houve efeito de suplementação no pós-parto apenas para proteína do leite (PL) ($P = 0,003$). Houve um efeito da suplementação no pós-parto sobre nitrogênio ureico sérico, glicose, ácidos graxos não esterificados, β -hidroxibutirato e progesterona ($P < 0,05$). Todos os metabólitos foram influenciados ($P < 0,05$) no dia pós-parto. As vacas suplementadas no pós-parto apresentaram maior taxa de concepção do que a observada em animais não suplementados ($P = 0,005$). A suplementação com 1 kg/dia de suplementação com 28,6% de PC no pré-parto melhorou algumas das características produtivas, no entanto, a suplementação no pós-parto permite efeitos mais expressivos sobre a eficiência produtiva, metabólica e reprodutiva das vacas. Portanto, a suplementação no pós-parto é recomendada quando os animais têm escore de condição corporal

(ECC) adequada ao parto. As bezerras das vacas do primeiro experimento foram utilizadas no segundo trabalho para avaliar estratégias de suplementação no sistema creep-feeding. Utilizaram-se quarenta e quatro bezerras Nelore, com idade e peso médio inicial, de quatro meses e $147,6 \pm 1,34$ kg, respectivamente. Um único suplemento com aproximadamente 20% de proteína foi fornecido em quantidades diferentes dependendo do peso corporal (PC). Os tratamentos consistiram no suprimento de 0,0%, 0,2%, 0,4% ou 0,6% do PC deste suplemento. Observou-se aumento do efeito linear ($P < 0,05$) do peso corporal final e ganho médio diário de novilhas com aumento da suplementação. A suplementação múltipla aumentou o consumo, em kg/dia, de matéria seca (MS), matéria orgânica (MO), proteína bruta (PB), matéria seca digerida (MSD) e nutrientes digestíveis totais (NDT). Não houve efeito da suplementação ($P > 0,05$) nas concentrações de AGNE. Houve um efeito linear crescente ($P < 0,05$) de suplementação sobre as concentrações de insulina. Conclui-se que a suplementação melhora o desempenho, as características nutricionais e metabólicas dos animais, sendo o suprimento de 0,6% do PC de suplemento com 20% de PB o tratamento mais efetivo. As mesmas novilhas foram utilizadas no terceiro experimento após o desmame para avaliar os efeitos da suplementação estratégica de novilhas Nelore após o desmame à concepção. As estratégias avaliadas foram: BAAL - suplementação com 0,2% de PC/animal/dia de suplementação nos primeiros 90 dias e suplementação com 0,6% de PC/animal/dia nos 90 dias subsequentes; MEME - suplementação com 0,4% de PC/animal/dia durante 180 dias; ALBA - suplementação com 0,6% de PC/animal/dia de suplementação nos primeiros 90 dias e suplementação com 0,2% de PC/animal/dia nos 90 dias seguintes; e MM - apenas mistura mineral *ad libitum* durante os 180 dias. Observou-se que a suplementação melhorou o desempenho dos animais durante os primeiros 90 dias de experimento, o que pode ser verificado pelo ganho médio diário (GMD) das fêmeas ($P = 0,001$). O mesmo fato foi observado na fase de transição seca/água, onde a suplementação melhorou o peso corporal final (PCf) ($P = 0,002$) e GMD ($P = 0,001$). Verificou-se também que a suplementação múltipla aumentou o consumo de matéria seca (MS), matéria orgânica (MO), proteína bruta (PB), matéria seca digestível (MSD) e nutrientes digestíveis totais (NDT) durante todo o experimento e fibra em detergente neutro digestível (FDNd) e fibra em detergente neutro corrigida para cinzas e proteínas (FDNcp) apenas na transição seca/água. A suplementação aumentou o coeficiente de digestibilidade aparente de MS, MO, PB,

FDNcp e NDT ($P < 0,05$), ou seja, de todos os parâmetros analisados. Os níveis de nitrogênio ureico sérico (NUS), glicose (GLIC), insulina (INS) e progesterona (PROG) foram maiores em novilhas suplementadas do que em novilhas não suplementadas ($P < 0,05$). Por outro lado, a suplementação reduziu as concentrações de ácidos graxos não-esterificados (AGNE) ($P = 0,001$). Finalmente, foi observado que a taxa de concepção foi maior em novilhas suplementadas ($P = 0,002$). Concluiu-se que os melhores níveis de NUS, INS, GLIC, AGNE e PROG das novilhas suplementadas associadas ao maior PCf, digestibilidade e consumo dos componentes da dieta proporcionaram melhor desempenho reprodutivo para as novilhas suplementadas, independentemente da suplementação.

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GENERAL INTRODUCTION

Reproduction is the main limiting factor in meat production efficiency. According to Coulter et al. (1994), this is ten times more important than breeding and five times more important than carcass improvement. Therefore, nutritional management should be adequate, jointly attending the energy demands for production and reproduction, so that the animals present a favorable body condition for the initiation of ovarian activity.

To improve the biological efficiency of the herd, it is necessary for heifers to reach puberty and mating earlier, and the importance of this characteristic increases as the production system becomes more intensive and competitive (Menegaz et al., 2008). The search for increased productive and reproductive efficiency in herds of beef cattle has been the objective of recent research. Higher fertility rates and sexually precocious herds have higher numbers of animals and, consequently, higher rates of enjoyment. In addition, they may be subjected to higher levels of selection intensity, resulting in greater genetic progress (Martín Nieto et al., 2003).

The sooner the female produces a calf and it is slaughtered, the greater the rate of enjoyment of the herd. In Brazil, the age of zebu heifers at first calf is close to 4 years. According to Fries (1991), this system leads to a rate of enjoyment of the herd at levels lower than 20%, due to the maintenance of several harvests of rearing females. This low performance is a reflection of low fertility rates and also of late age at puberty of replacement heifers.

Even though the knowledge that the matrices represent the basis of the system of production of beef cattle, this category is still neglected by the majority of cattle ranchers, being often allocated in pastures of worse quality, since they do not represent an immediate form of capital production, as animals in termination for example. When a good nutritional condition is provided for such an animal category, interesting results can be obtained, either in productive or reproductive response.

Working with beef cows with high and low body condition scores (BCS), Bohnert et al. (2013) concluded that high BCS animals had calves with higher calf weight and with lower mortality rate, as well as higher pregnancy rate when compared to animals with low BCS.

The BCS evaluation is a practical way to determine what is occurring in animal metabolism, but to be sure, it is of great importance to evaluate other

parameters, such as measurement of non-esterified fatty acids (NEFA), which are derived from the mobilization of adipose tissue, which occurs mainly in negative energy balance (NEB) conditions. NEFA are considered good indicators of the intensity of this energy balance (Caldeira, 2005), reflecting the nutritional condition in which the animal is found. The metabolism of these fatty acids in plasma is dynamic, since these compounds are readily available sources of metabolic fuel (Palmquist & Mattos, 2011).

The quantification of ketone bodies can also corroborate for a more accurate evaluation of the metabolic condition of the animal. In contrast to non-ruminants, serum / plasma levels of ketone bodies are relatively constant in ruminants, due to their origin from the volatile fatty acids (VFAs) produced in the fermentation of carbohydrates in the rumen (Fernandes et al., 2012). Butyrate (the main precursor of ketogenesis) undergoes a series of oxidation reactions catalyzed by specific enzymes in the rumen epithelial cells, where it is converted to β -hydroxybutyrate (BHB) and released into plasma (Bruss, 2008). The production of ketone bodies can also occur in the liver through long chain fatty acids from the mobilization of fat reserves, which are converted into acetoacetate and then into BHB, which can be used as energy source and also in the synthesis of fat in milk (Wittwer, 2000).

Beef cows also go through the so-called NEB after calving, which contributes to the manifestation of endogenous signals that inhibit reproduction (Beam & Butler, 1999), and one of these signals is the high concentration of BHB. In dairy cows the decrease in BHB concentration was associated with an increase in the pregnancy rate after the first insemination (Walsh et al., 2007) and a decrease in the interval from calving to first ovulation (Reist et al., 2000). Mulliniks et al. (2013) concluded that the study of BHB in the serum of beef cows when done between calving and conception was a good indicator of energy status and correlated with the competence to design a new calf.

When working with beef cows the ultimate objective is that this animal be reproductively successful, that is, restart its estrous cycle as soon as possible after calving. To perform such evaluation a hormone can be used, progesterone. Progesterone is a steroid hormone produced by the ovarian corpus luteum, and its presence at plasma levels above 1ng/mL (Nogueira et al., 2011) represents that the bovine female has already left the anestrous and started a new reproductive cycle after calving.

Exemplified the importance and consequences of a good nutritional condition of beef matrices, it is understood that this should be advocated. The use of a mound season is a practice that meets this interest, because when using it we allow the calves to be concentrated at the beginning of the rainy season, when the pastures begin to grow, allowing an adequate supply of potentially digestible dry matter (pdDM). However, the interval between calving and the next conception should not exceed 75 days (considering a 12-month calving interval), which is inappropriate for BCS increase in a matrix consuming only pasture and having to produce milk to feed its offspring newborn. Therefore, it is important to ensure the adequate supply of nutrients so that the cows can reach conditions to conceive again, and this can be achieved through the provision of multiple supplementation.

By providing multiple supplements, have the objective to provide nutrients and still allow greater utilization of the basal resource, which is the pasture. In the case of matrices, it is not yet clear which is the most appropriate period for supplementation, whether before, before and after or just after calving. If supplied immediately before, this will occur in the dry season of the year, a phase considered strategic due to less availability of quantity and quality of the forage. However, this is the phase in which the highest fetal development occurs, about 75% in the final third of gestation, and the idea that the input of nutrients at this stage would be mainly directed towards fetal growth, little influencing the maternal metabolic state. 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).

By providing multiple supplements to grazing beef cows in pre- and/or postpartum, these animals are expected to have a better productive and reproductive performance, which can be measured by assessing the metabolites already mentioned, the BCS assessment, and also by weighing of these animals.

The age at which first coverage and conception occur are primary determinants of heifer productivity, and age at puberty is the main factor determining the female's competence in her first mating season. The age at which heifers reach puberty varies, depending on numerous factors including body weight, nutrition and management (Day, Gasser, Grum & Pires, 2010).

The supplementation of the heifers in creep-feeding is intended not to reduce the growth momentum of calves, which normally occurs after two months of age due

to the drop in the mother's milk production. Several studies have reported a reduction in puberty age with greater preweaning body weight gain (Patterson et al., 1992; Buskirk et al., 1995; Arije and Wiltbank, 1971; Greer et al., 1983; Patterson et al., 1991).

The increase in dietary intake anticipates age at puberty of heifers (Yelich et al., 1996; Shamay et al., 2005), in addition to resulting in increased luteinizing hormone (LH) concentrations, increased insulin growth factor (IGF-1) concentrations (Granger et al. (Greer et al., 1983), increased body condition score (Short and Bellows, 1971; Simpson et al., 1998), improved pregnancy rates (Short and Bellows, 1971), in addition to decreasing age at first conception (Simpson et al., 1998).

Among the characteristics associated with reproductive efficiency in beef cattle, age at first calving (AFC) is one of the easiest to measure (Martins Filho et al., 1994; Pádua et al., 1994; Lôbo, 1998; Ferraz e Eler, 2000; Mercadante et al., 2000; Lôbo et al., 2000; Garnerio et al., 2001). According to Pereira et al. (1991), the AFC is a reflection of age at puberty, which in turn is related to the growth rate of the female.

The time that animals take to reach puberty also influences the cost of the breeding system. The development of puberty is delayed when growth is restricted in mammals. This observation is also associated with body weight, growth rate, percentage of fat and body protein. The priority for nutrients and energy varies between the organs and the physiological state of the animal. The reproductive system has a low priority, while the nervous system has a high priority during growth, for example. Therefore, a nutrient deficient diet will harm the organs and tissues of low priority.

The effects of nutrition on reproduction of beef female cattle are not mediated by a single nutrient, metabolite or hormone. Therefore, infertility observed in a herd may be due to one or more nutritional factors and these multiple factors must be considered in order to have a better understanding of this interaction.

The nutritional status of the animals has positive and negative reproductive effects, mediated directly by dietary nutrients or indirectly via the endocrine system, which acts primarily on the hypothalamic-pituitary-ovarian axis.

Follicular growth, ovulation and luteal function are regulated by the hypothalamic-pituitary-ovarian axis and, by a feedback mechanism, the ovarian hormones control gonadotrophic secretion. According to McDonald

(1989), gonadotrophin releasing hormone (GnRH) is a high molecular weight glycoprotein hormone produced in the hypothalamus under stimuli that reach the central nervous system. The pulsating release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) is controlled by the action of GnRH on the pituitary gland and, by feedback, according to serum levels of ovarian hormones. These, estrogen and progesterone, are produced from cholesterol in response to the action of gonadotrophins. Estrogens, responsible for differentiating the dominant follicle, are produced by internal theca cells and granulosa cells of the ovarian follicle. Nutritional deficits result in inadequate GnRH secretion and consequent decrease in follicular development and inhibition of ovulation rate.

Nutrition may alter the level and duration of FSH-dependent gonadotrophin follicle exposure. The effect of nutrition on circulating FSH concentration suggests that deficiency of nutrients such as glucose, amino acids and metabolic hormones such as insulin, growth hormone, IGF-1, and proteins compromise activity reducing the production of estrogen and FSH required for pulsatile release of GnRH to be maintained.

Insulin is an important marker of nutritional effects on follicular dynamics in cattle (Webb et al., 2004) and, combined with glucose, can stimulate the release of GnRH through the hypothalamus. The binding of insulin to its receptor results in a number of metabolic effects, the most important being the stimulation of glucose transport into the cells, used as the main energetic source for the ovary (Rabiee et al., 1997; Al., 2007). In the ovaries, insulin may also stimulate cell proliferation and steroidogenesis (Wettemann and Bosis, 2000) and, in the liver, the production of IGF-1 (Webb et al., 2004).

The occurrence of puberty in the female is the final apogee of a series of events, which result in an ovulation accompanied by estrus and normal luteal function (Moran et al., 1989). Puberty, however, is often immediately preceded by short estrus cycles, which may or may not be accompanied by ovulation or estrus (Kinder et al., 1995), but usually result in short periods of high progesterone of variable duration (Berardinelli et al., 1979, 1980). Regression of the short-lived luteal structure is followed by an increase in LH and estradiol secretion and is terminated by a pre-ovulatory LH peak.

Smith et al. (1976) reported that age and body weight at puberty have a positive correlation, considering their influence on puberty. In general, it has been

recommended that heifers reach approximately 65% of their estimated adult body weight at the start of their first mating season (Fox et al., 1988; Patterson et al., 2000). The zebu herd is intensely selected for sexual precocity.

The mating system for females aged fourteen to fifteen months assumes the use of accelerated and rapid growth at the expense of the development of the bony and muscular tissues that occurs from birth to puberty, activated by the release of growth hormones. For this to be feasible, nutritional management should enable continuous growth, with magnitude around 600g/animal/day (Paulino et al., 2000). It has been shown that increasing the rate of weight gain in heifers during the post-weaning period anticipates puberty (Buskirk et al., 1995; Hall et al., 1995, Lammers et al., 1999; Quintans et al., 2004) , As well as increasing conception rates in the first coverage (Fleke et al., 1980; Buskirk et al., 1995). Thus, pasture supplementation is a decisive factor in improving the reproductive rate of a herd at higher stocking rates (Olson, 2005).

With genetic potential, as weight and weight gain ensure sexual maturity, females exhibit better physiological conditions to demonstrate estrus and reproduce.

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CHAPTER 1

PRE AND POSTPARTUM SUPPLEMENTATION STRATEGIES FOR NELLORE COWS

ABSTRACT - The objective of this work was to evaluate the effect of strategic supplementation 90 days before calving (pre-partum) and 90 days after calving (postpartum) on productive and reproductive efficiency, hormonal and metabolite parameters in zebu beef cows in *Uruchloa decumbens* pastures. Were used 80 pregnant Nellore cows (6 months gestation) with initial age of six years and mean initial weight and initial body condition score of 515.5 ± 1.34 kg and 4.68 ± 0.15 , respectively. The experimental design was completely randomized, with four treatments and twenty repetitions. The four strategies evaluated were supplementation with 1 kg/cow/day of supplementation only in the pre-partum, only in the postpartum, in the pre and postpartum, and *ad libitum* mineral mixture during the pre and postpartum. The effect of supplementation on the body weight (BW) of cows at calving and calf weight at birth ($P < 0.05$) was observed. There was also effect of pre and postpartum supplementation ($P < 0.05$) on the final BW, mean daily gain and final body condition score of the animals. There was a trend of postpartum supplementation on milk production from the matrices ($P = 0.065$) and, for its components, there was post-partum supplementation effect only on milk protein (MP) ($P < 0.05$) and trend of the interaction on milk fat (MF) ($P = 0.057$) and total solids (TS) ($P = 0.074$). There was an effect of postpartum supplementation on serum urea nitrogen, glucose, non-esterified fatty acids, β -hydroxybutyrate and progesterone ($P < 0.05$). All metabolites were influenced ($P < 0.05$) by the postpartum day. Cows supplemented postpartum had a higher conception rate than that observed in non-supplemented animals ($P < 0.05$). Supplementation with 1 kg/day supplementation with 28.6% of CP in pre-partum improved some of the productive characteristics, however, postpartum supplementation allows more expressive effects on the productive, metabolic and reproductive efficiency of cows. Therefore, postpartum supplementation is recommended when the animals have adequate body condition score (BCS) at calving.

Key words: Postpartum, Pre-partum, Supplementation

1. INTRODUCTION

The low number of calves weaned from the number of mated cows is a bottleneck in the beef cattle breeding sector. Currently, the breeding sector produces about 50 million calves a year in Brazil, but we have 70 million cows (ANUALPEC, 2014). However, the theoretical objective for satisfactory production would be to produce and wean one calf per cow per year (Gottschall, et al., 2008).

The main factor that influences the fertility of these cows is nutrition, through the provision of specific nutrients necessary in the process of ovulation, fertilization, embryonic survival and development. The reproductive performance of beef cows is directly associated with productive parameters, such as body weight and BCS (Selk et al., 1988; Morrison et al., 1999). Body weight gain and BCS are necessary for the resumption of the estrous cycle after nutritionally induced anestrus (Richards et al., 1989). In addition, indirectly, nutrition has an impact on the circulation of hormones and metabolites that are needed in these processes (Robinson et al., 2006). It is estimated that 50% of beef cows in extensive systems do not receive adequate nutritional management (Madureira et al., 2014).

Thus, protein-energy supplementation is often beneficial, if not essential, for cows with calves on pasture, particularly those grazing tropical and subtropical forages (Mass, 1987; Moore et al., 1991). It has been demonstrated in beef cows that increased energy intake reduces postpartum anestrus (Roberts et al., 1997). In addition, inadequate energy intake is associated with delayed onset of puberty, prolonged postpartum intervals, and decreased conception rate (Santos and Amstalden, 1998; Loooper et al., 2003; Pescara et al., 2010).

Studies have shown different results on when is the best time to supplementation bovine females. Some authors have reported the benefits on production characteristic and conception rate of pre-partum supplementation (Graham et al., 2001; Bellows et al., 2001; Alexander et al., 2002; Small et al., 2004; Funston et al., 2008), while others observed in the postpartum (Williams et al., 1989; De Fries et al., 1998; Hawkins et al., 2000; Webb et al., 2001; Bottger et al., 2002; Funston, 2004). However, most of these studies were performed evaluating supplementation in only one phase (pre or postpartum), with the exception of studies evaluating the two phases sequentially.

This study was based on three hypotheses: there is an effect of the interaction between preand postpartum supplementation on improvement in the productive, reproductive and metabolic characteristics; postpartum supplementation improves milk production and animal performance; and pre-partum supplementation improves productive and reproductive traits in Nellore cows. The objectives of this study were to explore the best phase for supplementation (pre-partum, postpartum or pre and postpartum) in Zebu beef cows and to investigate how to improve the status of hormones (mainly progesterone), metabolites (glucose, β -hydroxybutyrate and non-esterified fatty acids) in reproductive performance.

2. MATERIAL AND METHODS

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV 08/2015).

Animals, experimental design and supplements

The experiment was conducted at the Universidade Federal de Viçosa, located in the municipality of Viçosa-MG (20°45'S and 42°52' W) between July and December, referring to the period of dry and transition dry-water. The experimental area is located in mountainous region, with 670 m of altitude, and presents annual precipitation of 1300 mm. An experimental area of 70 hectares was assigned to the animals, consisting of four 17.5 ha paddocks, covered uniformly with the *Uruchloa decumbens* grass, equipped with drinking fountains and troughs, which are covered and accessible from both sides.

The experiment lasted 180 days, being 90 days before calving (pre-partum) and 90 days after calving (postpartum).

Were used 80 pregnant Nellore cows (six months of gestation), with initial age of six years and mean initial weight and initial body condition score of 515.5 ± 1.34 kg and 4.68 ± 0.15 , respectively.

The experimental design was the completely randomized in factorial scheme, with four treatments, and twenty repetitions. The strategies evaluated were: PRMM - supplementation with 1 kg/cow/day supplement only in pre-partum; MMPS - supplementation with 1 kg/cow/day supplement only in the postpartum period; PRPS - supplementation with 1 kg/cow/day supplement before and after delivery; MM -ad

libitum mineral mixture during pre and postpartum. 1 kg/cow/day of a single supplement (Table 1), was provided through the food composition data provided by BR-CORTE 2.0 (Valadares et al., 2010), with 28.6% protein (supplementary centesimal composition: ground corn grain, 24.65; ground sorghum grain, 24.65, soybean meal, 45.7, mineral mixture, 5). The centesimal composition of the mineral mixture: dicalcium phosphate, 50.00; sodium chloride, 47.2; zinc sulfate, 1.50; copper sulfate, 0.70; cobalt sulfate, 0.05; potassium iodate, 0.05; and manganese sulphate, 0.5.

The supplement was provided daily at 10 am. The animals were rotated between the pickets every 7 days, aiming to control the possible effects of pickets on the treatments (pasture availability, water and trough location, relief, shading and others).

Experimental procedures and sampling

Cows were weighed at beginning, five days pre-partum, five days postpartum, and at the end of the experiment for performance evaluation. Similarly, suckling calves were weighed at birth and at the end of the experiment. All weighings were performed at 7:00 a.m.

The cow body condition score was evaluated at the beginning, at delivery and at the end of the experiment. The scoring scale from 1 to 9 was used, as recommended by the NRC (1996). The final score was obtained by means of the average of four duly trained evaluators and the assignment of the scores occurred independently among the evaluators.

The average cow milk yield (MY) was evaluated by sampling the production at 45 days postpartum. The cows were separated from their offspring at 5:30 p.m. and remained on the picket, being milked at 5:30 a.m. and 5:30 p.m. on the following day. Milking was performed mechanically by a trained employee. The milk secretion was stimulated with 2 mL oxytocin (10 IU/mL, Ocitovet®, Brazil) in the mammary artery, initiating milking immediately after oxytocin administration. Samples were collected for analysis of milk protein (MP), milk fat (MF), milk lactose (ML), total solids (TS) and non - greasy solids (NGS) after homogenization in the bucket. Samples were preserved with the use of one bronopol tablet per sample.

Forage samples were randomly taken, every 30 days, in order to evaluate the forage mass per hectare. In each plot, six forage samples were randomly selected by

using a metal square (0.5 x 0.5 m) and cut at approximately 1 cm above the soil. After that, forage subsamples (200 g) were dried at 60°C for 72 hours and ground to pass through a 1 and 2-mm screen.

Every fifteen days, a manual grazing simulation was performed simultaneously to the observation of grazing behavior of the animals in order to obtain samples to evaluate chemical composition of the forage consumed by the animals. All samples were dried at 60°C for 72 hours, grounded to pass through 1 and 2-mm screen, and proportionally sub-sampled to a composite sample per period.

Blood samples were collected 30, 45, 60 and 75 days postpartum. At 7:00 a.m., the jugular vein was punctured using vacuum tubes with clot accelerator and separator gel (BD Vacuntainer® SSTIIAdvance®, São Paulo, Brazil). After collection, the blood was centrifuged at 3600×g for 20 minutes and stored at -20°C. Subsequently, the levels of serum urea nitrogen (SUN), glucose (GLUC), non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) were quantified. In addition, progesterone concentrations (PROG) were measured at days 30, 45 and 60 postpartum.

On the 80th postpartum day, the cows were synchronized using the protocol: in d 0, an intravaginal progesterone release device (Tecnopec Primer®, São Paulo, Brazil) was inserted and was applied 2.0 mg of Estradiol benzoate (Tecnopec RIC-BE®, São Paulo, Brazil). At d 7, the intravaginal device was removed and the cows received an injection of 2 mL of sodium cloprosterol (MSD Saúde Ciosin® Animal, São Paulo, Brazil). Finally, on d 8, cows received 0.5 mL of estradiol cypionate (Zoetis-Pfizer E.C.P., Campinas, Brazil). Fixed-time artificial insemination (AIrt) was performed 46 to 52 h after removal of the intravaginal device (d 10). Semen doses of five Nellore bulls were randomly assigned to each cow. The diagnosis of gestation was determined by transrectal ultrasonography 30 days after the AIrt. The conception rate was calculated considering the cows that conceived in the AIrt.

Chemical analysis

Samples of forage and supplement were analyzed following procedures described by Detmann et al. (2012) for dry matter (DM; method INCT-CA G-003/1), crude protein (CP; method INCT-CA N-001/1), ether extract (EE; method INCT-CA G-004/1), ash (method INCT-CA M-001/1), neutral detergent insoluble nitrogen (NDIN; method INCT-CA N-004/1), neutral detergent insoluble fiber (apNDF;

method INCT-CA F-002/1) corrected for ash residue (method INCT-CA M-002/1) and residual nitrogen compounds (method INCT-CA N-004/1). The indigestible neutral detergent insoluble fiber (iNDF; method INCT-CA F-009/1) was evaluated using F57 (Ankon®) bags incubated in rumen by 288 h. Milk was analyzed for protein, fat, lactose, and total solids content, using spectroscopy (Foss MilkoScan FT120, Hillerød, Denmark).

The non-fibrous carbohydrates of the supplements were estimated according to the recommendations of Hall (2000), using the following equation: $NFC = 100 - (\% CP + \% \text{apNDF} + \% EE + \% \text{ash})$. The composition of the supplement and the forage obtained is shown in Table 1.

The pdDM was estimated according to the following equation (Paulino et al., 2008): $\text{pdDM} = 0.98 \times (100 - \text{NDF}) + (\text{NDF} - \text{iNDF})$, where: NDF = neutral detergent fiber (%); iNDF = indigestible neutral detergent fiber (%); pdDM = potentially digestible dry matter (%); 0.98 = true digestibility of the cell contents.

Serum concentrations of urea (K056) e glucose (K082) were measured using kits from Bioclin Diagnostics (Belo Horizonte, Brazil). Serum concentrations of NEFA was quantified by a colorimetric method (FA115, Randox Laboratories Ltd., São Paulo, Brazil) and BHB was evaluated by a kinetic enzymatic method based on oxidation of D-3-hydroxybutyrate to acetoacetate (RB1007; Randox Laboratories Ltd., Antrim, UK). An automatic biochemical apparatus (Mindray BS-200E; Shenzhen Mindray Bio-Medical Electronics Co. Ltd.) was used for all analyses. Serum urea nitrogen was estimated as 46.67% of the total serum urea.

Progesterone was analyzed by chemiluminescence using Access Progesterone Reagent (Ref. Number 33550), by Beckman Coulter®, (Brea, USA) in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA).

Statistical analyzes

Data were analyzed with analysis of variance (ANOVA), adopting the initial body weight as a co-variable when significant. A 2x2 factorial scheme was used, with or without supplementation, in the pre- and postpartum period. The PROC MIXED procedure of the SAS software (Statistical Analysis System, version 9.4) was used for all statistical analyzes and when more than one measurement was taken in the same animal (SUN, GLUC, NEFA, BHB and PROG), postpartum days when the measures were taken, were considered measures repeated over time. For all

statistical procedures, except for the conception rate, the Tukey test was used, using $\alpha = 0.05$ as the critical level of probability of Type I Error. The degrees of freedom of the denominator were calculated using the Kenward-Roger approximation.

A chi-square test was used for the conception rate data. The averages were tested by orthogonal contrasts, and the contrasts were: c1- inclusion of supplement in the pre, c2-inclusion of supplement in the post; and c3- interaction of pre and postpartum supplementation. For the critical level of probability for type I error, $\alpha = 0.05$ was used.

3. RESULTS

The mean availability of total dry matter (TDM) of the *Uruchloa decumbens* forage was 4933 and 4012 kg/ha before and after calving, and the forage pdDM was 3145 and 3109 kg/ha before and after calving, respectively. The *Uruchloa decumbens* forage, obtained by manual grazing simulation, had a mean content of 71.0 and 98.0 g of CP/kg DM before and after calving (Table 1), respectively.

Supplementation increased body weight (BW), BCS at calving, and also influenced the higher calf birth weight (Table 2) ($P < 0.05$).

There was no interaction effect in all the studied variables, so the effects of pre and postpartum supplementation were analyzed separately ($P > 0.05$). Pre or postpartum supplementation increased the final BW, final average daily gain (ADG), and final BCS of the cows ($P < 0.05$) (Table 3).

There was a tendency of post-partum supplementation to increase the milk yield (MY) of the cows (Table 3, $P = 0.065$). In the milk components, there was an effect of postpartum supplementation on an increase in crude protein ($P < 0.05$) and a trend between pre and postpartum supplementation on MF ($P = 0.057$) and TS ($P = 0.074$). The mean MY at 45 days postpartum was 7.97 kg/day, with 3.06% MP, 4.66% MF, 4.54% L, 13.33% TS and 8.67% NGS. Postpartum supplementation increased the ADG of calves at 90 days of age ($P < 0.05$).

All metabolites were influenced ($P < 0.05$) by the postpartum day (Table 4). There was an effect of postpartum supplementation on the increase on SUN, GLUC and PROG concentrations, and on the reduction in BHB and NEFA concentrations ($P < 0.05$). There was an effect of postpartum day interaction with treatments on BHB, NEFA and PROG concentrations ($P < 0.05$). Pre-partum supplementation reduced postpartum NEFA concentrations ($P < 0.05$).

Cows supplemented postpartum had a conception rate of 84.6% after the AIrt protocol (Table 4), higher than the 56.6% observed in non-supplemented animals ($P<0.05$).

4. DISCUSSION

Paulino et al. (2008), in order to associate production per animal and per area, suggested a supply between 4 and 5% of BW in pdDM (between 40 and 50 g of pdDM/kg of BW) of pasture for satisfactory animal performance of the animals under grazing conditions. In this study, the mean weight of pdDM was 91.7 and 75.6 g/kg BW in pre and postpartum, respectively, values above that recommended by Paulino et al., (2008), demonstrating that the amount of forage did not compromise animal performance. The percentage of CP of forage (Table 1), in the pre and postpartum period, was above the minimum value of 7% CP in the basal diet, reported by Lazzarini et al. (2009) as necessary for adequate utilization of the neutral detergent fiber (NDF) of basal forage, which is the main source of energy for grass-fed animals. However, these values are below the 10% reported by Sampaio et al. (2009) as a level that optimizes the use of energetic substrates of forage, which justifies pre and postpartum supplementation with nitrogen compounds to optimize forage utilization and, consequently, animal performance.

It is believed that body energy reserves at calving are the most important factors in the calving-conception interval in beef cows (Wettemann et al., 2002). In this study, the BCS improvement of pre-partum supplemented animals allowed the animals supplemented to have a mean BCS of 5.11 (Table 2). According to Looper et al. (1997) and Lents et al. (2000), cows with BCS of five or higher at calving reduced the number of calving days at first estrus and ovulation between 15 and 35% in relation to cows born with a BCS less than 5.

On the other hand, the cows that were not supplemented in the pre-partum period, due to high availability of pdDM, did not present a BCS (4.28) characteristic of lean animals (Table 2). This variation in BCS between supplemented and non-supplemented cows most likely did not affect pre-partum supplementation on milk production and its components, average daily gain of calves (caADG) (Table 3) and conception rate (Table 4). According to Wettemann et al. (2002), very lean cows at calving do not have satisfactory reproductive performance and, even if they gain a lot

of BW after calving, they have a longer postpartum anestrus time and a lower reproductive performance than cows with good body condition at calving.

The use of strategic supplementation in the pre-partum period allowed an improvement in the performance of the cows (Table 2), in which the BW ranged from 499.1 kg in the non-supplemented cows to 527.9 kg in the supplemented cows. This difference was most likely due to the higher consumption of nitrogen compounds by supplemented cows, which led to the optimization of the energy:protein adequacy in the diet and to the greater use of latent forage energy (Paulino et al., 2004).

In the same perspective, the increase in the nutritional level of the cows and the greater partition of nutrients in response to supplementation in the final third of gestation (Astestiano et al., 2012; Winterholler et al., 2012) may have resulted in the increase of calf birth weight by approximately 4 kg (Table 2). Previous studies with cattle (Loerch, 1996; Radunz et al., 2010) have already provided evidence that supplementation systems during the last third of gestation could alter the subsequent birth weight of the progeny (Radunz et al., 2011). This higher birth weight is crucial for tolerance to stress caused by cold, resistance to pathological agents and ability to overcome the stress at calving, being of vital importance for meat production efficiency.

At the end of the experiment, cows supplemented in the pre-partum period (PRMM and PRPS) presented a mean of 26.5 kg of BW and 0.7 of BCS (Table 3) higher than the animals not supplemented at this stage. This reinforces the importance of pre-partum supplementation for the maintenance or improvement of BCS and BW at 90 days postpartum, usually when AIrt occurs in the system of beef cattle production, aiming at one calf per year. However, the absence of postpartum supplementation for PRMM treatment was determined to obtain conception rate (CR) lower than that obtained by supplemented animals (Table 4). This result countered our hypothesis of the homeorrhetic effect of postpartum supplementation only for milk production and performance of the animals, showing that postpartum supplementation is fundamental to ensure a better energetic status of the dam, characterized by the lower concentration of BHB and NEFA postpartum (Table 4), and indicators of the animal's energy balance (Mcart et al., 2012; Mulliniks et al., 2013).

The cows not supplemented in the postpartum period (PRMM and MM) presented on average 1/3 (+6.85 kg) of the BW acquired by the supplemented cows (+21.7 kg), in addition to 0.5 point average BCS (4.7) lower than the supplemented animals (5.2) (Table 3). Based on the magnitude of postpartum BW and BCS change and allied to the BHB and NEFA indicators, postpartum supplementation was a more efficient strategy to reduce the effects of negative energy balance (NEB) in lactating beef cows.

The higher milk production of the cows supplemented in the postpartum period supported the hypothesis of nutrient targeting for milk production, however, the availability of at least 4 to 5% of pdDM, with 9.8% of CP and ad libitum mineral salt was determinant for them to express close to their productive genetic limit. As to its components, the higher percentage of MP produced by the supplemented cows allowed their calves, with a high requirement of CP for muscle growth, to present higher ADG (0.916 kg/day) to the non-supplemented calves (0.858 kg/day). In addition, the effect of the interaction of pre and postpartum supplementation on MF and TS in milk occurred due to lower MM production, showing that supplementation in at least one phase (pre or postpartum) already promotes increases in the production of these components.

The cows not supplemented in postpartum presented higher mean concentrations of NEFA and BHB (Table 4). The higher plasma concentrations of NEFA and BHB are indicative of adipose tissue mobilization due to the negative energy balance associated with inadequate nutrition, or a combination of both (Ospina et al., 2010; Chapinal et al., 2011; Mulliniks et al., 2013). The behavior in the NEFA (Figure 1a) and BHB (Figure 1b) concentrations along the postpartum days shows a similar rate of lipolysis of the adipose tissue (Lucy et al., 2002), but more pronounced in the animals not supplemented, which can be attributed to the lower postpartum BW and BCS gain. The increase in the concentrations of NEFA and BHB from day 30 to day 45 postpartum is most likely due to the reduced intake of feed after calving and the progressive increase in energy demand up to the peak of milk production characteristic of NEB, compensated by the mobilization of these adipose tissue metabolites (Bell, 1980; Baird, 1982). The decrease observed after this period may be indicative of the recovery of the nutritional status of beef cows (Ndlovu et al., 2007), since the results of NEFA suggest basal levels similar to those found in young animals with ADG of 0.700 kg per day (Unpublished data). In milk

cows, the decrease in BHB concentration, as seen in this study after 45 days postpartum, was associated with increased CR after the first insemination (Walsh et al., 2007) and a decrease in the delivery interval until the first ovulation (Reist et al., 2000).

The increase in SUN concentrations (Table 4) resulted from the increase of protein equivalents (Valadares Filho et al., 1997, 1999), assuming that these values were derived from the increase of ammoniacal nitrogen of the ruminal content of the animals that received higher amounts of supplement (Lazzarini et al., 2009b; Figueiras et al., 2010; Cabral et al., 2014). The mean SUN concentration of the postpartum supplemented group (11.55 mg/dL) is below 13.52-15.15 mg/dL, suggested by Valadares et al. (1997), which corresponds to the maximum microbial efficiency (ME) in steers fed with 62.5% of digestible organic matter, showing the need for a higher protein intake, through forage or supplement, in order to improve ME and, consequently, performance of these animals.

In the same sense, postpartum supplementation was the determining factor for the increase of glucose concentrations from 48.5 (animals not supplemented) to 53.5 mg/dL (Astessiano et al., 2013; Cappellozza et al., 2014; Van Cleef et al., 2014), since concentrations are directly associated with nutrient intake (Vizcarra et al., 1998). However, both values are between 45 and 75 mg/dL, suggested by Kaneko et al. (2008). The reduction in serum glucose concentration at day 45 postpartum was due to peak lactation, in which glucose requirements for ruminants are higher (Busato et al., 2002). The mean GLUC concentrations found for 30, 45, 60 and 75 days were 54.1, 44.5, 54.6, 55.1 mg/dL, respectively.

Finally, supplemented animals had higher levels of PROG from 45 days postpartum (Figure 1c). Progesterone is a steroid hormone produced by the ovarian corpus luteum and its plasma concentration is minimal at birth (Henricks et al., 1972; Smith et al., 1973), preceded by an increase after the first ovulation (Lauderdale, 1986; Perry et al., 1991; Werth et al., 1996) or luteinization (Donaldson et al., 1970; Corah et al., 1974) of a follicle. The higher plasma levels of PROG in the supplemented animals is an indication that the bovine female has already left the anestrous and began a new reproductive cycle after calving. In summary, postpartum supplementation reduced the intensity of the negative effects of BHB and NEFA and increased PROG concentrations, in addition to improving BCS and BW. This allowed a higher incidence of pregnant animals after the AIrt protocol, corresponding

to 56.6% for non-supplemented cows and 84.6% for cows supplemented in the postpartum period (Table 4).

5. CONCLUSION

Supplementation with 1kg/day of a supplement with 28.6% CP in pre-partum of cows with BCS greater than 4 does not improve the response to postpartum supplementation. Pre-partum supplementation is indicated for increased cows performance and calf weight at birth. To improve reproductive efficiency should be supplemented in the postpartum.

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Table 1 - Chemical composition of the supplement and forage.

Item	Supplement	Forage (Days after calving)						Pre ⁵	Pos ⁶
		-75	-45	-15	+15	+45	+75		
DM ^{1,2}	88,7	45,5	58,8	46,9	35,6	27,5	25,2	50,4	29,4
OM ^{1,3}	91,9	91,8	91,3	92,9	91,5	92,1	92,4	92,0	92,0
CP ^{1,3}	28,6	8,02	6,24	7,04	8,66	9,85	10,9	7,10	9,80
NDIN ^{1,4}	37,2	29,5	35,4	28,4	28,1	26,2	22,8	31,1	25,7
EE ^{1,3}	2,50	0,85	0,77	1,08	1,15	1,21	1,24	0,90	1,20
apNDF ^{1,3}	15,6	61,3	65,2	63,7	62,2	60,9	58,7	63,4	60,6
NFC ^{1,3}	45,2	21,6	19,1	21,1	19,5	20,1	21,6	20,6	20,4
iNDF ^{1,3}	2,80	32,1	40,2	34,2	25,3	21,4	18,4	35,5	21,7

¹/DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDIN, neutral detergent insoluble nitrogen; apNDF, neutral detergent fiber corrected for residual ash and protein; NFC, non-fibrous carbohydrates; iNDF, indigestible neutral detergent fiber. ²/In % of natural matter. ³/In % of dry matter. ⁴/In % of total nitrogen. ⁵/Mean values of the samples obtained through manual simulation of grazing in pre-partum ⁶/Mean values of the samples obtained through manual simulation of grazing in postpartum.

Table 2 - Performance of Nellore cows not supplemented or supplemented in pre-partum.

Item ¹	Supplementation		SEM ²	P-value
	Without	With		
BW (kg)	499,1	527,9	2,2	0,001
ADG (kg)	-0,182	0,138	0,1	0,001
BCS	4,284	5,109	0,1	0,001
caBW (kg)	30,64	34,28	0,7	0,001

¹/BW, cow body weight; ADG, average daily gain; BCS, body condition score; and caBW, calf birth weight. ²/SEM, standard error mean.

Table 3 - Performance and production of milk and its components of Nellore cows non-supplemented or supplemented in pre or postpartum period.

Item ¹	Supplementation strategy ²					P-value ³		
	MM	MMPS	PRMM	PRPS	SEM	Pre	Pos	Pre x Pos
iBW (kg)	473,5	467,0	486,4	493,5	-	-	-	-
fBW(kg)	475,0	487,3	498,6	516,6	4,6	0,001	0,001	0,532
fADG(kg)	0,016	0,204	0,136	0,257	0,1	0,003	0,001	0,245
fBCS	4,339	4,917	5,046	5,459	0,1	0,001	0,001	0,203
caADG(kg)	0,897	0,920	0,819	0,912	0,1	0,129	0,008	0,217
MY (kg/dia)	8,019	8,320	7,470	8,084	0,4	0,263	0,065	0,626
MF(%)	4,333	4,742	4,932	4,637	0,1	0,118	0,716	0,057
MP(%)	2,981	3,170	2,953	3,145	0,1	0,237	0,003	0,487
ML (%)	4,596	4,510	4,556	4,482	0,1	0,473	0,104	0,896
TS (%)	13,04	13,51	13,48	13,29	0,2	0,547	0,428	0,074
NGS (%)	8,702	8,771	8,546	8,656	0,1	0,101	0,282	0,811

¹iBW, body weight after calving; fBW, postpartum final body weight; fADG, postpartum average daily gain; fBCS, postpartum BCS; caADG, average daily gain of calves; MP, milk yield; MF, milk fat; MP, milk protein; ML, milk lactose; TS, total solids; e NGS, non-greasy solids. ²PRMM - supplementation with 1 kg/cow/day supplement only in pre-partum; MMPS - supplementation with 1 kg/cow/day supplement only in the postpartum period; PRPS - supplementation with 1 kg/cow/day supplement before and after delivery; MM - only ad libitum mineral mixture during pre and postpartum. ³Pre, effect of pre-partum supplementation; Pos, effect of postpartum supplementation; Pre x Pos, interaction between pre-partum supplementation and interaction between postpartum supplementation.

Table 4 - Concentration of metabolites and hormones in Nellore cows non-supplemented or supplemented in pre or postpartum period.

Item ¹	Supplementation strategy					P-value ²						
	MM	MM PS	PR MM	PR PS	SEM	Pre	Pos	Pre x Pos	Day	Pre x Day	Pos x Day	Pre x Pos x Day
SUN	9,18	11,2	8,87	11,9	0,7	0,412	0,001	0,113	0,001	0,956	0,105	0,564
GLIC	45,9	53,9	51,2	53,2	1,6	0,172	0,003	0,106	0,001	0,871	0,719	0,864
NEFA	0,42	0,31	0,30	0,21	0,1	0,001	0,001	0,449	0,001	0,117	0,001	0,627
BHB	0,57	0,37	0,51	0,35	0,1	0,103	0,001	0,442	0,001	0,402	0,001	0,895
PROG	1,51	3,28	2,20	3,60	0,4	0,210	0,001	0,638	0,001	0,702	0,001	0,791
CR ³	50,0	84,2	63,2	85,0	-	0,407	0,005	0,494	-	-	-	-

¹SUN, serum urea nitrogen (mg/dL); GLIC, glucose (mg/dL); NEFA, non-esterified fatty acids (mmol/L); BHB, β -hydroxybutyrate (mmol/L); PROG, progesterone (ng/mL); e CR, conception rate (%).² Pre, effect of pre-partum supplementation; Pos, effect of postpartum supplementation; Pre x Pos, interaction between pre-partum supplementation and postpartum supplementation; Day, effect of postpartum days; Pre x Day, interaction between pre-partum supplementation and postpartum days; Pos x Day, interaction between postpartum

supplementation and postpartum days; Pre x Pos x Day, interaction between pre x day and pos x day.³Performed by the chi-square test (P<0,05).

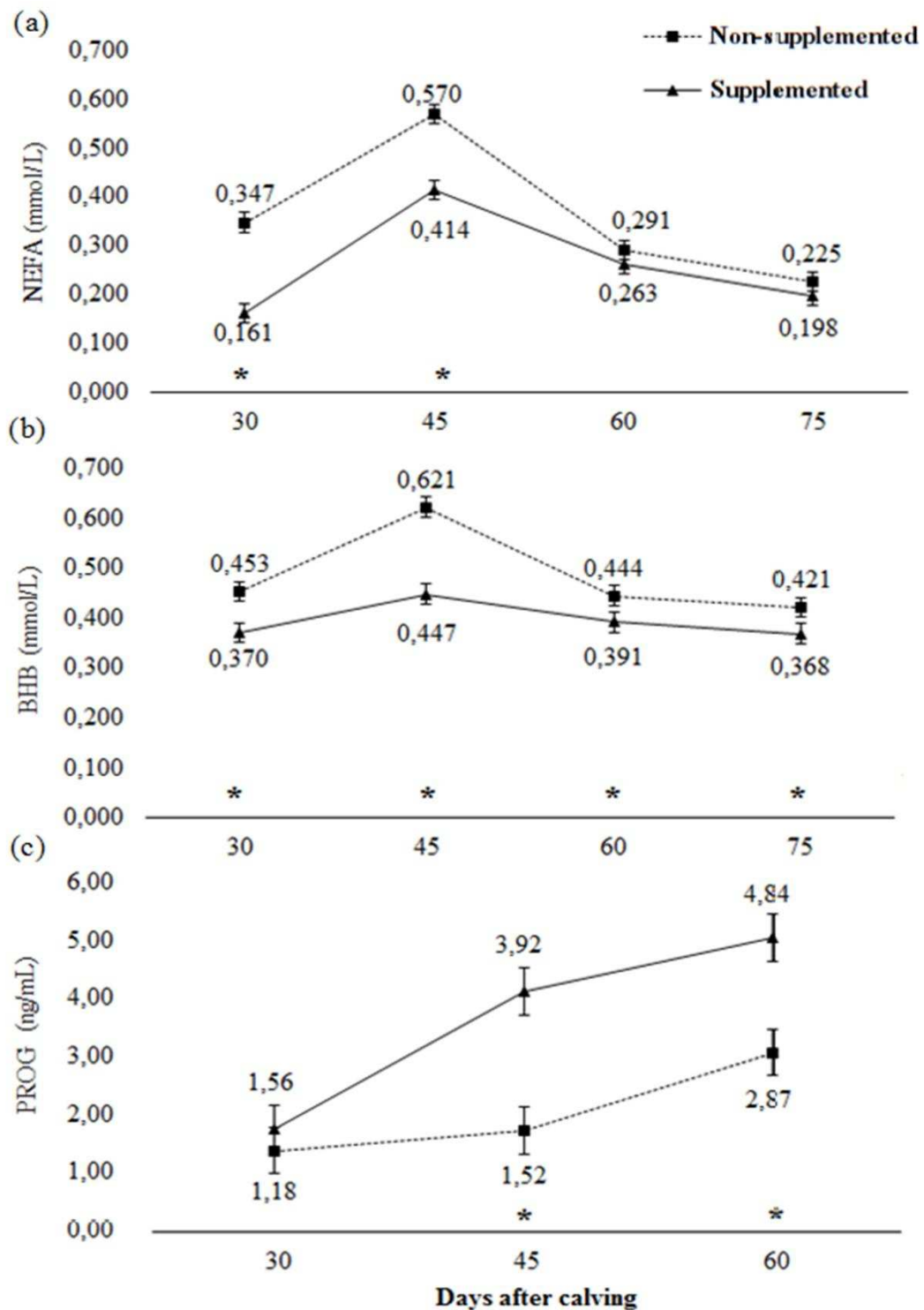


Figure 1 - Concentrations of NEFA (a), BHB (b) and Progesterone (c) in supplemented or non-supplemented beef cows in the postpartum period due to the postpartum days. */Indicative of significance (P<0.05).

CHAPTER 2

SUPPLEMENTATION STRATEGIES FOR NELLORE FEMALE CALVES IN CREEP-FEEDING

ABSTRACT - The objective of this study was to evaluate the effects of supplementation strategy on performance, nutritional and metabolic characteristics of Nellore suckling female calves, grazing *Uruchloa decumbens* during the dry-rainy transition period. Forty-four Nellore female calves, averaging 147.6 ± 1.34 kg (four months), were used. Calves were accompanied by their respective mothers, averaging 453.2 ± 10.3 kg (six years). The experimental design was a completely randomized design, with four treatments and eleven repetitions. A single supplement 20% of crude protein was provided, consisting of daily supplement at 0.0%, 0.2%, 0.4% or 0.6% of BW. Increasing linear effect ($P < 0.05$) of final body weight and average daily gain of female calves were observed with increased supplementation. Multiple supplementation increased consumption, in kg/day, of dry matter (DM), organic matter (OM), crude protein (CP), digested dry matter (dDM) and total digestible nutrients (TDN). There was an increasing linear effect in total DM and DM in g/kg of BW ($P < 0.05$). There was no effect of supplementation on the consumption in kg/day of neutral detergent fiber corrected for ash and protein (aNDF) ($P > 0.05$). Supplementation increased, in a quadratic way, the total apparent digestibility coefficient of DM, OM, CP, aNDF and TDN ($P < 0.05$). There was no effect of supplementation ($P > 0.05$) on microbial efficiency (micEF, g micP/kg of TDN consumed). There was a quadratic effect of the supplement increment on serum urea nitrogen (SUN) content ($P < 0.05$). There was no effect of supplementation ($P > 0.05$) on NEFA concentrations. There was an increasing linear effect ($P < 0.05$) of supplementation on insulin concentrations. It is concluded that supplementation improves the performance, nutritional and metabolic characteristics of the animals, being the supply of 0.6% of BW of supplement with 20% of CP the most effective treatment.

Key words: creep-feeding, female calves, insulin.

1. INTRODUCTION

Weaning heavier cattle is important because it allows the slaughter of males and the mating of heifers at ages less than 16 months (Paulino et al., 2012). In the tropics, calving is generally concentrated in the month of October before the first rains begin. Suckling calves remain with their dams during the wet period and the wet/dry transition period. At this stage, the animals have better food efficiency and potential for higher rates of weight gain.

However, due to physiological changes in calves and matings during the suckling phase, the performance of the calf can be compromised. During this period, calves effectively transform into a ruminant animal due to changes in the digestive tract, and protein requirements become proportionally higher than their energy requirements, due to the lower accumulation of fat and the high deposition of muscle tissue (Ørskov, 1987).

In addition, the milk production of the dam is decreased after reaching the lactation peak and the quality of the pasture may be compromised, due to the period of wet-dry transition, characterized by the lower incidence of rainfall and, consequently, reduction of growth and quality of forage. These factors may limit the animal's performance, since the availability of nutrients from milk and pasture may not be sufficient to meet the animal's demands (Porto et al., 2009).

In the case of heifers, the occurrence of puberty depends on the rate of growth and development of the animal to support the mechanisms of the endocrine system that result in first ovulation (Maquivar and Day, 2009). Studies have shown different results on when is the best time to accelerate growth in bovine females. Some authors report the occurrence of precocious puberty with increased rate of gain at early stages of development (Wiltbank et al., 1969; Arije e Wiltbank, 1971; Buskirk et al., 1995; Patterson et al., 1992; Gasser et al., 2006; Cardoso et al., 2014; Rodríguez- Sánchez et al., 2015).

Traditionally, creep-feeding systems have been used to increase weaning weight (Faulkner et al., 1994, Lardy et al., 2001, Valente et al., 2012), reduce grazing pressure, rest the dam, and improve the calf feed intake at weaning (Taylor and Field, 1999). However, most of the results from these studies were obtained using male calves.

Brito and Sampaio (2001) suggested that age and level of consumption are factors that affect the performance of the animals. Thus, it is possible that varied amounts of supplementation may increase the BW of heifers more than the supplementation of fixed amounts of supplement over a long period of time.

In this way, we hypothesized that supplementation of suckling zebu female calves would improve their performance and nutritional and metabolic characteristics. The objective of this study was to evaluate the effects of supplementation in increasing amounts, varied as a function of BW, on the performance, nutritional and metabolic characteristics of Nellore suckling female calves.

2. MATERIAL AND METHODS

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV 08/2015).

Animals, experimental design and supplements

The experiment was conducted at the Universidade Federal de Viçosa, in Viçosa-MG (20°45 'S and 42°52' W), between March and July, referring to the water-dry transition period. The experimental area is located in mountainous region, with 670 m of altitude, and presents annual precipitation of 1300 mm. The experiment lasted 123 days, divided in three experimental periods with 41 days each.

Forty-four Nellore female calves, with age and initial mean weight, of four months and 147.6 ± 1.34 kg, respectively, were followed, accompanied by their respective mothers, with age and initial mean weight of six years and 453.2 ± 10.3 kg, respectively.

An experimental area of 35 hectares was assigned to the animals, consisting of five 7.0 ha paddocks, covered uniformly with the *Uruchloa decumbens* grass, equipped with drinking fountains and troughs, which are covered and accessible from both sides. The supplement was provided daily at 10 a.m. Every seven days the animals were rotated between the paddocks, aiming to control possible paddock effects on the treatments (pasture availability, watering and trough location, relief, shading and others).

The experimental design was a completely randomized design, with four treatments, all treatments with eleven replications. A single supplement (Table 1), formulated (supplemented composition: corn grain, 63.0, soybean meal, 34.0, molasses, 3.0) was provided through the food composition data provided by BR-CORTE 2.0 (Valadares et al., 2010), with 20% of protein, in different amounts as a function of body weight. All animals received mineral mix ad libitum (MM; mineral composition of the mineral mix: dicalcium phosphate, 50.0, sodium chloride, 47.2, zinc sulfate, 1.5, copper sulfate, 0.7, cobalt sulfate, 0.05, potassium iodate, 0.05, and manganese sulphate, 0.5). Thus, 0.0%, 0.2%, 0.4% or 0.6% of the BW of this supplement were provided. Cows, in addition to the mineral mix at will, received 100 g/day of corn meal as a way to stimulate the demand and the longer stay near the troughs, and thus ensure the best supplement consumption by the female calves.

Experimental procedures and sampling

Evaluations of the average milk yield of the cows were performed by two samples of the production at 41 and 82 days after the beginning of the experiment. Cows were separated from their offspring at 5:30 p.m. and remained on the paddock, being milked at 5:30 a.m. and 5:30 p.m. on the following day, being the total production constituted by the sum of the collections at both times. Milking was performed mechanically by a trained employee. The milk secretion was stimulated with 2 mL oxytocin (10 IU / mL, Ocitovet ®, Brazil) in the mammary artery, initiating milking immediately after oxytocin administration. Samples were collected for analysis of milk protein (MP), milk fat (MF), milk lactose (ML), and total solids (ST) after homogenization in the bucket. Samples were stored at 4°C in a refrigerator using a bronopol tablet per sample as a preservative.

The animals were weighed at the beginning and at the end of the experiment after a fasting period of 12 hours. The average daily gain of the animals was estimated by the difference between the final weight and the initial weight, divided by the number of experimental days. All weightings were performed at 7:00 a.m.

Forage samples were randomly taken, every 15 days, in order to evaluate the forage mass per hectare. In each plot, four forage samples were randomly selected by using a metal square (0.5 x 0.5 m) and cut at approximately 1 cm above the soil. After that, forage subsamples (200 g) were dried at 60°C for 72 hours and ground to pass through a 1 and 2mm screen.

Every seven days, a manual grazing simulation was performed simultaneously to the observation of grazing behavior of the animals in order to obtain samples to evaluate chemical composition of the forage consumed by the animals. All samples were dried at 60°C for 72 hours, grounded to pass through 1 and 2mm screen, and proportionally sub-sampled to a composite sample per period.

To evaluate the nutritional characteristics of female calves from the 60th day of the experimental period, a digestibility assay was performed with a duration of nine days. To estimate the fecal excretion, the external chromium oxide (Cr₂O₃) indicator, packed in paper cartridges (Detmann et al., 2001), corresponding to 10 g per female calf/day, was applied to the animals using a metal probe directly in the esophagus, always at 10:00 a.m. To estimate the individual intake of supplementation by female calves, titanium dioxide (TiO₂), provided via supplement, was used in the proportion of 10 g of indicator/kg of supplement (Titgemeyer et al., 2001). To estimate the DM intake of pasture, the iNDF was used as an internal indicator (Detmann et al., 2001).

From the nine days of the test, six were destined to the adaptation of the animals to Cr₂O₃ and TiO₂. During the last three days, feces samples were collected at different times, at 3:00 p.m., 11:00 a.m. and 7:00 a.m., respectively. Feces samples were collected immediately after defecation or directly into the rectum of the animals, in an approximate amount of 200 g. They were identified by animal and oven dried with forced air circulation (60°C/72 hours) and, after drying, milled with a knife mill (1 and 2 mm). On the fifth day of the test, a manual grazing simulation was performed on each paddock separately, and these samples were used to estimate the intake and the digestibility coefficients.

On the last day of the digestive test, were obtained spot samples of urine in spontaneous urine, and blood, via jugular vein puncture, performed four hours after supplementation. After collection, urine samples were diluted (1:5) in H₂SO₄ (0.036 N) and frozen at -20°C for further evaluation of creatinine, urea and purine derivatives (allantoin and uric acid). Blood samples were collected at the end of the urine collection period to measure the levels of serum urea nitrogen (SUN), non-esterified fatty acids (NEFA), glucose (GLUC) and insulin (INS).

Chemical analysis

The forage and supplement samples, ground in 1 mm sieves, were analyzed according to the standard analytical procedures of the Instituto Nacional de Ciência e

Tecnologia em Ciência Animal (INCT-CA; Detmann et al., 2012) for dry matter (dried overnight at 105°C, INCT-CA method n° G-003/1), ethereal extract (Goldfisch method, INCT-CA method n° G-004/1), ashes (complete combustion in a muffle furnace at 600°C for 4 hours, INCT-CA method n° M-001/1), neutral detergent fiber corrected for ash and protein (apNDF; using a thermostable α -amylase, omitting sodium sulfite and correction of residual ash and proteins, INCT-CA n° F-002/1) and neutral detergent insoluble nitrogen content (NDIN; INCT-CA n° N-004 /1). From the forage and supplement samples that were processed through the 2 mm sieve, the indigestible neutral detergent fiber (iNDF) content was quantified as residual neutral detergent fiber (NDF) remaining after 288 hours in situ ruminal incubation, Using F57 filter bags (Ankom Technology Corp., Macedon, NY), according to Valente et al. (2011).

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

The non-fibrous carbohydrates of the supplements were estimated according to the recommendations of Hall (2000), using the following equation: apNFC = 100 - (% CP +% apNDF +% EE +% ash). The composition of the supplement and the forage obtained is shown in Table 1.

The pdDM was estimated according to the following equation (Paulino et al., 2008): $pdDM = 0,98 \times (100 - NDF) + (NDF - iNDF)$, where: NDF = neutral detergent fiber (%); iNDF = indigestible neutral detergent fiber (%); pdDM = potentially digestible dry matter (%); 0,98 = true digestibility of the cell contents.

A composite sample of feces based on air dry weight per animal of the three days of collection was elaborated, which were stored in polyethylene containers, duly identified and later analyzed for the contents of chromium and titanium using atomic absorption (Souza et al., 2013) and colorimetry (Titgemeyer et al., 2001), respectively. Were also evaluated the contents of DM; CP; EE; apNDF; iNDF and MM, as previously described.

Excretion of fecal MS was estimated using the chromic oxide indicator, and it was estimated by the ratio between the amount of indicator provided and its concentration in feces.

The estimation of individual supplement consumption by female calves was obtained through the following equation: $ICSup = (FE \times CIFI) / IFG$, where: ICSup = individual supplement consumption (kg/day); FE = fecal excretion (kg/day); CIFI =

concentration of the indicator in the feces of the animal (kg/kg); IFG = indicator present in the supplement provided to the group (kg/day).

The estimation of voluntary consumption of DM was performed using indigestible NDF as the internal indicator, according to the following equation: Dry matter intake (DMI) (kg/day) = $\{[(\text{FExFIC}) - \text{IS}] / \text{CIFO}\} + \text{SDMI} + \text{DMMI}$, where: FE = Fecal excretion (kg/day); FIC = fecal indicator concentration (kg/kg); IS = indicator consumption from the supplement (kg); CIFO = indicator concentration in the forage (kg/kg); SDMI = supplementary dry matter intake (kg/day); and DMMI = dry matter milk intake (kg/day).

The daily urinary volume was calculated using the relation between daily creatinine excretion (CE), adopting the equation proposed by Silva et al. (2012), and its concentration on spot samples: $\text{UCE (g/day)} = 0.0345 \times \text{FBW}^{0.9491}$, where FBW is the fasting body weight.

Allantoin analyzes were performed using the colorimetric method (Chen & Gomes, 1992). The total excretion of purine derivatives was calculated by the sum of the amounts of allantoin and uric acid excreted in the urine, expressed in mmol/day.

The absorbed purines (Y, mmol/day) were calculated from the excretion of purine derivatives (X, mmol/day) by the equation: $Y = (X - (0.301 \times \text{PC}^{0.75})) / 0.80$, In which 0.80 is the recovery of purines absorbed as purine derivatives and $(0.301 \times \text{PC}^{0.75})$ the endogenous contribution to purine excretion (Barbosa et al., 2011).

The ruminal synthesis of nitrogen compounds (Y, g micN/day) was calculated as a function of absorbed purines (X, mmol/day) using the equation described by Barbosa (2011): $Y = 70X / (0.93 \times 0.137 \times 1000)$, where 70 is the purine N content (mg N/mol); 0.137, the ratio N purines: total N in bacteria; and 0.93, the digestibility of bacterial purines.

The microbial efficiency (g micP/kg TDN) was obtained by the ratio between the production of microbial protein (micP), expressed in grams, and the amount of total digestible nutrients (TDN) consumed expressed in kilograms.

Metabolites were analyzed in accordance with manufacturer's instructions in an automatic biochemistry analyzer (Mindray BS200E, Shenzhen, China). The concentrations of glucose (K082), creatinine (K067), uric acid (K0139) and urea (K056), were measured using kits from Bioclin Diagnostics (Belo Horizonte, Brazil). Serum concentrations of non-esterified fatty acids (NEFA) was quantified by a

colorimetric method (FA115, Randox Laboratories Ltd., São Paulo, Brazil). An automatic biochemical apparatus (Mindray BS-200E; Shenzhen Mindray Bio-Medical Electronics Co. Ltd.) was used for all analyses. Insulin was analyzed by chemiluminescence using Access Ultrasensitive Insulin Reagent (Ref. Number 33410, Beckman Coulter Brea, USA) in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA). Serum and urinary urea N (SUN) was estimated as 46.67% of the total serum urea.

Statistical analyzes

The results were submitted to analysis of variance, adopting the initial body weight as co-variable when significant. The linear and quadratic effects of the multiple supplement amounts were evaluated by decomposing the sum of the squares of the treatments using orthogonal contrasts (Steel et al., 1997). The PROC MIXED procedure of the SAS (Statistical Analysis System, version 9.4) software was applied for all statistical analyzes. For all statistical procedures, $\alpha = 0.05$ was adopted as the critical level of error probability type I.

3. RESULTS

The mean total dry matter (TDM) and pdDM availability of forage *Uruchloa decumbens* throughout the experiment was 3297 kg/ha and 2468 kg/ha, respectively. The forage *Uruchloa decumbens* was obtained by manual grazing simulation and presented an average content of 78.0 g of CP/kg of DM (Table 1).

Supplementation improved the performance of the heifer calves and this fact can be verified by the increasing linear effect ($P < 0.05$) on the final body weight (fBW) and the average daily gain (ADG) of the female calves with increased supplementation (Table 2).

Supplementation of female calves did not affect the milk yield (MY) of their dams ($P > 0.05$; Table 3). In this study, the average milk yield was 5.43 kg/day, and included 3.61% of crude protein, 4.58% of fat, 4.31% of lactose and 13.36% of total solids.

Multiple supplementation increased intake (kg/day) (Table 4) of DM, OM, CP, dDM and TDN. There was an increasing linear effect in total DM and DM in g/kg of BW ($P < 0.05$). Supplementation supported a quadratic effect on forage DM intake in g/kg of BW and was higher in non-supplemented female calves. There was

no effect of supplementation on consumption in kg/day of apNDF ($P > 0.05$). However, supplementation supported a quadratic effect on the consumption of apNDF in g/kg of BW and dNDF, with the maximum consumption point being 9.96 g/kg of BW of NDFc and 1.37 kg/day of dNDF, both values observed for the treatment 0.2. Multiple supplementation allowed a linear effect on the intake and digestibility of DM ($P < 0.05$; Table 4).

Supplementation had a quadratic effect on the apparent digestibility coefficient of DM, OM, CP, NDF and TDN ($P < 0.05$; Table 5). The maximum digestibility point of DM (69.78%), OM (66.68%) and TDN (70.71%) was observed for the treatment 0.6. In addition, the maximum point of CP (62.48%) and apNDF (66.27%) was observed for the treatment 0.4.

There was a quadratic effect on SUN concentration ($P < 0.05$; Table 6). Supplementation did not affect micEF (g micP/kg of TDN consumed) ($P > 0.05$). Supplementation allowed a linear increase ($P < 0.05$) in the flow of microbial nitrogen compounds (micN) and there was also a quadratic effect on the excretion of urea nitrogen (urinary nitrogen(UN)/nitrogen intake (NI)), with a maximum point of 39.28% for the treatment 0.4.

Supplementation did not affect ($P > 0.05$) the concentrations of NEFA. However, there was an increasing linear effect ($P < 0.05$) of supplementation on INS and GLUC concentrations ($P < 0.05$) (Table 7).

4. DISCUSSION

Paulino et al. (2008), aiming to associate production per animal and per area, suggested the supply of 4 to 5% of BW in pdDM (between 40 and 50 g of pdDM/kg of BW), from pasture to satisfactory animal performance of animals under grazing conditions. In this research, the mean mass of pdDM was 57.3 g/kg of BW, a value above that recommended by Paulino et al. (2008), demonstrating that the amount of forage available did not compromise animal performance. The percentage of CP of the forage (Table 1), was above the minimum of 7% of CP in the basal diet, reported by Lazzarini et al. (2009) as necessary for adequate utilization of the neutral detergent fiber (NDF) of basal forage, which is the main source of energy for grass-fed animals. However, this value was below the 10% reported by Sampaio et al. (2009) as the level that optimizes the use of energetic substrates of the forage, which

justifies the supplementation with nitrogen compounds to optimize the use of the forage and, consequently, the animal performance.

The increase of ADG and fBW of the female calves (Table 2) confirmed our hypothesis that improvement in the performance of the heifers can be attributed to the higher consumption of nitrogen compounds (Table 4) and to the greater availability of readily available energy with the increase of the supplement supply. This may have had a beneficial effect on the ruminal environment, maximizing ammonia utilization and improving the synthesis of micN (Table 6) and dNDF (Table 5), resulting in more efficient use of the diet consumed, with an increase in energy latent extraction of the pasture, by the supplemented animals and, consequently, greater weight gain.

This reaffirms the importance of the supplementation of suckling heifer calves and the value of complementing nutrient intake and nutritional attributes, optimizing the productive performance of these calves, providing a greater weight at weaning, and contributing to the reduction of the duration of the rearing phase. In tropical regions, the consumption of milk and forage alone is not enough to allow the female calves to express their genetic potential (Valente et al., 2012).

Similar to the results reported in this work, Gelvin et al. (2004), Cardenas et al. (2012), and Valente et al. (2012) found no difference in milk production and its components (Table 3) among cows that had their calves supplemented or did not receive multiple supplements. Therefore, the milk production of cows did not influence the consumption and performance of heifer calves, with the differences observed in the ADG and fBW due substantially to the supply of multiple supplements.

Table 4 shows the estimates of intake by female calves. The higher the supplementation, the lower the forage dry matter (FDM) intake in kg/day due to the substitutive effect caused by the consumption of supplemental DM. However, supplementation allowed higher consumption of total DM by supplemented heifer calves.

The measurement of the ingested mass effectively digested allows the integration of the effects of the supplementation of the female calves on the consumption and the digestibility. In this study, supplementation increased dDM intake by female calves receiving multiple supplements (Table 4). This higher digestibility may be due in part, to the higher intake of nutritional compounds of easy digestion and, also, to the increase of the digestibility of the

grass. Additionally, increases in TDN content of the diet with supplementation appear to reflect the increase in the digestibility coefficients of CP and apNDF. This behavior is reinforced considering that the supplementation has increased the consumption of TDN, without, however, affecting the consumption of apNDF.

Table 5 shows the estimated total digestibility coefficients of the diet constituents as a function of the treatments received by the female calves. In the case of DM, OM and TDN, the increase in digestibility may be due to the progressive presence of more easily digestible compounds in the diet of animals receiving multiple supplements.

On the other hand, the significant increase in the digestibility of apNDF, may be due to the supply of the necessary substrates for the rumen microorganisms, in response to supplement consumption, allowing the increase of fibrolytic bacteria in number and the degradability of the fiber (Doyle et al., 2005).

The apparent digestibility coefficient of CP was higher in animals receiving multiple supplements, due to the higher CP intake and higher degradation, since the neutral detergent insoluble nitrogen content for the supplement was lower than for forage (Table 1). Moreover, the effect of the lower proportion of the fecal metabolic fraction in relation to the ingested nutrient occurs (Cabral et al., 2006; Barros et al., 2011), contributing to this higher digestibility.

In addition, supplementation enabled a higher concentration of SUN which can be attributed to the higher CP intake of these animals, since the SUN concentration is affected by the nutritional level, especially in ruminants, and is a sensitive and immediate indicator of protein intake (Gonzales and Scheffer, 2002). Thus, SUN estimates have been used to diagnose the adequacy of the use of nitrogenous compounds in the rumen as a function of the availability of degradable organic matter (Sampaio et al., 2009). In beef heifers, favorable SUN concentrations range from 11 to 15 mg/dL (Byers and Moxon, 1980). In this sense, only the level of 12.42 mg/dL presented by treatment 0.6 (Table 6) was within the suggested values. The lowest concentrations were observed for the non-supplemented animals (8.23 mg/dL, Table 6), indicating a possible compromise of the microbial activity and performance of these animals. Because of the higher intake of CP provided by supplementation, there was also higher urea nitrogen (UN/NI) excretion (Table 6).

According to data from Valadares et al. (2016), calves with a body weight of 200 kg, with ADG equal to 750 grams (results of treatment 0.6, Table 2), have a

requirement of 2.78 kg of TDN/day. In this study, TDN consumption by female calves, in kg/day, was higher than this value in all treatments that received supplementation (Table 4), and lower for MM treatment (2.65 kg / day). In regards to the requirement of CP for calves with 200 kg of body weight and ADG equal to 750 grams, the requirement is equal to 533 g/day. Only CP consumption by the female calves in the treatment 0.6 in g/day was higher than this value (580 g/day), allowing ADG of 749 g/day for this treatment. Although the TDN consumption was above the requirement for gain of 750 g/day in the treatments 0.2 and 0.4, the lower CP consumption limited the performance of these animals (Table 4). MM treatment had CP intake of only 410 g/day, not allowing these animals to have ADG above 650 g/day.

All treatments had similar NEFA concentrations, with mean values of 0.210 mol/L (Table 7). This is entirely plausible because, in young suckling animals with high gains, the mobilization of adipose tissue is not expected in order to supply energy deficiencies. However, this value is interesting, since it allows a reference of the basal levels of NEFA, for later use in studies of negative energy balance in beef cattle.

Roberts et al. (2009) demonstrated that puberty was more affected by the variation in the growth rate until about eight months of age, than in the subsequent growth. The results of Gasser et al. (2006) and Cardoso et al. (2014) indicate that, during the initiation of development, possibly between four and 6.5 months of age, heifers are more sensitive to nutritional programming, and may accelerate puberty, reinforcing the need of the supplementation of suckling female calves, aiming, mainly, at the anticipation of the age at the first conception. Most studies proving this positive effect of nutrition on heifers' development were conducted using feed levels in order to change the ADG. Most of them used pre-weaned female calves (Gasser et al., 2006; Cardoso et al., 2014) or controlled pre-weaning diets (Rodríguez-Sánchez et al., 2015).

In this study, supplementation increased the concentration of GLUC and INS due to the conjugated effect of increased energy from the supplement and increased digestibility of nutrients. Diets rich in starch increase the production of propionate, and this is converted to glucose in the liver, stimulating the release of insulin. Additionally, increased insulin concentration may be the result of amino acid stimulation in insulin secretion (Harmon, 1992). Insulin, in turn, as well as insulin-

like growth factor 1 (IGF-1), functions as a potent stimulus for cell proliferation and cell hypertrophy (Lawrence et al., 2012), indicating the metabolic, positive effect of increased supplementation on the higher ADG of these animals.

5. CONCLUSION

Supplementation, up to 0.6% of a female calf's BW, with 20% CP, improves the performance, nutritional and metabolic characteristics of the animals, being the supply of 0.6% of BW of supplement the most effective treatment.

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Table 1 - Chemical composition of the supplement and forage.

Item	Supplement	Forage			Dig ⁶	Mean
		(1-41 d) ⁵	(42-82 d) ⁵	(83-123 d) ⁵		
Dry matter ²	89,85	28,92	30,38	29,47	29,88	29,95
Organic matter ³	91,04	91,91	91,08	91,23	91,12	91,06
Crude protein ³	20,82	8,70	8,30	6,39	8,42	7,14

NDIN ^{1,4}	14,32	25,11	23,21	21,42	23,01	23,24
Ether extract ³	2,44	1,90	1,62	1,59	1,68	1,71
apNDF ^{1,3}	15,21	69,03	63,85	65,64	64,35	65,35
NFC ^{1,3}	52,57	12,28	17,31	17,61	16,67	16,86
iNDF ^{1,3}	2,64	24,83	19,33	25,15	21,45	24,44

^{1/} NDIN, neutral detergent insoluble nitrogen; apNDF, neutral detergent fiber corrected for residual ash and protein; NFC, non-fibrous carbohydrates; iNDF, indigestible neutral detergent fiber. ^{2/}In % of natural matter. ^{3/}In % of dry matter. ^{4/}In % of total nitrogen. ^{5/}Mean values of the samples obtained by manual grazing simulation. ^{6/}Sample obtained by manual grazing simulation in the digestibility assay.

Table 2 - Performance of supplemented and non-supplemented female calves during creep-feeding.

Item ¹	Supplementation strategy ²				SEM ³	P-value ⁴	
	0.0	0.2	0.4	0.6		L	Q
iBW	147,5	145,8	149,4	147,8	-	-	-
fBW	227,5	229,8	236,5	240,0	1,8	0,011	0,644
ADG	0,650	0,682	0,708	0,749	0,1	0,011	0,644

^{1/} iBW, initial body weight; fBW, final body weight; and ADG, average daily gain. ^{2/}MM, Mineral mix; T0,2, 0.2% of the supplement BW; T0,4, 0.4% of the supplement BW; T0,6, 0.6% of the supplement BW. ^{3/}SEM, standard error mean. ^{4/}Indicative of significance for linear (L) and quadratic (Q) effects of progressive increase of supplementation.

Table 3 –Milk yield and its components in function of the different treatments.

Item ¹	Supplementation strategy				SEM	P-value ²	
	0.0	0.2	0.4	0.6		L	Q
MY (kg)	5,72	5,37	5,20	5,42	0,44	0,591	0,444
MF (%)	4,70	4,69	4,38	4,53	0,14	0,188	0,369
MP (%)	3,52	3,59	3,72	3,60	0,09	0,384	0,225
ML (%)	4,30	4,35	4,22	4,38	0,06	0,719	0,503
TS (%)	13,65	13,18	13,51	13,11	0,52	0,576	0,826

^{1/}MP, milk yield; MF, milk fat; MP, milk protein; ML, milk lactose; and TS, total solids. ^{2/}Indicative of significance for linear (L) and quadratic (Q) effects of progressive increase of supplementation.

Table 4 - Consumption of supplemented or non-supplemented suckling female calves.

Item ¹	Supplementation strategy				SEM	P-value ²	
	0.0	0.2	0.4	0.6		L	Q
DM	4,01	4,25	4,36	4,48	0,17	0,046	0,343

FDM	3,14	3,10	2,99	2,70	0,13	0,006	0,673
MDM	0,87	0,85	0,81	0,86	0,04	0,354	0,554
OM	3,65	3,94	4,05	4,14	0,15	0,001	0,430
PB	0,41	0,47	0,51	0,58	0,02	0,001	0,176
apNDF	1,94	2,09	2,00	1,87	0,08	0,184	0,378
dDM	2,59	2,73	2,84	3,14	0,12	0,003	0,778
dNDF ²	1,10	1,37	1,36	1,13	0,07	0,344	0,001
TDN	2,65	2,89	2,97	3,05	0,11	0,001	0,335
	g/Kg of BW						
DM	19,1	20,28	20,43	20,67	0,36	0,004	0,194
FDM	14,91	14,55	13,93	12,11	0,35	0,465	0,043
apNDF ²	9,23	9,96	9,59	8,68	0,23	0,378	0,001

¹/DM, dry matter; FDM, forage dry matter; MDM, milk dry matter; OM, organic matter; CP, crude protein; apNDF, neutral detergent fiber corrected for residual ash and protein; dDM, digested dry matter; dNDF, digested apNDF; and TDN, total digestible nutrients. ²/Indicative of significance for linear (L) and quadratic (Q) effects of progressive increase of supplementation.

Table 5 - Total apparent digestibility of the constituents of the diet in function of the different treatments.

Item ¹	Supplementation strategy				SEM	P-value ²	
	0.0	0.2	0.4	0.6		L	Q
DM	64,59	64,01	64,98	69,78	0,76	0,567	0,001
OM	54,26	66,01	66,93	66,68	1,13	0,187	0,001
CP	53,95	58,35	62,48	61,02	1,31	0,221	0,034
apNDF	58,85	65,68	66,27	65,97	1,90	0,234	0,029
TDN	52,06	67,32	67,66	70,71	1,15	0,124	0,001

¹/DM, dry matter; OM, organic matter; CP, crude protein; apNDF, neutral detergent insoluble fiber corrected for ash and protein; and TDN, total digestible nutrients. ²/Indicative of significance for linear (L) and quadratic (Q) effects of progressive increase of supplementation.

Table 6 – Protein profile of supplemented and non-supplemented suckling female calves in creep feeding system.

Item ¹	Supplementation strategy				SEM	P-value ²	
	0.0	0.2	0.4	0.6		L	Q
micN	42,48	52,42	58,55	68,39	4,98	0,001	0,997

micEF	120,08	125,05	122,93	120,07	11,06	0,943	0,725
SUN	8,23	8,80	9,63	12,42	0,24	0,133	0,001
UN/NI	27,31	39,2	39,28	35,94	2,11	0,257	0,001

¹/SUN, serum urea nitrogen; micN, microbial nitrogen production; micEF, microbial efficiency; and UN/ NI, urinary nitrogen/nitrogen intake. ²/Indicative of significance for linear (L) and quadratic (Q) effects of progressive increase of supplementation.

Table 7 - Blood concentration of hormone and metabolites according to the different treatments.

Item ¹	Supplementation strategy				SEM	P-value ²	
	0.0	0.2	0.4	0.6		L	Q
NEFA (mmol/L)	0,212	0,210	0,209	0,208	0,02	0,854	0,965
GLUC (mg/dL)	67,75	75,12	80,89	82,41	2,79	0,001	0,289
INS (μIU/mL)	1,016	1,140	1,740	1,830	0,18	0,001	0,859

¹/NEFA, non-esterified fatty acids; GLUC, glucose; e INS, insulin. ²/ Indicative of significance for linear (L) and quadratic (Q) effects of progressive increase of supplementation.

CHAPTER 3

NUTRITIONAL PLANNING FOR NELLORE HEIFERS AFTER WEANING TO CONCEPTION AT 15 MONTHS OF AGE

ABSTRACT - The objective of this work was to evaluate the effects of strategic supplementation in the dry period and dry/water transition on the performance, nutritional, metabolic and conception rate of Nellore heifers in *Uruchloa decumbens* pastures. 48 Nellore heifers with age and mean body weight (BW) of eight months and 235.1 ± 3.34 kg, respectively, were used. The experimental design was a completely randomized design, with four treatments, all with twelve replications. The evaluated strategies were: BAAL- supplementation with 0.2% of BW/animal/day of supplement in the first 90 days and supplementation with 0.6% of BW/animal/day in the 90 days thereafter; MEME - supplementation with 0.4% of BW/animal/day for 180 days; ALBA - supplementation with 0.6% of BW/animal/day of supplement in the first 90 days and supplementation with 0.2% of BW/animal/day in the 90 days thereafter; MM - only mineral mixad *libitum* during the 180 days. Supplementation improved the performance of the animals during of dry period ($P = 0.001$) and dry/water transition ($P = 0.001$). Supplemented animals had higher longissimus muscle area (LMA) and subcutaneous fat thickness (SFT) at the end of the experiment ($P < 0.05$). Multiple supplementation increased intake of dry matter (DM), organic matter (OM), crude protein (CP), digestible dry matter (dDM) and total digestible nutrients (TDN) in kg/day throughout the experiment, and of digested neutral detergent fiber (dNDF) and neutral detergent fiber corrected for ash and protein (apNDF) in the dry/water transition. There was no effect of supplementation on the dry matter intake (kg/day) of forage (fDM), dNDF and apNDF ($P < 0.05$) during the dry period. The supplementation increased the total apparent digestibility coefficient of DM, OM, CP, apNDF and TDN ($P < 0.05$). Concentrations of serum urea nitrogen (SUN), glucose (GLUC), insulin (INS) and progesterone (PROG) were higher in supplemented heifers than in non-supplemented heifers ($P < 0.05$). Supplementation reduced the concentrations of non-esterified fatty acids (NEFA) ($P = 0.001$) and increased conception rate ($P = 0.020$). It is concluded that multiple supplementation improves performance and reproductive performance of heifers in grazing.

Key words: heifers, rearing, supplementation.

1. INTRODUCTION

Reproduction is a relevant economic impact component to obtain good productivity indexes in the beef cattle herd. Thus, special attention should be directed to females at all stages of the productive cycle so that there is no compromise of the meat production system.

To improve the biological efficiency of the herd, it is necessary that heifers reach puberty and mating earlier, and the importance of this characteristic increases as the production system becomes more intensive and competitive (Menegaz et al., 2008). The reduction of the age at first calving increases the reproductive life of the mother and the number of calves (Short et al., 1994), besides reducing the permanence of less productive categories in the production system, releasing areas for other categories and reducing the cost energy per unit of product (Beretta et al., 2001). The study of sexual precocity in zebu females is still recent in the scientific literature, so it is rare to cite mating Zebu females close to 14/16 months of age. One of the pioneers in this subject, Lobato (1995) reported reduction of the first service of Nellore females, from 26-27 months to 14-15 months of age, observing conception rate of 12%.

Nutrition is undoubtedly the management parameter that most changes the age of the animal at slaughter or at the first conception, that is, the precocity or the rate at which the animal approaches its adult weight is very sensitive to changes in the environment nutritional (Paulino et al., 2004).

Inadequately fed herds have low reproductive rates, delaying the resumption of cyclic luteal ovarian activity, as well as the onset of puberty and sexual maturity for heifers, which could be avoided or mitigated by the strategic supplementation of these animals during the post-weaning period.

Age and level of consumption are factors that affect the productive performance of the animals. Thus, it is possible that the indication of supplementation as a function of the increase of BW of the females in the rearing is more interesting than the supply of fixed amounts of supplement.

Thus, this study was carried out to evaluate the effects of strategic supplementation in the dry period and dry/water transition on the performance, nutritional, metabolic and conception rates of Nellore heifers.

2.MATERIAL AND METHODS

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV 08/2015).

Animals, experimental design and supplements

The experiment was conducted at the Universidade Federal de Viçosa, located in the municipality of Viçosa-MG (20°45'S and 42°52' W), between July and December, with a duration of 180 days, referring to the dry period and dry-water transition.

The experimental area of 32 hectares is located in mountainous region, with 670 m of altitude, and presents annual precipitation of 1300 mm. This is constituted by four paddocks of 8.0 ha, covered uniformly with the grass *Uruchloa decumbens*, provided with drinking fountains and troughs, these being covered and with access from both sides.

Nellore heifers with initial age of eight months and initial mean weight of 235.1 ± 3.34 kg were used.

The experimental design was a completely randomized design, with four treatments, all treatments with twelve replicates. The evaluated strategies were: BAAL - supplementation with 0.2% of BW/animal/day of supplement in the first 90 days and supplementation with 0.6% of BW/animal/day in the 90 days thereafter; MEME - supplementation with 0.4% of BW/animal/day for 180 days; ALBA - supplementation with 0.6% of BW/animal/day of supplement in the first 90 days and supplementation with 0.2% of BW/animal/day in the 90 days thereafter; MM - only mineral mix *ad libitum* during the 180 days. A single supplement was supplied, formulated through the food composition data provided by BR-CORTE 2.0 (Valadares et al., 2010), with approximately 20% of protein (supplementary composition: corn grain, 63.00; soybean meal, 32.0; mineral mix, 5.0). The mineral mix was composed of: dicalcium phosphate, 50.00; sodium chloride, 47.2; zinc sulfate, 1.50; copper sulfate, 0.70; cobalt sulfate, 0.05; potassium iodate, 0.05; and manganese sulphate, 0.5.

The supplement was provided daily at 10am. The animals were rotated between the paddocks every 15 days, with a balanced rotation for residual effect, aiming to control the possible effects of paddocks on the treatments (pasture availability, water and trough location, relief, shading and others).

Experimental procedures and sampling

Heifers were weighed at baseline at 90 days and at the end of the experiment after 12 hours of fasting for performance evaluation. The average daily gain (ADG) of the animals was estimated by the difference between the final weight and the initial weight, divided by the number of experimental days. All weighings were performed at 7:00 a.m.

At the beginning and at the end of the experiment ultrasonography of the rump and the region between the 12th and 13th ribs was performed to evaluate the subcutaneous fat thickness (SFT) and longissimus muscle area (LMA). The images were performed using the Aloka SSD 500. Vegetable oil was used to ensure adequate acoustic contact.

The forage samples were collected every 15 days for evaluation of the forage mass per hectare. At each paddock, four forage samples were randomly selected using a metal square (0.5 x 0.5 m) and cut approximately 1 cm above the ground. Subsequently, the forage samples (200 g) were immediately taken to the oven at 60 °C for 72 hours and milled knife mill with sieves of 1 and 2 mm.

The grass samples for analysis of the chemical composition were collected at intervals of 30 days by manual grazing simulation, then dried in a forced air circulation stove at 60°C for 72 hours and ground in a knife mill with sieves of 1 and 2 mm.

To evaluate the nutritional characteristics of heifers, two digestibility tests were carried out with a duration of nine days and beginning on the 45th and 135th days of the experimental period. To estimate fecal excretion, 20 grams per animal of the external chromium oxide indicator (Cr₂O₃) was supplied daily in paper cartridges (Detmann et al., 2001) with the aid of a metal probe. To estimate the individual consumption of supplement, titanium dioxide (TiO₂), supplied by supplement, was used in the proportion of 10 grams of indicator per animal (Titgemeyer et al., 2001). In order to estimate the DM intake of pasture, indigestible neutral detergent fiber (iNDF) was used as the internal indicator (Detmann et al., 2001).

From the nine days of the test, five were destined to adapt the animals to Cr₂O₃ and TiO₂. During the last four days, collections were held at the following times: 4:00 p.m., 1:00 p.m., 10:00 a.m. and 7:00 a.m. The feces samples were

collected directly in the rectum of the animals, in an approximate amount of 200 g and dried in an oven with forced air circulation stove (60 ° C / 72 hours) and, after drying, ground in a knife mill (1 and 2 mm). On the fifth day of the test, a manual grazing simulation was performed on each paddock separately, and these samples were used to estimate the intake and digestibility coefficients.

On the last day of the digestive test, spot samples of urine were obtained in spontaneous urine and blood via puncture of the jugular vein performed four hours after supplementation. After collection, urine samples were diluted (1: 5) in H₂SO₄ (0.036 N) and frozen at -20°C for further evaluation of creatinine, urea and purine derivatives (allantoin and uric acid).

Blood samples were collected 15, 45, 75, 105, 135 and 165 days after the start of the experiment. The collection was performed at 7:00 am via puncture of the jugular vein using vacuum tubes with clot accelerator and separator gel (BD Vacuntainer® SSTIIAdvance, São Paulo, Brazil). After collection, the blood was centrifuged at 3600 × g for 20 minutes and stored at -20 ° C. Subsequently, the levels of serum urea nitrogen (SUN), glucose (GLUC), insulin (INS) and non-esterified fatty acids (NEFA) were quantified. In addition, progesterone concentrations (PROG) on days 105, 135 and 165 were measured.

On the 170th day of the experiment, heifers were synchronized using the following protocol: in d 0, an intravaginal progesterone release device (Tecnopec Primer®, São Paulo, Brazil) was inserted and carried out the application of 2.0 mg of Estradiol benzoate (Tecnopec RIC-BE®, São Paulo, Brazil). At d 7, the intravaginal device was removed and heifers received an injection of 2 mL of sodium cloprosterol (MSD Health Ciosin® Animal, São Paulo, Brazil). Finally, at day 8, heifers received 0.5 ml of estradiol cypionate (Zoetis-Pfizer E.C.P., Campinas, Brazil). Fixed-time artificial insemination (AIrt) was performed 46 to 52 hours after removal of the intravaginal device (d 10). Semen doses of five Nellore bulls were randomly assigned to each heifer. The diagnosis of gestation was determined by transrectal ultrasonography 30 days after AIrt. The conception rate was calculated considering all heifers.

Chemical analysis

Samples of forage and supplement were analyzed following procedures described by Detmann et al. (2012) for dry matter (DM; method INCT-CA G-003/1),

crude protein (CP; method INCT-CA N-001/1), ether extract (EE; method INCT-CA G-004/1), ash (method INCT-CA M-001/1), neutral detergent insoluble nitrogen (NDIN; method INCT-CA N-004/1), neutral detergent insoluble fiber (apNDF; method INCT-CA F-002/1) corrected for ash residue (method INCT-CA M-002/1) and residual nitrogen compounds (method INCT-CA N-004/1). The indigestible neutral detergent insoluble fiber (iNDF; method INCT-CA F-009/1) was evaluated using F57 (Ankon®) bags incubated in rumen by 288 h.

The non-fibrous carbohydrates of the supplements were estimated according to the recommendations of Hall (2000), using the following equation: apNFC = 100 - (% CP +% apNDF +% EE +% ash). The composition of the supplement and the forage obtained is shown in Table 1.

The pdDM was estimated according to the following equation (Paulino et al., 2008): $pdDM = 0,98 \times (100 - NDF) + (NDF - iNDF)$, where: NDF = neutral detergent fiber (%); iNDF = indigestible neutral detergent fiber (%); pdDM = potentially digestible dry matter (%); 0,98 = true digestibility of the cell contents.

The feces samples were analyzed for chromium and titanium contents using atomic absorption (Souza et al., 2013) and colorimetry (Titgemeyer et al., 2001), respectively. The contents of DM, CP, EE, apNDF, iNDF and ashes were also evaluated, as previously described.

Excretion of faecal DM was estimated using the chromic oxide indicator, and it was estimated by the ratio between the amount of indicator provided and its concentration in feces.

The estimation of individual supplement consumption by female calves was obtained through the following equation: $ICSup = ((FExCIFI)/IFG)$, where: ICSup = individual supplement consumption (kg/day); FE = fecal excretion (kg/day); CIFI = concentration of the indicator in the feces of the animal (kg/kg); IFG = indicator present in the supplement provided to the group (kg/day).

The estimation of voluntary consumption of DM was performed using indigestible NDF as the internal indicator, according to the following equation: $Dry\ matter\ intake\ (DMI)\ (kg/day) = \{[(FExFIC) - IS] / CIFO\} + SDMI$, where: FE = Fecal excretion (kg/day); FIC = fecal indicator concentration (kg/kg); IS = indicator consumption from the supplement (kg); CIFO = indicator concentration in the forage (kg/kg); SDMI = supplementary dry matter intake (kg/day).

The daily urinary volume was calculated using the relation between daily creatinine excretion (CE), adopting the equation proposed by Silva et al. (2012), and its concentration on spot samples: $UCE \text{ (g/day)} = 0.0345 \times FBW^{0.9491}$, where FBW is the fasting body weight.

Allantoin analyzes were performed using the colorimetric method (Chen & Gomes, 1992). The total excretion of purine derivatives was calculated by the sum of the amounts of allantoin and uric acid excreted in the urine, expressed in mmol/day.

The absorbed purines (Y, mmol/day) were calculated from the excretion of purine derivatives (X, mmol/day) by the equation: $Y = (X - (0.301 \times PC^{0.75})) / 0.80$, In which 0.80 is the recovery of purines absorbed as purine derivatives and $(0.301 \times PC^{0.75})$ the endogenous contribution to purine excretion (Barbosa et al., 2011).

The ruminal synthesis of nitrogen compounds (Y, g micN/day) was calculated as a function of absorbed purines (X, mmol/day) using the equation described by Barbosa (2011): $Y = 70X / (0.93 \times 0.137 \times 1000)$, where 70 is the purine N content (mg N/mol); 0.137, the ratio N purines: total N in bacteria; and 0.93, the digestibility of bacterial purines.

The microbial efficiency (g micP/kg TDN) was obtained by the ratio between the production of microbial protein (micP), expressed in grams, and the amount of total digestible nutrients (TDN) consumed expressed in kilograms.

The concentrations of glucose (K082), creatinine (K067), uric acid (K0139) and urea (K056), were measured using kits from Bioclin Diagnostics (Belo Horizonte, Brazil).

Serum concentrations of urea (K056) e glucose (K082) were measured using kits from Bioclin Diagnostics (Belo Horizonte, Brazil). Serum concentrations of non-esterified fatty acids (NEFA) was quantified by a colorimetric method (FA115, Randox Laboratories Ltd., São Paulo, Brazil). An automatic biochemical apparatus (Mindray BS-200E; Shenzhen Mindray Bio-Medical Electronics Co. Ltd.) was used for all analyses. Serum urea N (SUN) was estimated as 46.67% of the total serum urea.

Progesterone and insulin were analyzed by chemiluminescence using Access Progesterone Reagent (Ref. Number 33550) and Access Ultrasensitive Insulin Reagent (Ref. Number 33410), by Beckman Coulter®, (Brea, USA) in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA).

Statistical analyzes

The results were submitted to analysis of variance, adopting a completely randomized design, being the evaluation periods included in the model with measures repeated in time. The PROC MIXED procedure of the SAS software (Statistical Analysis System, SAS University Edition) was applied for all statistical analyzes. In the case of significance of the effect of lactation thirds, lsmeans were compared using the student's t test, adopting $\alpha = 0.05$ as the critical level of type I error probability. The denominator degrees of freedom were calculated using the approximation Kenward-Roger.

Treatment effects on the priming rate were determined using the PROC FREQ of the SAS software (Statistical Analysis System, SAS University Edition). In case of significance, lsmeans were compared by the following orthogonal contrasts:

- Supplemented vs. not supplemented: $3 \times \text{MM} - \text{BAAL} - \text{MEME} - \text{ALBA}$
- Change in supplementation vs no change in supplementation: $2 \times \text{MEME} - \text{BAAL} - \text{ALBA}$
- High initial supplementation vs low initial supplementation: $\text{BAAL} - \text{ALBA}$

3.RESULTS

The mean total dry matter (TDM) and pdDM of the *Uruchloa decumbens* forage was 4112 kg/ha and 2680 kg/ha, respectively, during the dry season, and 3622 kg/ha and 2744 kg/ha, respectively, during the dry/water transition. The forage *Uruchloa decumbens* obtained by manual grazing simulation presented a mean content of 60.2 g of CP/kg of DM in dry period and 67.5 g of CP/kg of DM in the dry/water transition (Table 1).

Supplementation improved the performance of the animals during the first 90 days of the experiment, and this fact can be verified by the heifers' average daily gain (ADG) ($P < 0.05$) and the trend in the highest final body weight (fBW) ($P = 0.077$) with increased supplementation (Table 2). The same fact was observed in the dry/water transition phase, where supplementation improved fBW ($P < 0.05$) and ADG ($P < 0.05$) (Table 2).

In the present study, for the initial LMA and SFT, there was no difference ($P > 0.05$) between the supplementation strategies (Table 2). However, the supplemented animals had higher LMA and SFT at the end of the experiment ($P < 0.05$; Table 2).

Multiple supplementation increased intake (Table 3) in kg/day of DM, OM, PB, dDM and TDN throughout the experiment and of dNDF and apNDF only in the dry/water transition. There was no effect of supplementation on the consumption in kg/day of FDM, dNDF and apNDF ($P < 0.05$) during the dry period. The lowest FDM consumption in the dry/water transition period was observed for BAAL treatment. MEME and ALBA treatments obtained higher apNDF intake than the other treatments in the dry/water transition period. The DM intake in g/kg of BW ($P < 0.05$) was higher in the supplemented heifers, being higher for the ALBA treatment in the dry period and BAAL in the dry/water transition. However, FDM intake in g/kg of BW was higher in non-supplemented heifers and the consumption of apNDF in g/kg of BW was higher for MM and BAAL treatments ($P < 0.05$).

The supplementation increased the total apparent digestibility coefficient of DM, OM, CP, apNDF and TDN ($P < 0.05$), that is, of all analyzed parameters (Table 4).

The supplementation allowed increase ($P < 0.05$) on the flow of microbial nitrogen compounds (micN). No influence of supplementation ($P > 0.05$) on micE expressed in g micP/kg of TDN consumed was observed. Supplementation caused a higher excretion ($P < 0.05$) of urea nitrogen (urinary nitrogen(UN)/nitrogen intake (NI)) during the dry period. There were no differences in the excretion of urea nitrogen in the dry/water transition period ($P > 0.05$) (Table 5).

There was interaction effect of the treatment with the day of collection on the SUN, NEFA and INS concentrations ($P < 0.05$). There was effect of the day of collection on the concentrations of SUN, NEFA and GLUC ($P < 0.05$). Concentrations of SUN, GLUC, INS and PROG were higher in supplemented heifers compared to those not supplemented ($P < 0.05$). On the other hand, supplementation reduced NEFA concentrations ($P < 0.05$). The conception rate was higher for supplemented heifers ($P < 0.05$) (Table 6).

4.DISCUSSION

Paulino et al. (2008), aiming to associate production per animal and per area, suggested the supply between 4 and 5% of BW in pdDM (between 40 and 50 g of pdDM/kg of BW) of pasture for satisfactory grazing performance. In this work, the average mass of pdDM, during the first 90 days, was 41.4 g/kg of BW, a value that was recommended by this author, demonstrating that the amount of forage did not compromise animal performance. The percentage of CP of forage (Table 1) was below the minimum of 7% of CP in the basal diet (6.02%), reported by Lazzarini et al. (2009) as necessary for adequate utilization of the apNDF of basal forage, which is the main source of energy for animals raised on pasture. This resulted in the lower ADG reached by the MM group, to the detriment of the supplemented animals that had higher consumption of nitrogen compounds (Table 3), which probably led to an improvement in the energy: diet protein adequacy, allowing a significant increase in performance (Table 2). During the dry period of the year, weight loss of animals raised in tropical regions has been observed, due to decreases in forage quality, especially digestibility and CP levels (Lazzarini et al., 2009).

In mature forage-based diets, such as during the dry season, inadequate supply of nitrogen to the rumen would be the main limiting factor for ingestion, which in turn would lead to a limited energy intake by the animal (Table 4) (Detmann et al., 2010). Supplementation with 0.6% of BW (ALBA) in dry period with the supplementary of 25% of CP, allowed CP level in the diet higher than the 10% reported by Sampaio et al. (2009) as a level that optimizes the use of energetic substrates of the forage and this reflected in the higher performance of this treatment (0.345 kg/day, table 2), justifying supplementation with nitrogen compounds in greater quantities to optimize forage utilization.

In the last 90 days of experiment, the mean mass of pdDM was 30.3 g/kg of BW and the percentage of CP of the forage was 6.75 (Table 1). Since pdDM is the basal resource in the feeding of this production system, its availability below the recommended level promotes the need for additional resources. Thus, the animals remained a prolonged period with a diet of poor protein quality and with a marked neutral detergent insoluble nitrogen (NDIN) content (31%), protein slowly available to the animal, and iNDF, especially in the first 60 days of the dry/water transition phase (Table 1), reducing the use of apNDF by microorganisms (Paulino et al., 2008), which is determinant for weight loss of the MM group in this phase (-0.155 kg/day). On the other hand, the synchrony between protein adjustment and the

insertion of rapidly fermentable carbohydrates provided by supplementation was sufficient to meet the needs of heifers for maintenance (ALBA, 0.002 kg/day) or weight gain (MEME, 0.211 kg/day; BAAL, 0.264 kg/day), varying according to the level of supplementation adopted (Table 2).

Therefore, the amount of supplement provided in each period was determinant for the ADG of each treatment due to the low CP content of the forage, however, regardless of the nutritional plan adopted, the supplemented animals obtained even fBW and these were, on average, 46,9 kg higher than the control group (Table 2). This reinforces the thesis that when the CP content of the fodder is less than about 70 g/kg, the supply of protein supplement improves the animal performance, improving the microbial activity of the rumen and the digestibility of the diet (Table 4). In this sense, it is worth mentioning that nutrient intake and growth rate influence age at puberty, but do not affect weight at puberty. The animal at an earlier age, reaching the necessary weight, will enter puberty. Heifers who have a slow growth rate will need a longer time to reach the required weight and, as a result, will initiate the process of transition to puberty at a later age (Emerick et al., 2009).

The absence of difference at the beginning of experiment of the LMA and SFT parameters (Table 2), can be explained by the fact that the animals were nine months old, therefore, very young and from the breeding phase, infants and with grazing in quantity and quality. This reinforces the higher values of LMA and SFT at the beginning when compared to the values observed at the end of the experiment (Table 2).

The LMA analysis allowed us to infer that the supplemented animals, regardless of the amount offered, had less marked muscle tissue growth due to the optimization of gain when infants. However, MM treatment animals had LMA reduction (Table 2), probably because caloric restriction tends to increase protein turnover, increasing the metabolism of amino acids to glucose via gluconeogenesis (Weindruch et al., 2001). MEME and BAAL treatment obtained higher LMA at the end of the experiment.

Body fat reserves can be used to establish the body condition score and define nutritional status (Bruckmaier et al., 1998), have been related to the maintenance of estrous cycle in cattle (Imakawa et al., 1986, Richards et al., 1989) and can act as a marker of available energy for reproductive activity (Hall et al., 1995), acting as a permissive signal that allows ovulation and future conception. In the present study,

animals that received a high level of supplementation after weaning presented higher SFT at the end of the experiment (Table 2), an interesting indicator when working with heifers. According to Semmelmann et al. (2001), Nellore heifers that became pregnant after sexual maturation, 16 to 18 months of age, were heavier and had better body condition.

In some studies, there has been an increase in the total DM and FDM consumption with the protein supplementation of low quality tropical forages, especially when the forage presents levels of CP lower than 70 g/kg of DM (Delcurto et al., 1990; Lazzarini et al., 2009; Sampaio et al., 2010). However, although supplementation allowed higher consumption of total DM by supplemented heifers (Table 3), this was not observed in the consumption of FDM in kg/day. FDM intake is related to digestibility, which primarily reflects the fermentation and feed passage rates of the rumen. Thus, as the cell wall of the diet increases, both rates decrease. Thus, the amount of plant cell wall accounts for a large proportion of the variation in consumption (Van Soest, 1982; Waldo, 1986). Heifers receiving only mineral mix had FDM consumption similar to the consumption of animals supplemented during the dry season. In addition, the BAAL treatment obtained lower intake than the other treatments in kg/day and g/kg of BW in the dry/water transition period, probably due to the substitutive effect caused by the higher consumption of supplemental DM by these heifers (Table 3). Considering the consumption in g/kg of BW of FDM, MM treatment animals obtained higher intake, however, it was observed that animals supplemented with low level of supplementation in the dry period (BAAL), obtained similar consumption, possibly due to the positive effect on the rumen with catalytic supplement doses (0.2% of BW), greatly increasing the performance of this group (0.088 x 0.227).

The average consumption of FMS g/kg of BW was lower than the values of 20.84 g/kg of BW (Couto et al, 2010) and 20.66 g/kg of BW (Valente et al., 2012), perhaps due to the low quality of the forage, not allowing the maximization of pasture consumption, making the effects of multiple supplementation on FDM consumption less evident.

According to Detmann et al. (2003) the filler effect is expected to be the main controlling mechanism of the consumption of animals fed with low quality fodder, justifying the lower DM consumption of MM treatment animals. The iNDF content, which has been attributed a high portion of the ruminal repletion effect of tropical

forages (Vieira et al., 1997), causing a reduction in consumption, was on averaged 27 and 32.6% in the dry season and dry/water transition, respectively (Table 1). Protein supplementation had a positive effect on DM intake, probably increasing the rate of passage of the indigestible residue.

The higher CP intake by the supplemented animals, as supplementation levels increase, may be due to the higher concentrations of these nutrients in the multiple supplement in relation to the forage (Table 3). The apparent digestibility coefficient of CP was higher in animals receiving multiple supplements (Table 4), due to the higher CP intake of higher degradation, since the NDIN content in the supplement was lower than for forage (Table 1). The low digestibility of CP of the animals that received only mineral mixture may be a reflection of the high fiber content and low protein content in the forage used in the present study (Table 1), resulting in a significant proportion of nitrogen in feces (Bohnert et al. 2002).

The increase in total digestibility can be expected with the inclusion of concentrate in the diet of grazing animals, as they have higher digestibility than forage. However, the interaction between digestion of the concentrates and the pasture may or may not alter the digestion of the fiber. In this work, the higher intake of NDF by supplemented animals in the dry / water transition period signals the improvement in latent energy extraction from the supplemented animals (Table 4), and their higher digestibility confirms this better utilization efficiency (Table 5). This result can be attributed to the higher intake of nitrogen compounds by the supplemented animals, which may have had a beneficial effect on the ruminal environment (Dixon & Stockdale, 1999), increasing the microbial growth on the fibrous carbohydrates and increasing the digestibility of these components that promote high filling effect (Lazzarini et al., 2009). This provided a better use of the fodder consumed by the supplemented animals, increasing the availability of the energy of low cost for maintenance and animal growth.

In addition, Mertens (1994) suggested that DM consumption is maximized when the consumption of apNDF is approximately 12.5 g/kg of BW, and is frequently observed with C4 grasses (Bohnert et al., 2011). Data from the present study appear to be in agreement with the above assumptions only in the dry/water transition period for supplemented animals. In the dry period, animals from the MM and BAAL groups had a mean apNDF intake of 12.45 g/kg of BW, but DM consumption was lower than the other treatments that received supplementation,

emphasizing that this relationship should be adopted with caution, analyzing the influence of the quality of the apNDF and the potential of its use with the use of the supplementation on total DM consumption.

Supplementation increased dDM intake by heifers receiving multiple supplements (Table 4). This higher digestibility may be due, in part, to the higher intake of easily digestible nutritional compounds and also to increased digestibility of the pasture (Table 5).

Increases in the TDN content of the diet with supplementation appear to reflect the increase in the digestibility coefficients of CP and apNDF (Table 5). This behavior is reinforced, especially in the dry season, considering that supplementation has increased TDN consumption, without, however, affecting apNDF intake (Table 4).

The digestibilities of DM, OM, CP, apNDF and TDN were higher in the dry period, due to the higher availability of pdDM in this period. In general, the digestibility of all variables was greater, the larger the supplement quantities offered, regardless of the period (Table 4).

The levels of serum urea nitrogen (SUN) are affected by the nutritional level, especially in ruminants, and is a sensitive and immediate indicator of protein intake (Gonzales et al., 2002). Thus, SUN estimates have been used to diagnose the adequacy of the use of nitrogenous compounds in the rumen as a function of the availability of degradable organic matter (Sampaio et al., 2009). On days 15, 45 and 75 the ALBA treatment had higher concentrations of NUS, consistent with the higher CP consumption of the same in the dry season. At day 165, the observed increase was influenced by the higher CP content of the forage (Figure 1). The ideal concentrations of SUN in beef heifers range from 11 to 15 mg/dL (Byers and Moxon, 1980), indicating that heifers, regardless of treatment, were deficient in CP consumption. This fact may have compromised the ruminal microbial activity, preventing greater performance. However, the higher SUN concentration of the supplemented heifers (Table 6) can be attributed to the higher CP intake.

Because of this higher CP intake, there was also a higher excretion of urea nitrogen (urinary nitrogen(UN)/nitrogen intake (NI))by heifers supplemented during the dry season (Table 5). In the dry/water transition, there was no difference in excretion, possibly due to the higher efficiency of N. The higher availability of nutrients required by ruminal microorganisms, such as carbohydrates, ammonia,

peptides, amino acids, sulfur and branched chain fatty acids (Van Soest, 1994), affected microbial growth, explaining the higher microbial production of the supplemented animals (Table 5).

The higher plasma concentrations of NEFA in the non-supplemented animals (Table 6) are indicative of adipose tissue mobilization due to inadequate nutrition (Ospina et al., 2010, Chapinal et al., 2011; Mcart et al., 2012). The behavior in the NEFA concentrations over the days (Figure 1) shows a similar rate of lipolysis of the adipose tissue (Lucy et al., 2002), but more pronounced in the non-supplemented animals, which can be sustained by the lower fBW and SFT in the end of the experiment (Table 2). The increase in NEFA concentrations from day 75 to day 135 in non-supplemented animals is possibly due to the ingestion of low quality forage, and is compensated by the mobilization of these metabolites of adipose tissue (Bell, 1980; Baird, 1982). The NEFA peak at day 135 coincides with the worst quality of forage (Table 1, figure 1). The decrease observed after this period may be indicative of the recovery of the pastures through the first rains, however, the supplemented animals presented mean NEFA of 0.360 against 0.577 of the animals not supplemented, this fact may be associated with the higher conception rate after the Airt for the animals supplemented (Walsh et al., 2007).

The supplemented heifers had higher intake of CP and OM and, consequently, energy, due to the better quality of the supplement over forage. This explains the higher levels of glucose presented by these animals (Table 6), since starch-rich diets increase the production of propionate, which is converted to glucose in the liver, and stimulates the release of insulin.

The concentration of insulin in the blood is often associated with the nutritional status of the animal, since animals under restricted diet have lower blood levels of insulin compared to animals fed without food restriction (Pires et al., 2010). This explains the behavior of the data of the present experiment, since DM consumption of the supplemented animals was higher both in the dry period and in the dry / water transition period (Figure 1). It was observed that the levels of this metabolic hormone varied according to the days of collection, and this variation followed the amount of supplement provided in each period, however the MM treatment animals remained with mean insulin levels 53.3% below the levels presented by the animals supplemented, reflecting the improvement in the energy balance of the animals supplemented during the experimental period (Table 6).

The binding of insulin to its receptor results in a number of metabolic effects, the most important being the transport of glucose into the cells, which is the main source of energy for the ovary (Rabiee et al., 1997, Lawrence et al. ., 2007). In addition, insulin also stimulates steroidogenesis by bovine ovarian cells (Spicer & Echternkamp, 1995) and also regulates the synthesis of GnRH neurotransmitters and, consequently, by controlling the secretion of gonadotrophins, mainly in the release of luteinizing hormone (LH) by the pituitary gland, a major hormones linked to the onset of puberty. Alternatively, insulin can act directly on the ovaries by increasing progesterone production by corpus luteum and granulosa cells (Robinson, 1990). The behavior of the insulin levels is also in agreement with the circulating levels of the hormone progesterone, the increase of insulin levels was accompanied by higher levels of progesterone (Table 6).

Physiologically, puberty is characterized by an increase in the concentration and pulsatile frequency of LH and a decrease in the sensitivity of the hypothalamus to gonadal steroids, which results in the first ovulation (Hafez & Hafez, 2004) and consequent elevation of serum progesterone levels to values greater than 1 ng/ml. Although all treatments had progesterone values above 1 ng/ml, 105 days before AIrt, the values for the supplemented animals were higher than the non-supplemented animals (Table 6), indicating that the supplemented animals were reproducible. The mean PROG concentration of the MM treatment was 1.76 ng/ml, however, this was not enough for these heifers to become pregnant.

Supplemented heifers had a conception rate of 47.2% while heifers of the MM treatment did not conceive (0.0%, table 6). The percentage of heifers, considering all treatments, was 35.4%, a value above those described in the literature involving Nellore animals, which vary between 10 and 18% (Eler et al., 2002; Silva et al. 2003; Eler et al., 2004, Silva et al., 2005). However, according to Lammoglia et al. (2000) if the animal does not undergo selection pressure for precocious puberty, dietary supplementation will not anticipate puberty until the animals acquire a chronological age inherent in the limitation of the breed itself.

5.CONCLUSION

It is concluded that multiple supplementation improves the performance, metabolic status and reproductive performance of heifers in grazing.

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Table 1 - Chemical composition of the supplement and the forage.

Item	Supl.	Forage						<90 ⁵	>90 ⁶
		+15	+45	+75	+105	+135	+165		
Dry matter ²	88,63	49,2	59,8	68,9	66,6	34,5	26,2	59,3	42,4
Organic matter ³	91,13	91,8	91,6	93,6	92,6	91,8	92,4	92,3	92,3
Crude protein ³	20,01	7,15	6,76	4,16	4,72	5,64	9,88	6,02	6,75
NDIN ^{1,4}	30,51	30,5	34,4	35,4	36,1	31,2	21,7	33,4	29,7
Ether extract ³	02,93	1,66	0,41	0,86	0,99	1,06	1,22	0,97	1,09
apNDF ^{1,3}	14,89	70,1	78,9	80,6	79,6	68,8	59,7	76,5	69,4
NFC ^{1,3}	53,30	12,9	5,53	7,98	7,29	16,3	21,6	8,80	15,1
iNDF ^{1,3}	02,48	23,9	26,6	30,6	42,6	36,1	19,2	27,0	32,6

¹/NDIN, neutral detergent insoluble nitrogen; apNDF, neutral detergent fiber corrected for ash and protein; NFC, non-fibrous carbohydrates; iNDF, indigestible neutral detergent fiber. ²/In % of natural matter.³/In % of dry matter.⁴/In % of total nitrogen.⁵/Mean values of the samples obtained by manual grazing simulation in the first 90 days of the experiment. ⁶/Mean values of the samples obtained by manual grazing simulation after 90 days.

Table 2 - Performance and carcass characteristics of heifers supplemented and non-supplemented during the dry season and dry/water transition.

Item ¹	per	Nutrition plans ²				SEM ³	P-value ⁴		
		MM	BAAL	MEME	ALBA		treat	per	treat*per
iBW	1-90	228,3	230,4	239,1	242,6	9,06	0,196	0,001	0,197
	91-180	236,0	250,4	262,3	272,9				
fBW	1-90	236,0	250,4	262,3	272,9	9,92	0,009	0,185	0,049
	91-180	225,6	268,1	276,4	273,1				
ADG	1-90	0,088	0,227	0,263	0,345	0,03	0,001	0,001	0,001
	91-180	-0,155	0,264	0,211	0,002				
LMA	1-90	43,6	45,2	46,8	48,2	1,76	0,042	0,001	0,001
	91-180	38,0	47,2	48,0	43,2				
SFT	1-90	3,13	3,36	3,35	3,50	0,24	0,14	0,001	0,049
	91-180	2,23	3,14	2,88	2,89				

¹/iBW, initial body weight (kg); fBW, final body weight (kg); ADG, average daily gain (kg); LMA, longissimus muscle area (cm²); and SFT, subcutaneous fat thickness (mm).²/MM, mineral mix; BAAL, 0.2% of the BW of supplement in the dry season and 0.6% of the BW of supplement in the dry/water transition; MEME, 0.4% of BW of supplement in dry season and dry/water transition; ALBA, 0.6% of BW of supplement in dry season and 0.2% of BW of supplement in the dry/water transition.³/SEM, standard error mean. ⁴/Indicative of significance (P<0.05).

Table 3 - Consumption of Nellore heifers supplemented or non-supplemented in the dry season and dry/water transition.

Item ¹	per	Nutrition plans				SEM	P-value ²		
		MM	BAAL	MEME	ALBA		treat	per	treat x per
DM	1-90	4,24	4,81	4,95	5,56	0,20	0,003	0,143	0,001
	91-180	4,23	5,07	5,26	5,16				
FDM	1-90	4,24	4,39	4,00	4,09	0,17	0,574	0,087	0,001
	91-180	4,23	3,74	4,26	4,67				
OM	1-90	3,94	4,47	4,62	5,19	0,19	0,002	0,012	0,001
	91-180	3,95	4,77	4,94	4,84				
CP	1-90	0,21	0,30	0,39	0,50	0,02	0,001	0,001	0,001
	91-180	0,38	0,60	0,58	0,52				
apNDF	1-90	2,82	2,98	2,80	2,94	0,12	0,427	0,001	0,001
	91-180	3,00	2,86	3,17	3,39				
dDM	1-90	2,41	2,91	3,16	3,68	0,13	0,001	0,001	0,001
	91-180	2,10	3,00	2,80	2,61				
dNDF	1-90	1,76	1,85	1,81	1,96	0,08	0,147	0,402	0,003
	91-180	1,69	1,74	1,87	2,02				
TDN	1-90	2,37	2,83	3,14	3,69	0,13	0,001	0,001	0,001
	91-180	2,12	2,98	2,89	2,66				
g/Kg of BW									
DM	1-90	18,61	19,97	19,59	20,52	0,19	0,001	0,306	0,001
	91-180	19,27	20,08	19,91	19,84				
FDM	1-90	18,61	18,23	15,83	15,09	0,15	0,001	0,181	0,001
	91-180	19,27	14,84	16,12	17,97				
apNDF	1-90	12,37	12,53	11,08	10,85	0,10	0,001	0,001	0,001
	91-180	13,66	11,34	12,02	13,03				

¹/DM, dry matter; FDM, forage dry matter; OM, organic matter; CP, crude protein; apNDF, neutral detergent fiber corrected for ash and protein; dDM, digested dry matter; dNDF, digested neutral detergent fiber; and TDN, total digestible nutrients. ²/Indicative of significance (P<0.05).

Table 4 - Total apparent digestibility of the constituents of the diet in function of the different treatments.

Item ¹	per	Nutrition plans				SEM	P-value ²		
		MM	BAAL	MEME	ALBA		treat	per	treat x per
DM	1-90	56,80	60,41	63,69	66,07	0,35	0,001	0,001	0,001
	91-180	49,43	59,24	53,05	50,61				
OM	1-90	59,77	62,42	66,70	70,00	0,46	0,001	0,001	0,001
	91-180	53,59	62,56	58,48	55,89				
CP	1-90	40,47	46,45	59,34	62,49	1,13	0,001	0,005	0,001
	91-180	44,72	59,20	50,50	47,16				
apNDF	1-90	62,58	62,02	64,50	66,55	0,59	0,001	0,001	0,001
	91-180	56,42	60,80	58,75	59,53				
TDN	1-90	55,98	58,70	63,24	66,21	0,65	0,001	0,001	0,001
	91-180	49,97	58,67	54,81	51,50				

¹/DM, dry matter; OM, organic matter; CP, crude protein; apNDF, neutral detergent fiber corrected for ash and protein; and TDN, total digestible nutrients. ²/Indicative of significance (P<0.05).

Table 5 - Protein profile of Nellore heifers supplemented and non-supplemented in the dry season and dry/water transition.

Item ¹	per	Nutrition plans				SEM	P-value ²		
		MM	BAAL	MEME	ALBA		treat	per	treat x per
micN	1-90	47,39	53,12	57,97	66,01	3,74	0,022	0,016	0,012
	91-180	43,85	55,36	55,92	52,19				
micEF	1-90	125,2	118,2	115,1	111,7	5,63	0,164	0,256	0,689
	91-180	128,3	116,5	120,0	122,7				
UN/NI	1-90	28,86	44,64	35,83	53,71	5,83	0,349	0,962	0,060
	91-180	42,12	40,97	43,00	37,71				

¹/micN, microbial nitrogen compounds (g/day); micEF, microbial efficiency (g/kg of TDN); and UN/NI, urinary nitrogen(UN)/nitrogen intake (NI)(%). ²/Indicative of significance (P<0.05).

Table 6 - Concentration of metabolites, hormones and reproductive performance in Nellore heifers non-supplemented or supplemented during the dry season and dry/water transition.

Item ¹	Nutrition plans				SEM	P-value ²		
	MM	BAAL	MEME	ALBA		treat	dia	treat x dia
SUN	8,98	9,79	9,95	9,91	0,58	0,017	0,001	0,001
NEFA	0,529	0,381	0,346	0,361	0,04	0,001	0,001	0,033
GLUC	64,73	70,86	70,19	69,73	1,82	0,001	0,040	0,094
INS	0,680	1,453	1,500	1,320	0,16	0,001	0,268	0,003
PROG	1,76	2,90	2,52	2,85	0,32	0,006	0,225	0,701
CR ³	0,00	33,3	58,3	50,0	-	0,020	-	-

¹/SUN, serum urea nitrogen (mg/dL); NEFA, non-esterified fatty acids (mmol/L); GLUC, glucose (mg/dL); INS, insulin (μ IU/mL); PROG, progesterone (ng/mL); and CR, conception rate (%). ²/ Indicative of significance ($P < 0.05$). ³Performed by the Chi-Square test ($P < 0.05$).

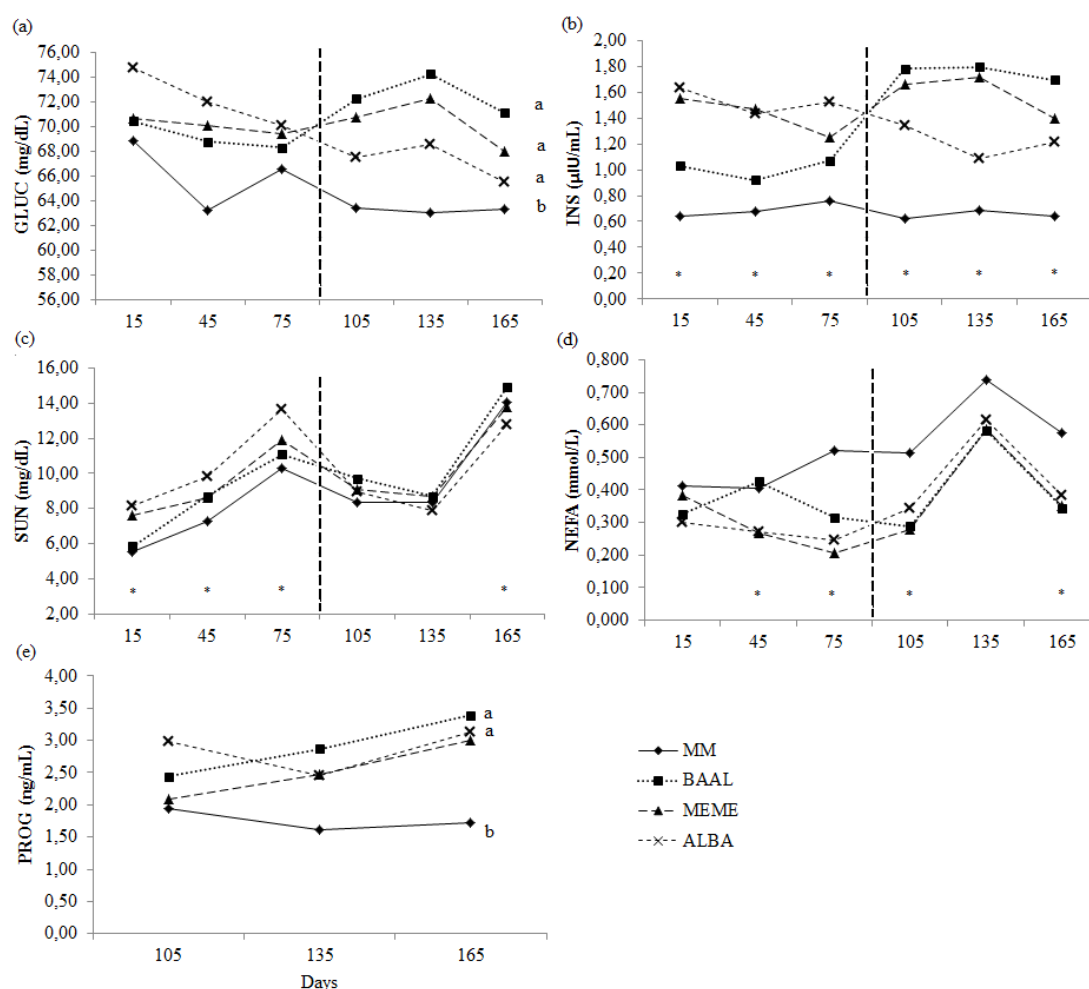


Figure 1 – Concentrations of GLUC (a), INS (b), SUN (c), NEFA (d) and PROG (e) in heifers supplemented or non-supplemented in the dry season and dry/water transition. a, b, means of treatment followed by different letters, differed by Student's t test ($P < 0.05$). */Indicative of significance of interaction treat x per ($P < 0.05$).

CHAPTER 4

ESTIMATION OF DAILY MILK YIELD OF NELLORE BEEF COWS IN GRAZING

ABSTRACT - Studies with lactating beef cows have only done one milking and doubled this production in order to determine daily milk production. The objective was to determine the correct relation between the morning and afternoon lactating Nellore cows in a pasture (*Uruchloa decumbens*). Eighty Nellore cows (six months of gestation) were used, with a mean initial weight of 515.5 ± 1.34 kg and an initial body condition score of 4.68 ± 0.15 , respectively. The experimental design was completely randomized in a factorial scheme, with four treatments, and twenty repetitions. The strategies evaluated were: PRMM - supplementation with 1 kg of supplement/cow/day only in prepartum; MMPS - supplementation with 1 kg of supplement/cow/day only in postpartum period; PRPS - supplementation with 1 kg of supplement/cow/day in pre and postpartum; MMMM - only mineral mix ad libitum during pre and postpartum. Milk samples were collected on days 45, 135 and 225 postpartum (initial, middle and final lactation, respectively). On each of these days, a milk collection was performed in the morning and another in the afternoon. No effects were observed of pre and postpartum supplementation or interaction on milk yield (MY), including milk yield in the morning, milk yield in the afternoon, afternoon/morning proportion, afternoon/total proportion and milk yield corrected to 4% fat (MY4F) ($P < 0.05$). The afternoon/morning proportion of 0.451 in the initial third of lactation was higher than the other thirds (mean and final), which had a proportion of 0.414 ($P > 0.05$). There was no effect of pre and postpartum supplementation or interaction on milk fat (MF) and milk lactose (ML) in the morning and afternoon ($P > 0.05$). Pre-partum supplementation or interaction did not affect milk protein (MP) and total milk solids (TS) in the morning and afternoon ($P > 0.05$). Postpartum supplementation increased P in the morning and afternoon milking, and increased TS values only at morning milking ($P < 0.05$). There was also no effect of pre and postpartum supplementation, or interaction on the afternoon/morning proportion of MF, MP, ML and TS ($P > 0.05$), showing that the content of each component analyzed was similar in both the morning milking and afternoon milking. It was concluded that the proportions found (afternoon/morning of 45.1% in the initial third and 41.4% in the remaining thirds) are recommended to determine the

daily milk yield of Nellore beef cows in grazing. Milk composition is similar in morning and afternoon milks.

Key words: Beef cows, Nellore, milk yield.

1. INTRODUCTION

In the literature, three methods have been used to estimate the daily milk yield in beef cows: 1) estimation of milk yield by the indirect method of calf suckling, in which calves are separated from cows at dusk, and after twelve hours after separation, they are weighed, suckled for 30 minutes, and weighed again. The difference in weight is then multiplied by two, estimating the milk production in the 24-hour period (Melton et al., 1967; Rutledge et al., 1971; Bartle et al., 1984; Beal et al., 1990; Pimentel et al., 2005); 2) direct method for milk yield, where the calves are separated from the cows for a period of 6 hours and put to suck again at 17:30. This procedure aims at the exhaustion of the udder. After calving, the calves are separated from the cows again at 18:00 and remain in the cattle shed. The cows are released in a picket line with grass and water. The next day at 6:00 a milking is performed, which is performed in two quarters of the udder (one front and one rear), and the production multiplied by 2 in order to obtain production of the udder, and again multiplied by 2 to estimate the production in 24 hours (Ribeiro et al., 1991; Restle et al., 2003); and 3) direct method for milk yield, in which the calves are separated from the cows at 18:00. The cows remain on the picket and are milked at 6:00 the next day. After obtaining the milk yield in 12 hours, it is multiplied by 2 for estimation of the milk yield in the 24-hour period (Valente et al., 2013; Lopes et al., 2014; Barros et al., 2015; Silva et al., 2016).

Although the methods cited are internationally accepted, the data found in the literature for milk yield in dairy cows indicate that they do not produce in the afternoon the same amount of dairy production observed at milking in the morning (Gilbert et al. Kendall et al., 2006).

Thus, we hypothesize that in beef cows, the daily distribution of milk yield follows the same behavior observed for dairy cows, and milk yield in the morning is different from that in the afternoon. Therefore, as no data were found in the literature on the distribution of beef cow's milk yield throughout the day, the objective was to

determine the afternoon/morning proportion of milk yield for use in research with lactating Nellore cows in the system of grazing.

2.MATERIAL AND METHODS

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV 08/2015).

The experiment was conducted at the Universidade Federal de Viçosa, located in the municipality of Viçosa-MG (20°45'S and 42°52' W), between July 2013 and June 2014, referring to the dry season, dry-water transition, water and water/dry transition. The experiment lasted 360 days, beginning 90 days before calving (prepartum) and 270 days after calving (postpartum).

Eighty Nellore cows (six months of gestation) were used, with a mean initial weight of 515.5 ± 1.34 kg and an initial body condition score of 4.68 ± 0.15 , respectively.

An experimental area of 70 hectares was assigned to the animals, consisting of four 17.5 ha paddocks, covered uniformly with the *Uruchloa decumbens* grass, equipped with drinking fountains and troughs, which were covered and accessible from both sides. The animals were rotated between the paddocks every 14 days, aiming to control the possible effects of paddocks on the treatments (pasture availability, location of water and trough, relief, shading and others).

This work was developed in parallel with a companion paper, which aimed to verify the effects of different strategies of the supply of pre and postpartum supplementation on reproductive efficiency and the productive, hormonal and metabolic parameters in Zebu beef cows (Almeida et al., 2017). The experimental design was completely randomized in a factorial scheme, with four treatments and twenty repetitions. The strategies evaluated were: PRMM - supplementation with 1 kg of supplement/cow/day only in prepartum (90 days); MMPS - supplementation with 1 kg of supplement/cow/day only in the 90 days postpartum; PRPS - supplementation with 1 kg of supplement/cow/day in the pre (90 days) and postpartum (90 days); MMMM –only mineral mix *ad libitum* during the pre (90 days) and postpartum (90 days). After 90 days postpartum, all treatments received only mineral mix *ad libitum* until the end of the experiment. 1 kg/cow/day of a single supplement of 25% crude protein was given at 10:00 a.m., formulated through the

food composition data provided by BR-CORTE 2.0 (Valadares et al., 2010), with 25% protein (centesimal composition of the supplement: ground corn grain, 24.65; ground sorghum grain, 24.65; soybean meal, 45.7; mineral mix, 5). The centesimal composition of the mineral mix: dicalcium phosphate, 50.00; sodium chloride, 47.2; zinc sulfate, 1.50; copper sulfate, 0.70; cobalt sulfate, 0.05; potassium iodate, 0.05; and manganese sulphate, 0.5.

The cows' milk yield was evaluated through three production samples, at 45 (initial third), 135 (middle third) and 225 (final third) days after calving. The cows were separated from their offspring at 2:00 p.m. and remained in the picket. At 5:00 p.m. the calves were fed for 30 minutes in order to deplete the milk produced by the cows, and then they were separated again, with the cows being released in the paddock. Milking was performed at 5:30 a.m. (12 hours after calving separation) and at 5:30 p.m. on the following day (24 hours after calving separation). The cows were trapped in the containment trunk and their legs were properly tied to facilitate handling. The milking was performed mechanically and the cows were milked in the same sequence in both periods. The milk secretion was stimulated with 2 mL of oxytocin (10 UI/mL, Ocitovet ®, Brazil) in the mammary artery, initiating milking immediately after oxytocin administration. After each milking, the milk was weighed and duly registered. The total production was made up of the sum of the collections in the two schedules. Individual samples of 50 mL of homogenized milk from each milking were taken for analysis of protein (MP), fat (MF), lactose (ML) and total solids (TS). Samples were stored at 4°C in a refrigerator using a bronopol tablet per sample as preservative. Milk samples were analyzed using spectroscopy (Foss MilkoScan FT120, Hillerød, Denmark).

The milk produced was corrected to 4% fat, calculated by the following equation (NRC, 2001): $\text{Milk 4\% (kg)} = 0.4 \times (\text{milk production}) + [15 \times (\text{fat production} \times \text{milk production}/100)]$.

Statistical analyzes

The results were submitted to analysis of variance, adopting a completely randomized design, in a factorial scheme 2x2, being the factors the inclusion or not of supplement in the pre and postpartum. In addition, the third of lactation was included in the model as time-repeated measures. The PROC MIXED procedure of the SAS software (Statistical Analysis System, SAS University Edition) was applied

for all statistical analyzes. In the case of significance of the effect of lactation thirds, lsmeans were compared using the student's t test, adopting $\alpha = 0.05$ as the critical level of type I error probability. The denominator degrees of freedom were calculated using the approximation Kenward-Roger.

3.RESULTS

There was no effect of pre and postpartum supplementation, or interaction on milk yield, milk yield in the morning, milk yield in the afternoon, afternoon/morning proportion, afternoon/total proportion and milk yield corrected for 4% fat (MY4F) ($P < 0.05$; Table 1).

The MP was higher in the initial third of lactation (7.22 liters / day), followed by the second third of lactation (5.00 liters / day) and finally, the final third of lactation (4.11 liters / day) ($P < 0.05$, Table 1, Figure 1). Milk yield in the morning and afternoon also followed the same disposition of total MP, with higher values in the first third of lactation, intermediate values in the second third, and lower yields in the third third ($P < 0.05$; Figure 1). This gradual reduction of milk yield with the advancement of lactation is a natural process, and has been reported in several studies with beef cows (Leal & Freitas, 1982; Alencar et al., 1988; Restle et al., 2003) and dairy cows (Gengler, 1996; Tekerli et al., 2000; Van Der Linde et al., 2000).

In agreement with our hypothesis, the afternoon/morning proportion followed the relationship close to that observed in dairy cows, so that the cows in the afternoon milking did not double the milk yield observed in the morning (Figures 1 and 2). The afternoon/morning proportion observed in the initial third of lactation was higher than the other thirds (mean and final), being represented by 0.451; that is, 45.1% ($P < 0.05$; Figure 2), while in the other thirds, the proportion did not differ, being represented by 0.414 ($P > 0.05$; Figure 2). These differences observed between morning and afternoon milk productions are a result of the circadian system, which affects daily milk production (Harvatine, 2012; Plaut and Casey, 2012). The biological mechanisms that lead to higher yields in the morning milking are not clear, but some points, such as higher incidence of nocturnal grazing (Kendall et al., 2006; Fisher et al., 2002) and a longer cooling period at night (Igono et al., 1992) observed in dairy cows may also explain these higher yields of beef cows in grazing. However, the lower milk yield observed in afternoon milking is a reflection

of diurnal rhythm (Klopčič et al., 2013), influenced mainly by high temperatures in the tropics (Davis et al., 1988; Spiers et al., 2004).

According to Schaeffer and Rennie (1976) and Lee and Wardrop (1984), the lactation third is an important source of variation in the estimation of the daily production in dairy cows. In this study, the difference observed in the afternoon/morning proportion (45.1% for the initial third and 41.4% for the middle and final third), shows that there is a variation in the lactation third for beef cows as well. Thus, the afternoon/total proportion observed in the initial third was also higher (0.309, $P < 0.05$, Figure 3) than in the last two thirds, which did not differ among each other (0.290, $P > 0.05$, Figure 3). Kendall et al. (2006) observed a 36.1% of afternoon/total proportion for dairy cows in the first third of lactation, which are values close to those found in this study.

There was no effect of pre and postpartum supplementation or interaction on milk fat (MF) and milk lactose (ML) in the morning and afternoon ($P > 0.05$; Table 2). Pretreatment supplementation or interaction did not affect milk protein (MP) and total milk solids (TS) in the morning and afternoon ($P > 0.05$). Postpartum supplementation increased MP in the morning and afternoon milking by the same magnitude and increased TS values only at the morning milking ($P < 0.05$; Table 2).

There was no effect of pre, postpartum supplementation or interaction on the afternoon/morning proportion of MF, MP, ML and TS ($P > 0.05$; Table 2), showing that each analyzed component was similar in both morning and afternoon milking. This may have happened due to the fact that beef cows have low milk yield when compared to dairy cow production, so possibly, these low volumes produced were not sufficient for variations in milk composition to be observed. In addition, similar values found for milk components reinforce the importance of afternoon/morning and afternoon/total proportion found in this work to estimate milk yield in Nellore cows.

The MF presented similar values in the initial and final third ($P > 0.05$), and these were higher than the mean value obtained in the middle third ($P < 0.05$; Figure 4). The values found were within the range observed in the NRC review (2001) (between 2.79 and 5.27%).

The MP presented an increasing behavior with the advancement of lactation ($P < 0.05$, Figure 4). Protein is one of the components of milk that can vary considerably, both between genetic groups of cows and between cows of the same

genetic group. These values can vary between 3.11 and 3.65% (NRC, 2001). In this study, mean values of the morning and afternoon milking found were between 3.12% in the initial third and 3.64% in the final third (Figure 4).

In the initial third of lactation, the ML had higher values than those observed in the middle and final lactation ($P < 0.05$, Figure 4). There was no difference between the values found in the middle and final lactation ($P > 0.05$). The observed ML contents were within the limits of the NRC (2001), which vary from 3.84% to 5.66%.

Regarding the TS, lower values were observed in the third of initial and medium lactation compared to those observed in the final third ($P < 0.05$). There was no difference between the values found in the initial and middle third ($P > 0.05$; Figure 4). These data corroborate the inferences of Rutledge et al. (1971), that this component increases its content with the advancement of lactation.

4.CONCLUSION

The proportions found in this work (afternoon/morning from 45.1% for initial third and 41.4% for medium and final third lactation) are recommended to determine the daily milk yield of Nelore beef cows in grazing. Milk composition is similar in morning and afternoon milks.

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Table 1 - Effects of supplementation on pre, postpartum or interaction on MY, MY morning, MY afternoon, MY afternoon/morning, MY afternoon/total and MY4F.

Item ¹	P-value						
	Pre	Pos	Pre x Pos	Third	Pre x Third	Pos x Third	Pre x Pos x Third
MY	0,561	0,185	0,970	0,001	0,269	0,903	0,468
MYm	0,900	0,058	0,772	0,001	0,353	0,427	0,467
MY a	0,181	0,934	0,626	0,001	0,275	0,414	0,631
MY a/m	0,310	0,053	0,370	0,012	0,848	0,121	0,812
MY a/t	0,252	0,074	0,355	0,009	0,888	0,117	0,770
MY4F	0,956	0,096	0,733	0,001	0,663	0,999	0,546

¹MY, daily milk yield; MY m, milk yield in the morning; MY a, milk yield in the afternoon; MY a/m, proportion of milk yield afternoon/morning; MY a/t, proportion of milk yield afternoon/total; and MY4F, milk yield corrected to 4% fat. ²Pre, effect of prepartum supplementation; Post, effect of postpartum supplementation; Pre x Post, interaction between prepartum supplementation and postpartum supplementation; Third, effect of the lactation third; Pre x Third, interaction between prepartum and lactation supplementation; Post x Third, interaction between postpartum and lactation supplementation; Pre x Post x Third, interaction between prepartum supplementation, postpartum supplementation and lactation third.

Table 2 - Effects of supplementation on pre, postpartum or interaction on milk components in morning and afternoon milking and afternoon/morning proportion.

Item ¹	P-value ²						
	Pre	Pos	Pre x Pos	Third	Pre x Third	Pos x Third	Pre x Pos x Third
Fm	0,679	0,125	0,183	0,006	0,087	0,938	0,145
F a	0,647	0,475	0,162	0,006	0,297	0,763	0,497
F a/m	0,870	0,059	0,667	0,591	0,052	0,398	0,117
P m	0,108	0,004	0,677	0,001	0,654	0,523	0,834
P a	0,067	0,003	0,675	0,001	0,208	0,571	0,124
P a/m	0,440	0,464	0,559	0,497	0,114	0,755	0,113
L m	0,829	0,329	0,073	0,001	0,402	0,133	0,873
L a	0,281	0,406	0,101	0,001	0,494	0,096	0,976
L a/m	0,111	0,330	0,858	0,002	0,305	0,395	0,764
TS m	0,719	0,022	0,515	0,001	0,151	0,822	0,329
TS a	0,490	0,092	0,413	0,001	0,267	0,657	0,697
TS a/m	0,472	0,072	0,202	0,179	0,075	0,542	0,053

¹F m, milk fat at milking in the morning; F a, milk fat at milking in the afternoon; Fa/m, afternoon/morning proportion of milk fat; P m, milk protein at milking in the morning; P a, milk protein at milking in the afternoon; P a/m, afternoon/morning proportion of milk protein; L m, milk lactose at milking in the morning; L a, milk lactose at milking in the afternoon; L a/m, afternoon/morning proportion of milk lactose; TS m, milk total solids at milking in the morning; TS a, milk total solids at milking in the afternoon; TSa/m, afternoon/morning proportion of milk total solids.²Pre, effect of pre-partum supplementation; Post, effect of postpartum supplementation; Pre x Post, interaction between pre-partum supplementation and postpartum supplementation; Third, effect of the lactation third; Pre x Third, interaction between pre-partum and lactation supplementation; Post x Third, interaction between postpartum and lactation supplementation; Pre x Post x Third, interaction between pre-partum supplementation, postpartum supplementation and lactation third.

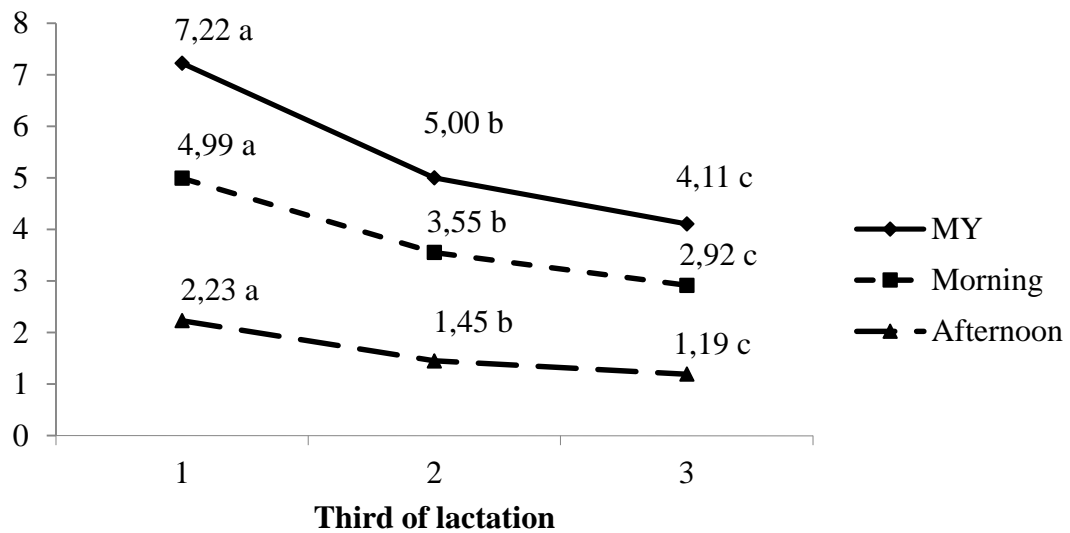


Figure 1 - Morning, afternoon and total milk yield. a, b, c; means followed by different letters, differed by Student's t-test ($P < 0.05$).

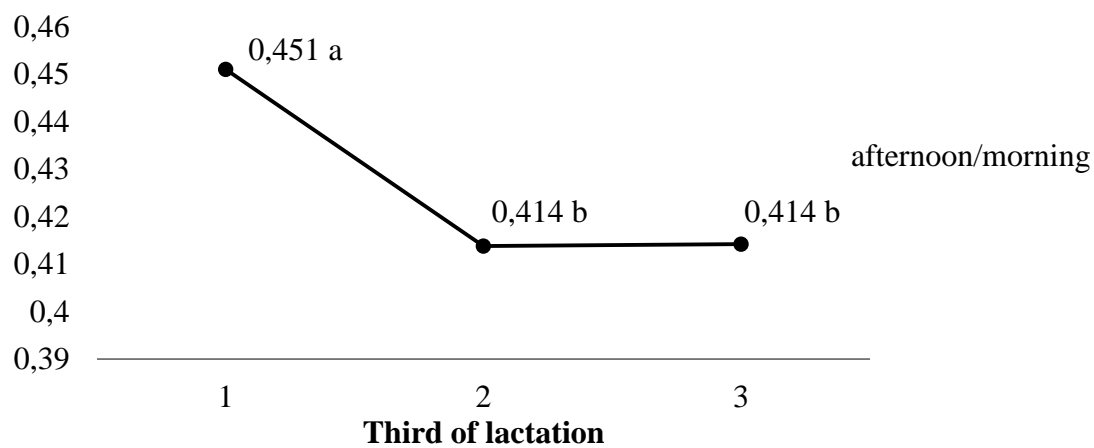


Figure 2 - Afternoon/morning proportion of milk yield. a,b; means followed by different letters, differed by Student's t-test ($P < 0.05$).

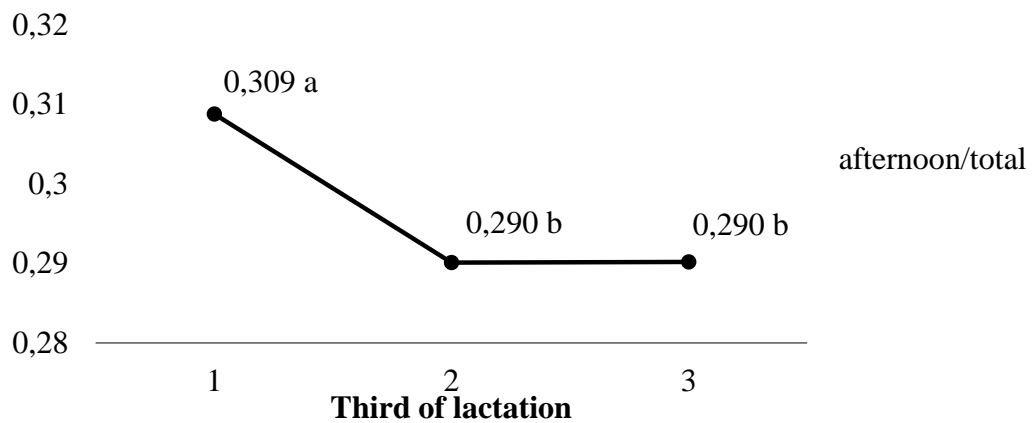


Figure 3 – Afternoon/total proportion of milk yield. a,b; means followed by different letters, differed by Student’s t-test (P<0.05).

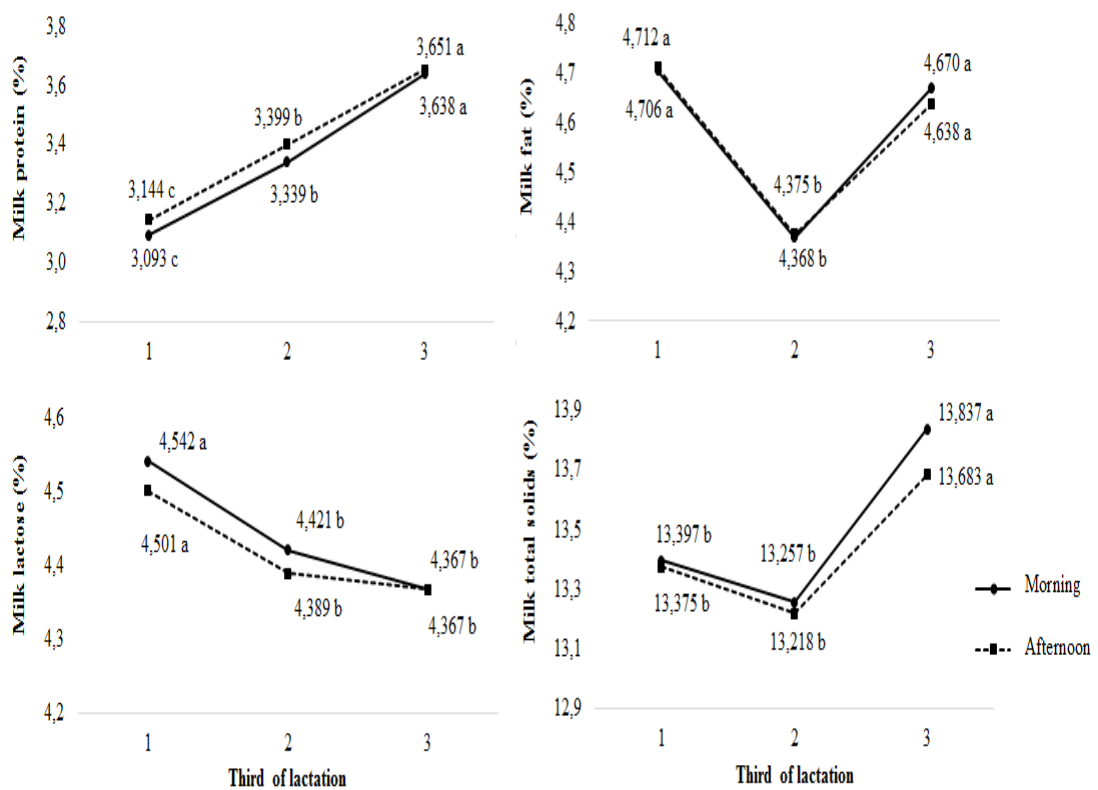


Figure 4 - Percentage values for protein, fat, lactose and total solids of milk. a, b, c; means followed by different letters, differed by Student’s t-test (P<0.05).