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Effects of nutritional strategies on metabolic and productive responses in Nellore heifers under grazing

Letícia Zamberlan Pistillo
Doctor Scientiae

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

Adviser: Claudia Batista Sampaio

Co-adviser: Edenio Detmann

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Assent:

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Claudia Batista Sampaio
Adviser

Dedication

To God, my parents, my husband,
and all those who supported me throughout this journey.

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First, I thank God for taking care of me and for giving me strength and direction at all times.
To my mother, Leda, for always believing in my potential, even when I did not believe in myself.
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ABSTRACT

PISTILLO, Letícia Zamberlan, D.Sc., Universidade Federal de Viçosa, May, 2026. **Effects of nutritional strategies on metabolic and productive responses in Nellore heifers under grazing**. Adviser: Claudia Batista Sampaio. Co-adviser: Edenio Detmann.

This thesis investigated the effects of different nutritional strategies during the rearing period on growth performance, metabolic responses, and developmental parameters of grazing Nellore heifers. Nutritional management during the post-weaning and prepubertal phases is essential to ensure adequate growth before the reproductive period, particularly in tropical grazing systems, where animal performance is strongly influenced by forage availability and seasonal variation in forage quality. Thus, two studies were conducted to evaluate supplementation strategies aimed at supporting growth efficiency. The first study evaluated a stair-step supplementation strategy (SST), comparing continuous gain (CONG) with compensatory gain (COMPG). Forty nulliparous heifers were assigned to CONG, receiving 5 g/kg of concentrate supplement for 120 days, or COMPG, receiving 2 g/kg for 60 days followed by 8 g/kg for 60 days. A treatment × day interaction was observed for BW and ADG ($p < 0.001$). CONG showed greater performance during the initial phase, but COMPG reached similar final BW by the end of the experiment. BCS, body measurements, and fat thicknesses were mainly affected by day ($p < 0.001$), whereas ribeye area showed a treatment × day interaction ($p < 0.005$). Metabolic responses showed treatment × day interactions for total protein, IGF-1, and SUN ($p < 0.036$, $p < 0.001$, and $p < 0.001$, respectively). These results indicate that SST promoted compensatory growth without impairing final development. The second study evaluated yeast supplementation during the prepubertal phase. Thirty-six nulliparous heifers were assigned to concentrate supplements containing yeast cell wall product (YCW), or live yeast, either Saccharomyces cerevisiae (SC) or Saccharomyces boulardii (SB). A treatment × day interaction was observed for BW ($p = 0.018$), with SB tending to show greater final BW than SC, whereas YCW was intermediate. ADG increased over time ($p = 0.016$), but no consistent treatment effect was observed. No treatment effects were detected for ribeye area ($p > 0.05$), whereas subcutaneous fat increased over time ($p < 0.001$). Glucose and SUN increased ($P = 0.024$ and $p = 0.043$), while IGF-1 decreased ($p = 0.046$) over time. Reproductive variables were not affected by treatments ($p > 0.05$). Overall, yeast-based additives had limited effects on performance, metabolic responses, and reproductive parameters under grazing conditions. Collectively, these findings show that nutritional strategies can be used during the rearing phase of Nellore heifers without compromising growth and development.

Keywords: *Bos indicus*; compensatory gain; prepubertal development; tropical grazing systems; yeast supplementation

RESUMO

PISTILLO, Letícia Zamberlan, D.Sc., Universidade Federal de Viçosa, maio de 2026. **Efeitos de estratégias nutricionais nas respostas metabólicas e produtivas de novilhas Nelore em regime de pastagem**. Orientadora: Claudia Batista Sampaio. Coorientador: Edenio Detmann.

Esta tese investigou os efeitos de diferentes estratégias nutricionais durante o período de recria sobre o desempenho produtivo, as respostas metabólicas e os parâmetros de desenvolvimento de novilhas Nelore em pastejo. O manejo nutricional durante as fases de pós-desmame e pré-púbere é essencial para garantir crescimento adequado antes do período reprodutivo, particularmente em sistemas tropicais de pastejo, nos quais o desempenho animal é fortemente influenciado pela disponibilidade de forragem e pela variação sazonal da qualidade da forragem. Assim, dois estudos foram conduzidos para avaliar estratégias de suplementação voltadas ao suporte da eficiência de crescimento. O primeiro estudo avaliou uma estratégia de suplementação stair-step (SST), comparando ganho contínuo (CONG) com ganho compensatório (COMPG). Quarenta novilhas nulíparas foram distribuídas em CONG, recebendo 5 g/kg de suplemento concentrado por 120 dias, ou COMPG, recebendo 2 g/kg por 60 dias, seguido de 8 g/kg por 60 dias. Foi observada interação tratamento × dia para peso corporal (BW) e ganho médio diário (ADG) ($p < 0{,}001$). O grupo CONG apresentou maior desempenho durante a fase inicial, mas o grupo COMPG alcançou BW final semelhante ao final do experimento. O escore de condição corporal (BCS), as medidas corporais e as espessuras de gordura foram influenciadas principalmente pelo dia de avaliação ($p < 0{,}001$), enquanto a área de olho de lombo apresentou interação tratamento × dia ($p < 0{,}005$). As respostas metabólicas apresentaram interações tratamento × dia para proteína total, IGF-1 e nitrogênio ureico sérico (SUN) ($p < 0{,}036$, $p < 0{,}001$ e $p < 0{,}001$, respectivamente). Esses resultados indicam que a SST promoveu crescimento compensatório sem prejudicar o desenvolvimento final. O segundo estudo avaliou a suplementação com leveduras durante a fase pré-púbere. Trinta e seis novilhas nulíparas foram distribuídas em suplementos concentrados contendo produto de parede celular de levedura (YCW) ou levedura viva, seja Saccharomyces cerevisiae (SC) Saccharomyces boulardii (SB). Foi observada interação tratamento × dia para BW ($p = 0{,}018$), com SB tendendo a apresentar maior BW final que SC, enquanto YCW foi intermediário. O ADG aumentou ao longo do tempo ($p = 0{,}016$), mas nenhum efeito consistente de tratamento foi observado. Não foram detectados efeitos de tratamento para área de olho de lombo ($p > 0{,}05$), enquanto a gordura subcutânea aumentou ao longo do tempo ($p < 0,001$). Glicose e SUN aumentaram ($p = 0,024$ e $p = 0,043$), enquanto IGF-1 reduziu ($p = 0,046$) ao longo do tempo. As variáveis reprodutivas não foram afetadas pelos tratamentos ($p > 0,05$). De modo geral, os aditivos à base de leveduras apresentaram efeitos limitados sobre desempenho, respostas metabólicas e parâmetros reprodutivos sob condições de pastejo. Coletivamente, esses achados demonstram que estratégias nutricionais podem ser utilizadas durante a fase de recria de novilhas Nelore sem comprometer o crescimento e o desenvolvimento.

Palavras-chave: *Bos indicus*; desenvolvimento pré-púbere; ganho compensatório; sistemas de pastejo tropical; suplementação com leveduras

SUMMARY

Introduction

Most beef cattle production systems in Brazil occur under grazing conditions, where climatic seasonality markedly affects forage availability and quality. The weaning period in southeastern region of Brazil often coincides with the dry season, when forage crude protein contents decline and lignin concentration increases, imposing nutritional limitations that restrict microbial growth and animal performance, ultimately compromising growth patterns and overall productivity (Detmann et al. 2009; Detmann et al. 2014). This phase represents a critical period that may influence long-term reproductive performance and the retention of females within the herd.

The performance of heifers during the post-weaning period has a direct impact on subsequent developmental and reproductive stages (Cardoso et al. 2014; Moriel et al. 2014; D'Occhio et al. 2019; Miszura et al. 2021; Paula et al. 2022), particularly in systems aimed at producing replacement females. Achieving adequate body and metabolic readiness before breeding is essential for reproductive success in *Bos indicus* heifers, which are expected to reach 60–65% of their mature body weight at the onset of the first breeding season (Whittier et al. 2005; Freetly et al. 2011).

Adequate growth patterns directly influence body composition, endocrine development, and consequently the timing of puberty in heifers and subsequent reproductive outcomes. Due to the seasonality in tropical conditions, often characterized by low-quality forage, supplementation programs during the post-weaning phase may maximize productivity (Detmann et al. 2009; Pereira et al. 2025).

Overall, nutritional status and metabolic hormone concentrations are tightly regulated in response to changes in growth patterns and body composition, initiating several signals at the onset of the reproductive phase (D'Occhio et al. 2019; Cardoso et al. 2020). Circulating signals such as leptin, kisspeptin, glucose, insulin-like growth factor 1 (IGF-1), insulin, among others, act as permissive and regulatory mediator linking energy balance to endocrine control, influencing key neuroendocrine pathways such as GnRH and LH secretion, which are essential for the onset of puberty (Pires et al. 2010; Hausman et al. 2012; Cardoso et al. 2020).

Therefore, implementing effective nutritional strategies during the post-weaning period represents a critical opportunity for management intervention in tropical grazing systems. Different approaches have been proposed to mitigate nutritional limitations and support adequate growth and physiological development. The stair-step strategy (SST) has been used during the post-weaning period, as an alternative to continuous gain, and is often associated with changes in several metabolites and hormones that allow animals to achieve similar outcomes (Luna-Pinto and Cronjé, 2000; Cardoso et al. 2014; Miszura et al. 2021). Currently, strategies using more sustainable and natural products have gained increasing attention and have been widely used. In this context, some studies evaluating yeast supplementation have reported improvements, mainly in the ruminal fermentation and stability. However, these effects may translate into improvements in performance (Chaucheyras-Durand et al. 2008; Batista et al. 2021; Baker et al. 2022).

Despite the recognized relevance of post-weaning nutrition, there is still limited understanding of how dynamic nutritional strategies, such as stair-step supplementation, interact with metabolic regulation in grazing *Bos indicus* heifers under tropical conditions. In addition, although yeast and yeast-derived products have shown variable effects on beef cattle performance. While live yeast supplementation has not consistently improved performance in previous studies (Sartori et al. 2017), positive responses have been reported under specific challenging conditions (Batista et al. 2021). Thus, ruminal changes induced by yeast supplementation are not always translated into improved animal performance. This inconsistency may be attributed to differences in experimental methodologies, dosage and yeast strains, among others factors (Baker et al. 2022). Therefore, the effects of these strategies on growth patterns, metabolic signaling, and physiological development of grazing heifers under tropical conditions have not been fully elucidated, particularly during early development.

In this context, this thesis evaluated two nutritional strategies during the post-weaning period: a stair-step supplementation strategy and yeast supplementation (live yeast and yeast-derived products). It was hypothesized that these supplementation strategies could support growth performance and metabolic responses, promoting adequate body development and physiological conditioning of grazing Nellore heifers under seasonal variation in forage quality.

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CHAPTER 1

STAIR-STEP NUTRITIONAL STRATEGY PROMOTES PERFORMANCE RECOVERY AND ENHANCES METABOLIC RESPONSES IN GRAZING NELLORE HEIFERS

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Chapter 1: Stair-step nutritional strategy promotes performance recovery and enhances metabolic responses in grazing Nellore heifers

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Abstract

This study evaluated the effects of a stair-step supplementation strategy (SST) on performance and metabolic responses of grazing Nellore heifers during post-weaning phase. Forty nulliparous (224 ± 22 kg; 9 ± 0.57 months) were assigned to two nutritional plans using concentrate supplements: continuous gain (CONG: 5 g/kg for 120 days) and compensatory gain (COMPG: 2 g/kg for 60 days followed by 8 g/kg for 60 days). Performance, body condition score (BCS), carcass traits, and metabolic responses such as glucose, serum urea nitrogen (SUN), total protein (TP), albumin, globulin, and insulin-like growth factor 1 (IGF-1) were evaluated. Data were analyzed using the GLIMMIX procedure of SAS ($p < 0.05$). A treatment × day interaction was observed for BW and average daily gain ($p < 0.001$). On day 60, CONG had the highest BW, but both groups achieved similar BW by the end of the experimental period. Differences in BCS occurred for day of evaluation ($p < 0.001$). Rump and withers heights increased over time ($p < 0.001$). Ribeye area showed A treatment × day interaction ($p < 0.005$), whereas fat thicknesses were affected by day ($p < 0.001$). Glucose decreased ($p < 0.001$) and globulin increased over time ($p < 0.001$), while TP, IGF-1, and SUN showed A treatment × day interaction ($p < 0.036$; $p < 0.001$; $p < 0.001$, respectively). TP and IGF-1 increased in COMPG but remained stable in CONG. SUN increased in both groups, with a greater increase in CONG. Albumin decreased ($p < 0.001$), less in COMPG. In conclusion, the SST strategy can be used without impairing performance and development of grazing heifers.

Keywords: Beef cattle; Compensatory gain; Females; Growth; Pasture; Supplementation

Introduction

Supplementation programs aimed at promoting continuous gain have been widely adopted in strategic nutrition programs to enable genetically superior animals to fully express their growth potential, particularly in systems focused on the development of precocious females without negatively affecting body composition, endocrine development, or the timing of puberty in heifers (Moriel et al. 2014; Moriel et al. 2017; Almeida et al. 2019).

Strategic supplementation programs during the post-weaning phase may mitigate the effects of seasonality in tropical systems and ensure adequate heifer development, which is essential for the establishment of hormonal and metabolic signals associated with the onset of reproductive function (D'Occhio et al. 2019). As an alternative to strategies that promote continuous gains, research has explored approaches such as the stair-step nutritional strategy (SST), which exploits the physiological effects of compensatory gain during specific phases of growth and metabolic conditioning before subsequent productive stages (Cardoso et al. 2014; Moriel et al. 2014; Rosasco et al. 2020; Miszura et al. 2021).

The SST consists of alternating periods of controlled nutritional restriction and realimentation, aiming to induce compensatory growth through metabolic adaptations that improve nutrient use efficiency (Park et al. 1987; Lynch et al. 1997; Cardoso et al. 2014; Miszura et al. 2021; Moriel et al. 2022). The magnitude of these responses is strongly dependent on the degree and duration of restriction, particularly in prepubertal heifers, which represent a critical window of metabolic and reproductive programming. When appropriately applied, this strategy may reduce feed inputs without compromising growth potential or reproductive development (Park et al. 1987; Lopes et al. 2018; Clariget et al. 2024).

This may represent a strategy to reduce supplementation levels during specific periods without compromising the hypothalamic–pituitary–ovarian axis activity (Luna-Pinto and Cronjé 2000), which is responsible for signaling within a complex neuroendocrine network that regulates diverse functions, including reproductive function, by sensing the availability of metabolic fuels and permitting reproductive processes (Luna-Pinto and Cronjé 2000; Cardoso et al. 2014; Miszura et al. 2021). From a metabolic perspective, compensatory gain triggered by SST is associated with changes in key hormones and metabolites, such as leptin, insulin, insulin-like growth factor-1 (IGF-1), and glucose (Luna-Pinto and Cronjé 2000; Cardoso et al. 2014; Miszura et al. 2021), which may influence neuroendocrine regulation and subsequent reproductive function (Armstrong et al. 2001; Schneider 2004).

Considering that the post-weaning period coincides with the dry season in southeastern Brazil, when forage availability and nutritive value are markedly reduced, we hypothesized that

SST would allow grazing Nellore heifers to maintain adequate BW development and metabolic status during the prepubertal period, while achieving final performance comparable to that of heifers under continuous supplementation. Therefore, we aimed to evaluate the effects of SST on growth performance and metabolic responses in grazing Nellore heifers as an alternative nutritional strategy under seasonal conditions of limited forage quality.

Materials and methods

Location, animals, management, and treatments

The experiment was conducted at the Research, Teaching and Extension Unit in Beef Cattle of the Federal University of Viçosa (UFV), Minas Gerais, Brazil. All animal care and handling procedures were approved by the Ethics Committee on the Use of Production Animals of UFV (protocol number 068/2023).

Forty nulliparous Nellore beef heifers with an initial BW of 224 ± 22.4 kg and 9 ± 0.6 months of age were used. Heifers were randomly divided into eight groups, each containing five to seven animals per paddock, which was considered the experimental unit. Groups were continuously stoked in eight paddocks covered with *Uruchloa decumbens* grass, each equipped with covered feed bunks and water troughs and measuring 3.2 hectares.

The experimental period lasted 120 days, from July to November, and two supplementation strategies were evaluated to impose distinct patterns of nutrient supply and growth. In the continuous gain treatment (CONG), heifers received 5 g/kg of BW of a commercial supplement (Probeef Nutripec, Cargill®, São Paulo, Brazil; 198.4 g CP/kg DM) throughout the entire experimental period, aiming to provide a constant moderate nutrient supply. In the compensatory gain treatment (COMPG), heifers received 2 g/kg of BW of a commercial supplement (Probeef Nutripec Sprint, Cargill®, São Paulo, Brazil; 432.3 g CP/kg DM) during the first 60 days, followed by 8 g/kg of BW of a mixture composed of 15% commercial supplement (Probeef Nutripec CP, Cargill®, São Paulo, Brazil) and 85% ground corn (138.3 g CP/kg DM) during the remaining 60 days. The greater CP concentration of the COMPG supplement during the first phase was mainly related to the greater contribution of non-protein nitrogen from urea, aiming to provide ruminal nitrogen under low forage CP conditions while maintaining a restricted total supplement supply. In the second phase, when forage CP concentration increased, the COMPG supplement had a lower CP concentration and lower urea contribution, but was offered at a greater level to increase total nutrient and energy supply and stimulate compensatory gain. Therefore, differences in supplement CP concentration and ingredient composition were intentional and defined according to the nutritional objective of each growth strategy and the changes in forage chemical composition over the experimental period.

A concentrate supplement (Table 1) was provided daily at 11h, and orts, if any, were weighed prior to the morning feeding. To adjust the supplement supply, heifers were weighed monthly, without fasting (Silva and Detmann 2025).

Experimental procedures and sampling

Heifers were weighed without fasting (Silva and Detmann 2025) at the beginning of the experiment, at 60 days (level change of supplementation in COMPG), and at 120 days (the end of the experiment), always at 8h and quantified average daily gain (ADG). The body condition score (BCS), rump height, and height at withers were recorded at the same time. The BCS was assessed by three experienced evaluators on a scale ranging from 1 to 9 (NRC 2001) and averaged across evaluators.

Carcass ultrasound measurements were also performed on day 60, and on day 120. The ribeye area (REA) and subcutaneous fat thickness between the 12th and 13th ribs (rib fat) were measured at the *Longissimus dorsi*. Subcutaneous fat thickness over the *Gluteus biceps* was also measured (rump fat). Carcass images were recorded using an Aloka SSD500II ultrasound device, equipped with an 18 cm linear probe and the BioSoft Toolbox® II for Beef software (Biotronics Inc., Ames, Iowa, USA).

Forage samples (Table 2) were collected on days 60 and 120 by hand-plucking sampling to evaluate the chemical composition of the forage. At the same time points, forage availability was estimated by collecting five random samples per paddock using a metal square (0.5 × 0.5 m), which were cut approximately 1 cm above the soil surface. All samples were oven-dried (55 °C) and ground in a knife mill (Model 2; Arthur H. Thomas, Philadelphia, PA, USA) to pass through a 2-mm sieve. Simultaneously, half of each ground sample was reground to pass through a 1-mm sieve. Samples were composited by paddock for subsequent analyses. Supplement samples were collected at the same time points and processed as previously described.

Blood samples were collected by jugular venipuncture using vacuum tubes with clot activator and serum separation gel (BD Vacutainer SST II Plus, São Paulo, Brazil) at 60 days and at 120 days, always at 08h, to quantify the blood concentrations of glucose, serum urea nitrogen (SUN), total protein, albumin, globulin and IGF-1. After collecting, the samples were centrifuged at 3,600 × g for 15 minutes, and the serum was frozen at -20 °C for later analysis.

Laboratory analysis and calculations

Supplement and forage samples processed through a 1-mm sieve were analyzed for dry matter (DM; method G-003/1), ash (method M-001/2), crude protein (CP; method N-001/2), ether extract (EE; method G-005/2), and neutral detergent fiber corrected for ash and protein (apNDF; methods F-013/1, M-002/2, and N-004/2, respectively. Indigestible neutral detergent fiber (iNDF) was evaluated in samples processed through a 2-mm sieve and incubated in situ for 288 hours (iNDF; method G-009/1). All analyses were performed according to the procedures described by Detmann et al. (2025).

Blood glucose concentration was quantified by the enzymatic-colorimetric method. Urea was quantified by the enzymatic method, and values were converted to serum urea nitrogen (SUN) using the factor 0.466. The concentrations of total protein and albumin were analyzed by the colorimetric method, and the concentration of globulin was calculated by subtracting albumin from total protein. Serum concentrations of IGF-1 were quantified by a chemiluminescent method. All analyses were performed in a commercial laboratory (Viçosalab®, Viçosa, Minas Gerais, Brazil).

The compensatory index was calculated according to Hornick et al. (2000), using the following equation:

$$\text{Compensatory index (\%)} = [(\text{A} - \text{B}) / \text{A}] \times 100$$

Where A represents the difference in body weight between groups at the end of the restricted growth period, and B represents the difference in body weight between groups at the end of the compensatory growth period.

Statistical analysis

The statistical analysis of the data followed the basic model:

$$Y_{ijk} = \mu + T_i + P_{(i)j} + \varepsilon_{(ij)k}$$

Where Y_{ijk} is the response measured in animal k, managed in paddock j submitted to treatment i, μ is the general constant, Ti is the fixed effect of the treatment, $P_{(i)j}$ is the random effect of the experimental unit (paddock) where j represents the paddock nested within treatment i, and $\varepsilon_{(ij)k}$ is the random error, assumed to be NIID $(0, \sigma^2_\varepsilon)$.

All variables measured were evaluated using the proposed model, considering the supplementation strategy, as repeated measures over time using the GLIMMIX procedure of SAS (Statistical Analysis System, version 9.4). When significant effects were detected, least-squares means were compared by pairwise comparisons. When the interaction was significant, simple effects were evaluated using the SLICE option. Differences were declared significant at $p < 0.05$.

Results

An interaction between treatment and day of evaluation ($p < 0.001$) was observed for BW. On day 60, treatments differed, with CONG showing the highest BW (Figure 1), being 16 kg heavier than COMPG. However, both treatments achieved similar BW at the end of the experimental period. A similar treatment × day interaction was observer for ADG ($p < 0.001$; Table 3). During the first 60 days, CONG had greater ADG than COMPG, with ADG values of 346 and 138 g/day, respectively. By day 120, this response pattern shifted: COMPG continued to show low weight gain, whereas CONG showed lost BW loss.

Furthermore, BCS was affected by a treatment × day interaction ($p = 0.015$; Figure 2). Both treatments showed a decrease in BCS between days 60 and 120, although, this reduction was less pronounced in COMPG. There was an effect of day on rump height and height at withers, with values increasing over the experimental period and following a similar pattern ($p < 0.001$; Figure 3 and 4). Similarly, carcass characteristics (Table 3), such as rib fat and rump fat, increased over time ($p < 0.001$). For REA, a treatment × day interaction was observed ($p < 0.05$), with both treatments showing increases over time, although COMPG showed an almost twofold greater increase than CONG (12.1 vs. 6.6 cm^2).

The blood parameters (Table 4) such as glucose and globulin were affected only by evaluation day ($p < 0.001$). Glucose decreased over time, whereas globulin showed the opposite pattern. Total protein ($p < 0.05$) and IGF-1 ($p < 0.001$) showed a treatment × day interaction. Heifers in COMPG showed a marked increase in IGF-1 concentrations between days 60 and 120, whereas CONG showed a slight decline. A similar response was observed in total protein, which increase in COMPG but remained stable in CONG. SUN ($p < 0.001$) and albumin ($p < 0.05$) were also affected by treatment × day interactions, although they followed distinct response pattens. SUN concentrations increased over time, particularly in CONG. In contrast, albumin concentrations decreased over time, whit a smaller reduction observed in COMPG.

Discussion

The nutritional strategy used in the present study, referred to as SST, was designed to simulate a controlled low-gain phase followed by a period of greater nutritional support, a pattern that commonly occurs in grazing systems during and after periods of limited forage quality. The treatment × day interactions observed for BW and ADG indicate that COMPG heifers were able to achieve similar final BW compared with CONG heifers, despite lower gains during the initial phase. Thus, the SST was effective in promoting compensatory growth and may represent an alternative nutritional strategy for systems in which heifers experience reduced gains during the dry season. The compensatory index calculated according to Hornick et al. (2000) was 56.25%, indicating a moderate compensatory response in the present study.

From a physiological perspective, this response may involve metabolic adaptations induced during the controlled low-gain phase, including reductions in maintenance energy requirements and metabolic rate, as well as changes in the gastrointestinal tract, which have been associated with improved nutrient-use efficiency during the subsequent refeeding period (Carstens et al. 1991; Yambayamba et al. 1996). As a result, animals may recover part of the growth not achieved during the initial phase and exhibit improved growth efficiency during the compensatory phase, as previously reported in studies evaluating compensatory growth and stair-step nutritional programs (Park et al. 1987; Lynch et al. 1997; Freetly et al. 2001; Cardoso et al. 2014; Rosasco et al. 2020; Miszura et al. 2021).

The initial reduction in growth rate, followed by subsequent compensatory gain, was evident in the ADG response. This pattern, together with the increase in total protein and IGF-1 concentrations in COMPG, supports the interpretation that the SST provided adequate nutritional conditions for recovery and continued development during the second phase. In contrast, continuous supplementation resulted in stabilization of the growth curve, suggesting the formation of a plateau in CONG, whereas COMPG maintained a more continuous growth trajectory throughout the experimental period (Figure 1).

Compensatory gain is commonly associated with changes in metabolic hormones and metabolites, including IGF-1, insulin, leptin, and glucose, which reflect nutrient availability and metabolic status (Luna-Pinto and Cronjé 2000; Cardoso et al. 2014; Miszura et al. 2021). In the present study, the marked increase in IGF-1 in COMPG between days 60 and 120 provides physiological support for the observed compensatory response. Because IGF-1 is closely related to growth and anabolic activity (Zulu et al. 2002; Velazquez et al. 2008; Sartori et al. 2013), its increase suggests that heifers under SST had greater metabolic support for tissue accretion during the higher supplementation phase. Therefore, the metabolic profile observed in COMPG is consistent with the recovery in ADG and the achievement of similar final BW compared with CONG.

Importantly, the objective of the SST during the prepubertal phase is not to suppress growth, but to promote a controlled growth trajectory in which animals maintain BW or achieve modest weight gain during the low-gain phase. This approach allows heifers to recover during the subsequent higher supplementation phase and reach adequate BW before the breeding season. Previous studies have indicated that heifers can reach 60–65% of mature BW at the onset of breeding, which is critical for early puberty, reproductive performance, and adequate development (Whittier et al. 2005; Freetly et al. 2011). In this context, the similar final BW observed between COMPG and CONG suggests that SST did not compromise the growth target required for subsequent reproductive development.

Rump height, height at withers, and carcass characteristics, such as rib fat and rump fat, increased over the experimental period, with no treatment effect. These findings indicate that SST did not impair structural growth or body reserve deposition. BCS and ribeye area showed treatment × day interactions. On day 120, COMPG heifers showed a greater increase in ribeye area than CONG heifers, with increases of 12.1 and 6.6 cm^2, respectively. This response suggests that the compensatory phase favored muscle development in COMPG. Although BCS slightly decreased in both treatments on day 120, the reduction was smaller in COMPG than in CONG (-0.25 vs. -0.59, respectively). The slight decrease in BCS may indicate that energy supply was not sufficient to promote increases in body reserves in either treatment. Nevertheless, BCS remained within adequate ranges throughout the experimental period, which is important because heifers should present adequate BCS at the beginning of the breeding season to optimize reproductive performance and productivity during the cow-calf phase (Cooke et al. 2021). Overall, these results indicate that the nutritional plan was sufficient to support structural growth, muscle accretion, and adequate body condition during this developmental phase.

Contrary to expectations, glucose concentrations decreased over time, regardless of supplementation plan, suggesting a temporal adjustment in energy metabolism. This response may be related to changes in forage conditions, energy availability from the diet, or greater peripheral use of glucose as heifers progressed through the experimental period. However, the decrease in glucose should not be interpreted alone as evidence of impaired energy status, since COMPG heifers maintained positive ADG and showed increased IGF-1 concentrations at day 120. Thus, glucose dynamics likely reflected the balance between hepatic glucose production and tissue utilization rather than a direct effect of supplementation plan.

SUN was affected by the treatment × day interaction, increasing in CONG from day 60 to day 120, whereas it remained relatively stable in COMPG. Although greater SUN could be expected in COMPG because the greater amount of supplement offered during the second phase resulted in an approximately 12% higher crude protein supply than in CONG, SUN concentration is not determined only by the amount of crude protein supplied through the supplement. Rather, it reflects the integration of nitrogen intake, ruminal nitrogen metabolism, hepatic urea synthesis, urea recycling, excretion, and tissue nitrogen demand (Hammond 1998). In this context, nitrogen utilization and animal performance in cattle consuming tropical forages are influenced by the interaction between forage quality, nitrogen supply, and available energy (Detmann et al. 2014). Additionally, increased SUN may also be associated with amino acid mobilization for gluconeogenesis (Ferreira et al. 2021). Thus, the lower SUN observed in COMPG may be related to the positive ADG and greater IGF-1 concentration observed at day 120, suggesting greater anabolic activity and possibly greater use of absorbed nitrogen during the compensatory phase. Conversely, the increase in SUN in CONG, together with the lack of sustained BW gain during the final period, may also reflect greater amino acid mobilization or protein catabolism relative to productive nitrogen use. Therefore, the SUN response appears to reflect differences in nitrogen metabolism between supplementation strategies rather than being explained by supplement allowance alone.

The concomitant decrease in albumin and increase in globulin indicate that circulating protein fractions changed over time. Albumin is considered an indicator of nitrogen status (Payne 1987), and an increase in its concentration could be expected, especially in COMPG heifers, because of the greater supplement allowance during the second phase. However, albumin is a relatively slower-response indicator of protein metabolism, and its decrease from day 60 to day 120, observed in both supplementation strategies, may not directly follow the improvement in forage crude protein observed during the final period. In CONG heifers, the greater decrease in albumin occurred simultaneously with increased SUN and lack of sustained BW gain, suggesting that the greater SUN concentration was not accompanied by better maintenance of serum albumin or sustained productive response. In contrast, COMPG heifers showed stable SUN, a smaller reduction in albumin, and increased total protein and globulin concentrations, which coincided with the gradual increase in BW and greater IGF-1 concentration at day 120. Together, these responses suggest a temporal modulation of protein metabolism and support greater anabolic activity during the compensatory phase.

Overall, these findings suggest that a controlled low-gain phase followed by a higher supplementation phase did not impair heifer development. Instead, COMPG heifers were able to compensate for the lower initial gains and achieve similar final BW compared with continuously supplemented heifers. This reinforces the potential efficiency of SST as a nutritional management strategy for grazing replacement heifers, particularly in systems where seasonal variation in forage quality can limit growth during specific periods. The results of the present study are consistent with previous findings demonstrating that compensatory growth can allow heifers to reach adequate BW before the breeding season, thereby supporting subsequent productive and reproductive phases (Cardoso et al. 2014; Miszura et al. 2021; Moriel et al. 2022).

Conclusion

The SST strategy can be used without impairing performance and development of grazing heifers. The results also suggest that metabolic and hormonal adaptations, indicated by changes in metabolites such as IGF-1 and total protein, may contribute to improved performance in subsequent productive phases. However, it is important to emphasize that this strategy was designed to simulate and manage production systems often characterized by low weight gain during the dry season under grazing conditions, rather than to intentionally restrict developmental potential during this critical growth phase.

Authors contribution:

Conceptualization, Claudia Batista Sampaio and Edenio Detmann; Methodology, Claudia Batista Sampaio and Edenio Detmann; Formal analysis, Letícia Zamberlan Pistillo; Investigation, Letícia Zamberlan Pistillo; Resources, Claudia Batista Sampaio; Data acuration, Letícia Zamberlan Pistillo, Claudia Batista Sampaio and Edenio Detmann; Writing—original draft preparation, Letícia Zamberlan Pistillo and Claudia Batista Sampaio; Writing—review and editing, Letícia Zamberlan Pistillo, Claudia Batista Sampaio and Edenio Detmann; Visualization, Claudia Batista Sampaio; Supervision, Claudia Batista Sampaio; Project administration, Claudia Batista Sampaio; Funding acquisition, Claudia Batista Sampaio.

Statements and declarations

Ethics approval

The animal care and handling procedures were approved by the Ethics Committee on the Use of Production Animals of the UFV (Protocol number: 068-2023).

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Conflict of interest

The authors declare no conflicts of interest.

Data availability

The data generated during the current study is available from the corresponding author upon reasonable request.

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Table 1 Chemical composition of the supplements offered to grazing Nellore heifers under continuous and compensatory gain strategies

	Supplementation strategy[a]		
	CONG	COMPG	
Item[a]	5 g/kg	2 g/kg	8 g/kg
Organic matter (g/kg DM)	807.4	728.8	900.5
Crude protein (g/kg DM)	198.4	432.3	138.3
Ether extract (g/kg DM)	29.1	23.4	37.0
apNDF (g/kg DM)	64.3	33.0	45.0

[a]DM: Dry matter; apNDF: Neutral detergent fiber corrected for ash and protein; CONG: Continuous gain; COMPG: Compensatory gain.

Table 2 Chemical composition and availability of the forage during the experimental period

	Sampling period	
Item[a]	Day 60	Day 120
Forage availability (ton DM/ha)	2.32	2.81
Dry matter (g/kg as fed)	808.5	255.0
Organic matter (g/kg DM)	936.1	902.2
Crude protein (g/kg DM)	26.8	113.2
apNDF (g/kg DM)	762.1	524.0
iNDF (g/kg DM)	406.5	236.6

[a]DM: Dry matter; apNDF: Neutral detergent fiber corrected for ash and protein; iNDF: Indigestible neutral detergent fiber.

Forage availability was sampled using a metal square, whereas all other variables were obtained using the hand-plucking method.

Table 3 Performance and *in vivo* carcass measurements of grazing Nellore heifers under continuous and compensatory nutritional strategies

	Treatments[a]						
	CONG		COMPG		*P*-value		
Item[a]	d60	d120	d60	d120	Treatment (T)	Day (D)	T × D
ADG, g/day	346±24.5	-39±30.2	138±23.	108±29.5	0.37	<0.001	<0.001
Ribeye area (cm^2)	39.3±2.28	45.9±1.95	30.7±2.24	42.8±1.88	0.061	<0.001	0.005
Rib fat (mm)	3.3±0.17	3.7±0.18	2.9±0.17	3.6±0.18	0.063	<0.001	0.089
Rump fat (mm)	2.7±0.14	3.0±0.16	3.0±0.14	3.5±0.16	0.83	<0.001	0.055

[a]ADG: average daily gain; CONG: Continuous gain; COMPG: Compensatory gain.

Values are presented as mean ± standard error of the mean.

Table 4 Blood parameters of grazing Nellore heifers under continuous and compensatory nutritional strategies

	Treatments[a]						
	CONG		COMPG		*P*-value		
Item[a]	d60	d120	d60	d120	Treatment (T)	Day (D)	T × D
Glucose (mg/dL)	62.0±1.80	54.4±1.62	62.4±1.77	59.0±1.60	0.28	<0.001	0.077
SUN (mg/dL)	8.5±0.43	13.9±0.52	10.1±0.42	10.4±0.51	0.12	<0.001	<0.001
Total protein (g/dL)	7.29±0.118	7.22±0.140	7.17±0.116	7.49±0.137	0.65	0.17	0.036
Albumin (g/dL)	3.5±0.07	2.8±0.06	3.4±0.07	2.9±0.06	0.70	<0.001	0.037
Globulin (g/dL)	3.8±0.09	4.4±0.13	3.7±0.07	4.5±0.10	0.80	<0.001	0.13
IGF-1 (ng/mL)	149.1±6.01	145.5±10.60	104.2±5.86	178.4±10.33	0.58	<0.001	<0.001

[a] SUN: Serum urea nitrogen; IGF-1: Insulin-like growth factor-1; CONG: Continuous gain; COMPG: Compensatory gain.

Values are presented as mean ± standard error of the mean.

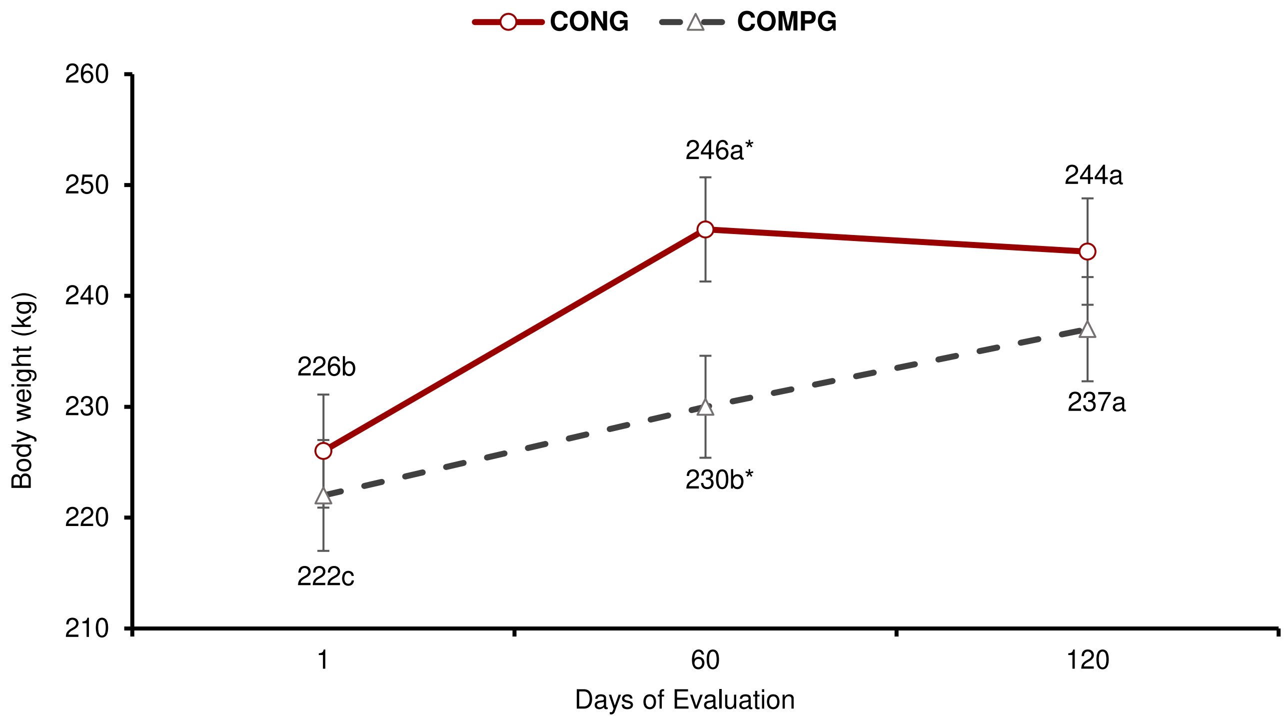

Fig. 1 Body weight of grazing Nellore heifers during the prepubertal phase in the dry under continuous and compensatory gain strategies: COMPG (compensatory gain; dashed line) and CONG (continuous gain; solid line). Means within each treatment followed by different letters differ across days of evaluation ($p < 0.05$), indicating differences over time within each strategy. Means on the same evaluation day marked with an asterisk (*) differ between treatments ($p < 0.05$). Effects: Treatment ($p = 0.21$); Day ($p < 0.001$); T × D ($p < 0.001$).

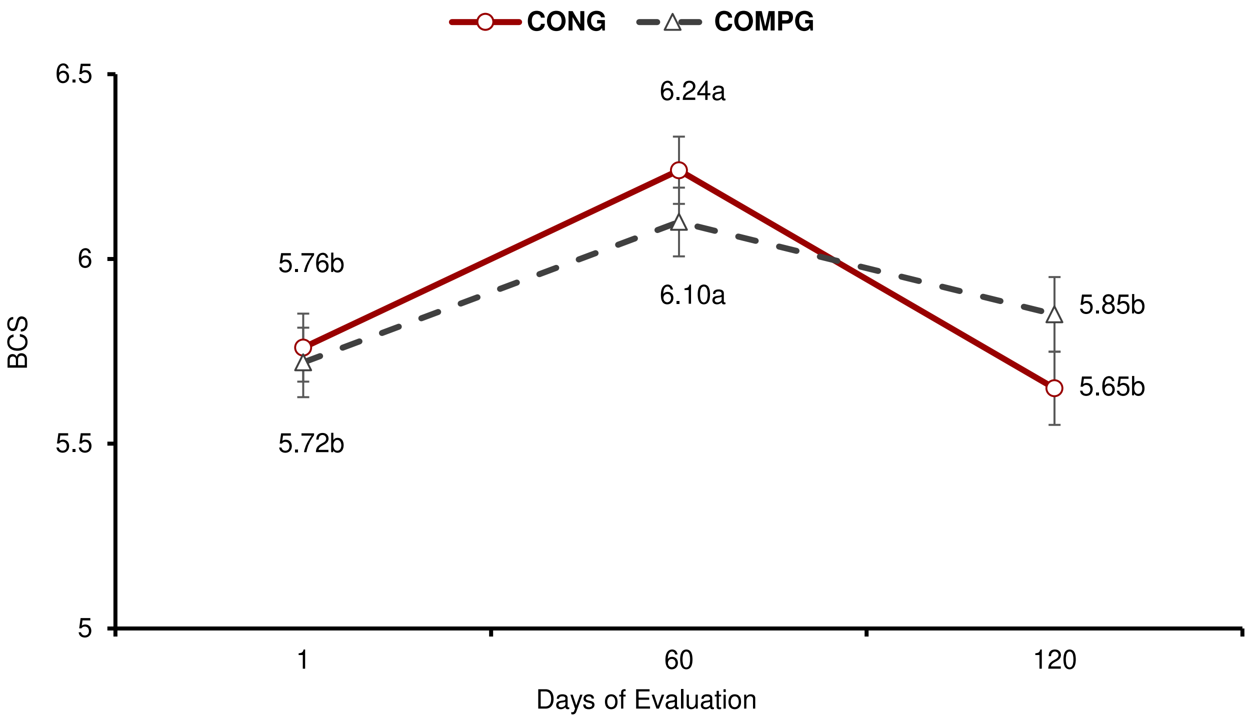

Fig. 2 Body condition score of grazing Nellore heifers during the prepubertal under continuous and compensatory gain strategies: COMPG (compensatory gain; dashed line) and CONG (continuous gain; solid line). Means within each treatment followed by different letters differ across days of evaluation ($p < 0.05$), indicating differences over time within each strategy. Effects: Treatment ($p = 0.95$); Day ($p < 0.001$); T × D ($p = 0.015$).

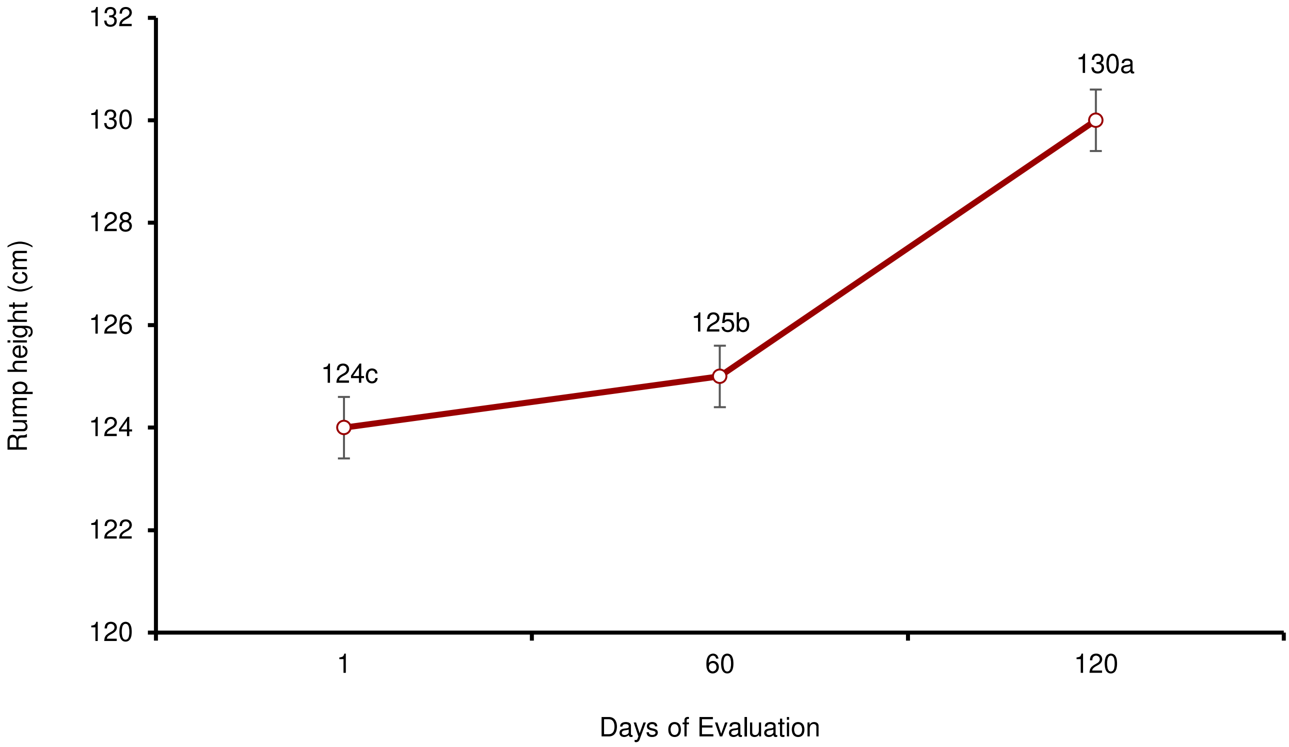

Fig. 3 Rump height of grazing Nellore heifers during the prepubertal phase under continuous and compensatory gain strategies. Means followed by different letters differ among days of evaluation ($p < 0.05$). Effects: Treatment ($p = 0.80$); Day ($p < 0.001$); T × D ($p = 0.100$).

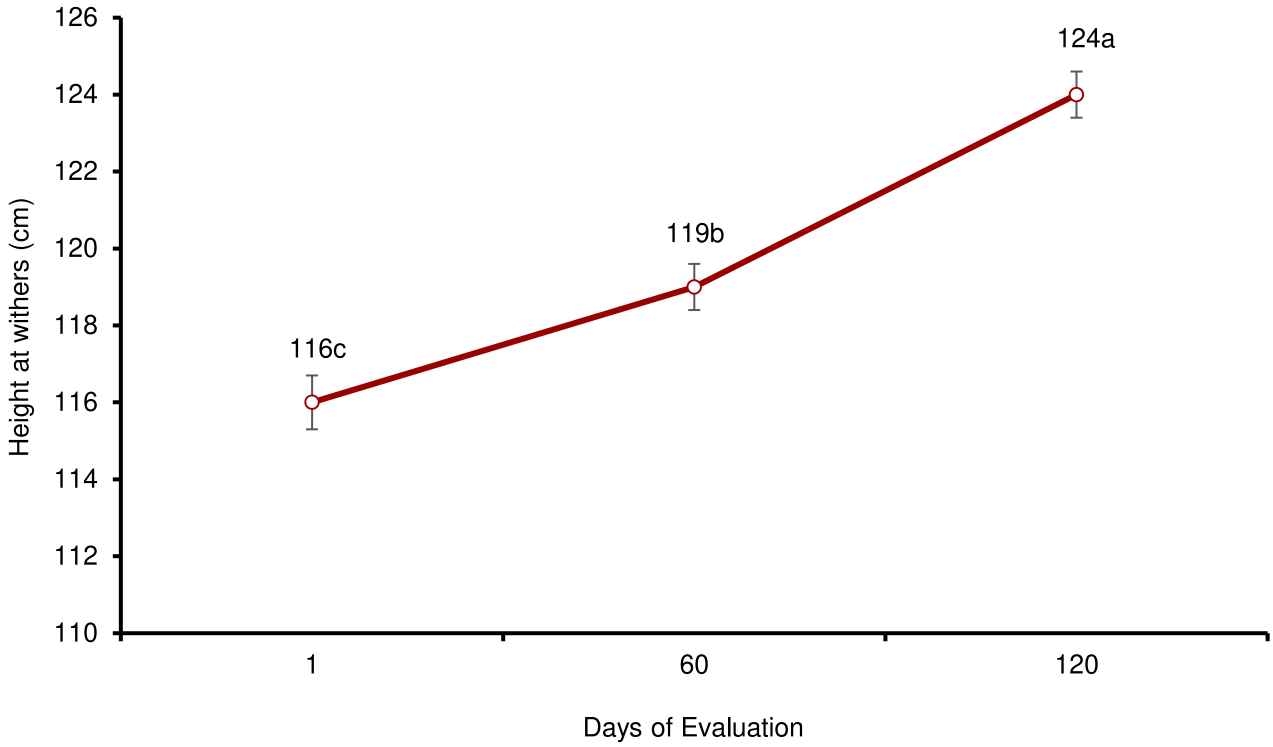

Fig. 4 Height at withers of grazing Nellore heifers during the prepubertal phase under continuous and compensatory gain strategies. Means followed by different letters differ among days of evaluation ($p < 0.05$). Effects: Treatment ($p = 0.70$), Day ($p < 0.001$); T × D ($p = 0.065$).

CHAPTER 2

EFFECTS OF LIVE YEAST OR YEAST-DERIVED PRODUCTS SUPPLEMENTATION DURING THE PREPUBERTAL PERIOD ON GROWTH PERFORMANCE AND METABOLIC RESPONSES OF GRAZING NELLORE HEIFERS

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Chapter 2: Effects of live yeast or yeast-derived products supplementation during the prepubertal period on growth performance and metabolic responses of grazing Nellore heifers

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ABSTRACT

This study evaluated the effects of different yeast-derived products during the prepubertal period on growth performance and metabolic responses of grazing Nellore heifers. Thirty-six nulliparous heifers (263 ± 2.7 kg; 12 ± 0.09 months) were assigned to three treatments consisting of concentrate supplements containing a yeast cell wall product (YCW) or live yeast, either *Saccharomyces cerevisiae* (SC) or *Saccharomyces boulardii* (SB). The experiment lasted 120 days, with each phase lasting 60 days during the prepubertal period. Performance, carcass traits, blood metabolites, and ovarian follicular dynamics were analyzed using the GLIMMIX procedure of SAS ($p < 0.05$). A treatment × day interaction was observed for BW ($p = 0.018$), with SB tending to show greater final BW than SC, whereas YCW was intermediate. ADG increased over time ($p = 0.016$). No treatment effects were detected for ribeye area ($P > 0.05$), whereas subcutaneous fat increased over time ($p < 0.001$). Glucose and SUN increased (p = 0.024 and 0.043), while IGF-1 decreased ($p = 0.046$) over time. Reproductive variables were not affected ($p > 0.05$) by treatments. The development of grazing Bos indicus beef heifers during the prepubertal phase was primarily determined by overall nutrient availability and forage quality rather than by the type of yeast-based additive supplied.

Keywords: adequate body weight; beef cattle; *Bos indicus*; feed additives; tropical conditions; rearing; reproductive readiness

Introduction

In grazing *Bos indicus* animals, the period from weaning to first breeding represents a critical window for nutritional interventions (Moriel et al. 2014; Almeida et al. 2019; Paula et al. 2022). Adequate nutrient supply during this phase is fundamental to support body growth, metabolic maturation, and physiological development before the onset of the first reproductive period (Cardoso et al. 2014). Readiness for reproduction in Zebu heifers is commonly assessed based on the proportion of mature body weight (BW), integrating growth, nutrient utilization, and physiological development (Moriel et al. 2014; Almeida et al. 2019; Paula et al. 2022).

In this context, nutritional strategies aimed at improving rumen function and nutrient utilization are essential to support adequate body development and metabolic preparedness before the reproductive season, particularly during periods of declining forage quality. Natural feed additives have emerged as alternatives to conventional nutritional approaches, and yeast supplementation is among the most widely studied options. Batista et al. (2021), in a meta-analysis, reported that yeast supplementation can enhance ruminant performance, although the magnitude of response is generally modest, likely due to improvements in ruminal environment, animal health, and nutrient digestibility. Thus, the potential benefits of yeast supplementation are mainly associated with its role in modulating ruminal dynamics and improving nutrient use efficiency.

Live yeast supplementation has been associated with improvements in ruminal dynamics. Based on a compilation of in vitro and in vivo studies reported by Chaucheyras-Durand et al. (2008), yeast supplementation can help control ruminal pH, preventing its decline, as well as reducing lactate accumulation through an increase in the number of lactate-utilizing species. Although lactate accumulation is less likely to be a major concern in grazing systems than in feedlot systems, these modifications in the ruminal environment may favor the growth and fiber degradation capacity of fibrolytic bacteria in rumen fermentation systems. Under grazing conditions, these improvements are particularly relevant, since enhanced fiber degradation may result in improved growth patterns and, consequently, improved animal performance.

Saccharomyces cerevisiae (SC) and *Saccharomyces boulardii* (SB) are yeasts commonly used in cattle supplementation. However, they may differ in their physiological and probiotic properties. Supplementation with SC is generally associated with modulation of the ruminal environment and enhanced fiber degradation (Sousa et al. 2017; Amin et al. 2021; Parra et al. 2021), whereas SB has been more closely linked to intestinal health and immune modulation (Hiltz et al. 2021; Cangiano et al. 2023). Although their mechanisms of action may differ, both yeasts may contribute to improved animal performance and development.

Differently from live yeast, products such as yeast cell walls do not exhibit metabolic activity. However, their mode of action is associated with structural components, mainly mannooligosaccharides (MOS), β-glucans and chitin (Lipke and Ovalle, 1998). These components may be used as substrates for selected microorganisms and can also modulate the immune system and microbial populations. As reported by Franklin et al. (2005), MOS supplementation near parturition was associated with increased immune response in both cows and their offspring. Improvements in health status may also translate into enhanced animal performance, reinforcing the link between ruminal modulation, immune function and productivity.

In this context, yeast-derived products may represent a nutritional tool for grazing heifers, not necessarily by preventing ruminal acidosis, but by supporting ruminal function, fiber utilization, gut health, and metabolic stability under pasture-based conditions. Because live *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, and inactive yeast cell wall products have distinct biological properties, their effects may differ according to the productive scenario and physiological stage of the animal. We hypothesized that these yeast supplementation strategies would modulate growth performance and metabolic responses in grazing Nellore heifers during the prepubertal period. Therefore, the present study aimed to evaluate the effects of different yeast-derived products on growth performance and metabolic responses, with reproductive-related traits evaluated as secondary indicators of subsequent reproductive development.

Materials and methods

Location, animals and diets

The experiment was conducted at the Research, Teaching and Extension Unit in Beef Cattle of the Federal University of Viçosa (UFV), Viçosa, Minas Gerais, Brazil. All animal care and handling procedures were approved by the Ethics Committee on the Use of Production Animals of UFV (protocol number 0124/2025).

Thirty-six nulliparous Nellore beef heifers at 263 ± 2.7 kg of initial body weight (BW) and 12 ± 0.1 months of age were used. Heifers were randomly divided into nine paddocks, each containing four animals per paddock, which was considered the experimental unit. Groups were continuously stoked in eight paddocks covered with *Uruchloa decumbens* grass, each equipped with covered feed bunks and water troughs and measuring 3.2 hectares.

The experimental period lasted 120 days (October to January) and was divided into two distinct phases: a 60-day peripubertal phase aimed at reaching adequate BW, followed by a 60-

day period dedicated to reproductive evaluations. The study included three treatments: yeast cell wall (YCW); *Saccharomyces cerevisiae* (SC), provided at a minimum concentration of 8.55 $\times 10^9$ CFU/kg; and *Saccharomyces boulardii* (SB), provided at a concentration of 2.86 $\times 10^9$ CFU/kg. These viable yeast concentrations were predetermined in the commercial premixes supplied by the manufacturer.

A concentrate supplement (Table 1) with crude protein (CP) concentrations for YCW, SC, and SB (201.5, 213.8, and 213.3 g/kg DM, respectively) was provided daily at 11h in a covered feed bunk within the paddocks. The supplement (20% CP) consisted of ground corn (69.12%), soybean meal (18.58%), and a commercial premix (12.30%) containing yeast additives specific to each treatment. All supplements were provided at a rate of 6 g/kg of BW throughout the experimental period, and to adjust the supplement supply, heifers were weighed monthly, without fasting (Silva and Detmann, 2025).

Experimental procedures and sampling

Heifers were weighed without fasting (Silva and Detmann 2025) at the beginning of the experiment, at 60 days (level change of supplementation in COMPG), and at 120 days (the end of the experiment), always at 8h and quantified average daily gain (ADG).

Carcass ultrasound measurements were also performed on day 60, and on day 120. The ribeye area (REA) and subcutaneous fat thickness between the 12th and 13th ribs (rib fat) were measured at the *Longissimus dorsi*. Subcutaneous fat thickness over the *Gluteus biceps* was also measured (rump fat). Carcass images were recorded using an Aloka SSD500II ultrasound device, equipped with an 18 cm linear probe and the BioSoft Toolbox® II for Beef software (Biotronics Inc., Ames, Iowa, USA).

Forage samples (Table 2) were collected on days 60 and 120 by hand-plucking sampling to evaluate the chemical composition of the forage. All samples were oven-dried (55 °C) and ground in a knife mill (Model 2; Arthur H. Thomas, Philadelphia, PA, USA) to pass through a 2-mm sieve. Simultaneously, half of each ground sample was reground to pass through a 1-mm sieve. Samples were composited by paddock for subsequent analyses. Supplement samples were collected at the same time points and processed as previously described.

Blood samples were collected by jugular venipuncture using vacuum tubes with clot activator and serum separation gel (BD Vacutainer SST II Plus, São Paulo, Brazil) at 60 days and at 120 days, always at 08h, to quantify blood concentrations of glucose, serum urea nitrogen (SUN), and insulin-like growth factor 1 (IGF-1). Additionally, IGF-1 concentrations were evaluated at pregnancy diagnosis (PD), including the first and second PD. After collection, the samples were centrifuged at 3,600 × g for 15 min, and serum was frozen at −20 °C for later analysis.

During the last four weeks before reproductive phase, heifers underwent ultrasonographic evaluations to measure the diameter of the dominant follicle and the antral follicle count (AFC) in both ovaries (left and right). During these evaluations, the presence of a *Corpus Luteum* (CL) was also assessed, and when detected, a blood sample would be collected for progesterone determination, with concentrations above 1 ng/mL indicating pubertal animals. However, no heifers presented a CL during this period, indicating absence of luteal activity. Therefore, progesterone concentrations were not evaluated. Once heifers reached adequate BW, they were subsequently submitted to a fixed-time artificial insemination (FTAI) protocol.

All heifers were subjected to a fixed-time artificial insemination (FTAI) protocol at the beginning of the reproductive phase (day 61 of the experimental period). On this day, all heifers received an intravaginal progesterone (P4) device (Primer®, Agener União, Brazil) and estradiol benzoate (EB; RIC-BE®, Agener União, Brazil). After eight days, the device was removed, and equine chorionic gonadotropin (eCG; Novormon®, Zoetis, Brazil), estradiol cypionate (ECP; E.C.P.®, Zoetis, Brazil), and prostaglandin F2α (PGF2α; Estron®, Agener União, Brazil) were administered. Forty-eight hours later, heifers were artificially inseminated with concomitant administration of GnRH (Tec Relin®, Agener União, Brazil).

Fourteen days after the first insemination, a very early resynchronization protocol was initiated with the insertion of a reused intravaginal P4 device and administration of EB. Twenty-two days after the first insemination, Doppler ultrasonography was performed to identify luteal activity. Heifers without luteal activity were submitted to a second FTAI following the same hormonal protocol described above, whereas those with luteal activity remained with the device until pregnancy diagnosis. Pregnancy diagnosis was performed by transrectal ultrasonography thirty days after each insemination.

Laboratory analysis and calculations

Supplement and forage samples processed through a 1-mm sieve were analyzed for dry matter (DM; method G-003/1), ash (method M-001/2), CP (method N-001/2), ether extract (EE; method G-005/2), and neutral detergent fiber corrected for ash and protein (apNDF; methods F-013/1, M-002/2, and N-004/2, respectively). Indigestible neutral detergent fiber (iNDF) was evaluated in samples processed through a 2-mm sieve and incubated in situ for 288 hours (iNDF; method G-009/1). All analyses were performed according to the procedures described by Detmann et al. (2025).

Blood glucose concentration was determined using an enzymatic-colorimetric method. Urea was quantified using an enzymatic method, and values were converted to serum urea nitrogen (SUN). Serum concentrations of insulin-like growth factor 1 (IGF-1) were measured using a chemiluminescent method. All analyses were conducted at a commercial laboratory (Viçosalab®, Viçosa, Minas Gerais, Brazil).

Statistical analysis

The statistical analysis of the data followed the basic model:

$$Y_{ijk} = \mu + T_i + P_{(i)j} + \varepsilon_{(ij)k}$$

Where Yijk is the response measured in animal k, managed in paddock j submitted to treatment i, μ is the general constant, Ti is the fixed effect of the treatment, P(i)j is the random effect of the experimental unit (paddock) where j represents the paddock nested within treatment i, and Ɛ(ij)k is the random error, assumed to be NIID (0, σ2ε).

Variables measured over time were analyzed as repeated measures using the GLIMMIX procedure of SAS (Statistical Analysis System, version 9.4). When required, data were log-transformed to meet model assumptions. When significant effects were detected, least-squares means were compared by pairwise comparisons. When the interaction was significant, simple effects were evaluated using the SLICE option. Differences were declared significant at $p < 0.05$.

Results

An interaction between treatment and evaluation day ($p = 0.018$) was observed for BW (Figure 1). Over the experimental period, heifers receiving SB, YCW, and SC gained 88, 80, and 70 kg, respectively, with a tendency for greater BW in SB compared with SC observed on day 120 ($p = 0.051$). Regarding ADG, only an effect of evaluation day was detected ($p = 0.016$), with greater values observed on day 120, during the reproductive evaluation period (Table 3).

REA was not affected by nutritional treatments ($p > 0.05$), indicating that the different dietary strategies did not influence *Longissimus dorsi* muscle development at the evaluated time points. In contrast, rib fat and rump fat increased over the evaluation period ($p < 0.001$; Table 3).

The blood parameters (Table 4) were affected only by evaluation day. Glucose and SUN, increased over the evaluation period in all treatments (p = 0.024 and 0.043, respectively). Conversely, IGF-1 concentrations decreased over time (p = 0.046), with lower values observed on day 120 than on day 60. No treatment × day interaction was detected for any of these variables.

Regarding the variables evaluated during the reproductive phase to provide insight into the biological events modulating reproduction, IGF-1 concentrations at pregnancy diagnosis did not differ among treatments (p = 0.24 and 0.84). Likewise, pregnancy rate was not affected by treatment (p = 0.65 and 0.87; Table 5). AFC was not affected by ovarian side, with no differences between the left and right ovaries (p = 0.070 and 0.056, respectively; Table 6). However, follicle diameter increased on day 14 of the evaluation period (p = 0.045; Figure 2).

Discussion

The treatment × day interaction observed for BW indicates that the effect of yeast-based supplementation on BW was dependent on the progression of the experimental period. Although no main effect of treatment was detected, heifers supplemented with SB showed a greater numerical increase in BW from day 1 to day 120 compared with those supplemented with SC. This suggests that possible differences among yeast-based products were not expressed as an immediate improvement in growth rate, but rather as a cumulative response over time. This interpretation is supported by the absence of treatment effects on ADG, indicating that the BW response should not be interpreted as a consistent increase in daily gain, but as a gradual change in the overall growth trajectory of the animals.

Yeast-based additives may influence animal performance through different mechanisms depending on the strain, product composition, diet, and production system (Baker et al. 2022). SC supplementation has been mainly associated with modulation of ruminal fermentation and improvement of fiber degradation, whereas SB and yeast-derived products have been more closely related to intestinal health, immune modulation, and gut functionality (Franklin et al. 2005; Chaucheyras-Durand et al. 2008; Sousa et al. 2017; Amin et al. 2021; Parra et al. 2021; Hiltz et al. 2021; Cangiano et al. 2023). However, the translation of these mechanisms into animal performance is indirect and variable, because it depends on the magnitude of the nutritional challenge and on how ruminal and intestinal changes affect nutrient use. This helps explain why performance responses to yeast supplementation are inconsistent among studies.

In the present study, the main nutritional challenge was not related to high concentrate intake, but rather to the seasonal changes in forage quality typically observed under grazing conditions. From day 60 to day 120, forage CP decreased from 124.0 to 84.1 g/kg DM, and iNDF increased from 161.9 to 196.8 g/kg DM. These changes indicate a reduction in forage nutritive value during the experimental period, which may have increased the importance of nutrient utilization efficiency for sustaining heifer development. In particular, forage CP concentration at the end of the period was below the approximate level associated with improved ruminal nitrogen availability in cattle fed low-quality tropical forage, as suggested by Detmann et al. (2009). Therefore, the use of different yeast-based products was based on the hypothesis that these additives could indirectly support performance through distinct mechanisms related to ruminal fermentation, fiber use, intestinal functionality, or metabolic adaptation. This approach is particularly relevant for prepubertal heifers, because adequate growth during this phase is essential before the beginning of the reproductive period.

The grazing conditions and the moderate level of supplementation may have limited the magnitude of the performance response. Yeast effects are often more evident when animals are exposed to greater ruminal challenges, such as diets with high concentrate inclusion, in which stabilization of ruminal fermentation and control of lactate accumulation may have a greater impact on nutrient utilization (Chaucheyras-Durand et al. 2008; Desnoyers et al. 2008). This may explain why Bomfim et al. (2024) reported greater final BW, total BW gain, ADG, and feed efficiency in animals supplemented with SB under feedlot conditions, whereas responses under forage-based systems are usually less pronounced. Similarly, Cabrera et al. (2020) observed no improvement in performance or digestibility with SC supplementation in grazing cattle, supporting the interpretation that yeast responses may be limited when the main challenge is associated with forage quality and availability rather than high ruminal fermentative pressure.

Therefore, although the BW interaction suggests a product-specific response over time, the absence of treatment effects on ADG, ribeye area, rib fat, and rump fat limits the interpretation of this result as a clear improvement in performance. The numerical advantage observed for SB may indicate some support for nutrient use or metabolic adaptation during the later phase of development, possibly associated with intestinal functionality or immune modulation during nutritional challenge, but this response was not strong enough to consistently affect daily gain or *in vivo* carcass measurements. Overall, yeast-based supplementation did not clearly improve the performance of grazing prepubertal Nellore heifers, although the BW trajectory suggests that SB may have promoted a modest cumulative response during development.

Despite these findings, studies simultaneously comparing different yeast strains, such as SB and SC, and different supplementation forms, such as viable cells and yeast cell wall components, remain scarce. This limits the understanding of their functional differences and makes it difficult to clarify the mechanisms underlying the responses observed in the present study. Although SB and SC are closely related, SB is currently classified within *Saccharomyces cerevisiae* as *S. cerevisiae* var. *boulardii*. Duffey et al. (2025) reported high overall genomic similarity between SB and SC, with average nucleotide identity >99%. Nevertheless, the same authors also reported distinct functional capacities between SB and SC related to probiotic activity, indicating that strain-level differences may result in distinct biological responses. Therefore, the tendency for greater BW in SB may reflect strain-specific effects rather than a general response to yeast supplementation.

Blood metabolites were affected mainly by day rather than by yeast-based supplementation, indicating that temporal changes in the nutritional environment had a greater influence on metabolic status than the type of additive supplied. Glucose and SUN concentrations increased on day 120, whereas IGF-1 decreased over the same period. The absence of treatment effects for these variables suggests that live yeast or yeast-derived products did not markedly alter energy or nitrogen metabolism under the conditions of the present study.

SUN is commonly used as an indicator of nitrogen metabolism, because inefficient ruminal nitrogen utilization can increase ammonia absorption and subsequent hepatic conversion into urea (Hammond 1998). In this context, Baker et al. (2022) suggested that reductions in ruminal ammonia without a concomitant reduction in dietary protein supply may indicate improved ruminal nitrogen utilization. In the present study, however, SUN increased over time and did not differ among treatments, suggesting that yeast supplementation was not sufficient to modify nitrogen use efficiency. This response may be related to the decline in forage nutritive value from day 60 to day 120, characterized by lower CP concentration and greater fiber and indigestible fiber fractions. Under grazing conditions, this scenario may reduce the synchrony between available nitrogen and fermentable energy in the rumen (Detmann et al., 2014), limiting microbial nitrogen capture and increasing the amount of nitrogen converted to urea.

The increase in glucose over time should be interpreted cautiously. In ruminants, circulating glucose is largely derived from hepatic gluconeogenesis, mainly from propionate, and yeast supplementation has been associated with changes in ruminal fermentation that may favor propionate production in some studies (Gunun et al. 2022; Pickett et al. 2025). However, because glucose was affected only by day and not by treatment, the increase observed in the present study was more likely associated with changes in diet, animal development, or adaptation to grazing conditions rather than a specific effect of yeast supplementation. Moreover, the concomitant increase in SUN indicates that the increase in glucose did not necessarily reflect a more efficient overall use of nitrogen by the animals.

The reduction in IGF-1 concentrations on day 120 further supports the interpretation that the metabolic response was not clearly anabolic. IGF-1 is closely related to nutritional status and growth regulation (Zulu et al. 2002; Velazquez et al. 2008; Sartori et al. 2013), and its decrease suggests that the increase in glucose was not accompanied by a proportional improvement in anabolic signaling. Therefore, the simultaneous increase in glucose and SUN, together with the reduction in IGF-1, may indicate a partial imbalance between energy and nitrogen metabolism during the later phase of the experiment. This imbalance was probably associated with the decline in forage quality rather than with the yeast products themselves, because no treatment effects were detected for glucose, SUN, or IGF-1.

Overall, these results suggest that yeast-based supplementation did not consistently affect blood markers related to energy metabolism, nitrogen utilization, or anabolic status in grazing prepubertal Nellore heifers. The day effects observed for glucose, SUN, and IGF-1 appear to reflect changes in the nutritional environment over time, particularly the reduction in forage CP and increase in fiber fractions, which may have limited the metabolic response to supplementation and masked potential differences among yeast-based products.

Reproductive function in developing heifers is closely linked to metabolic status, because signals related to energy and protein metabolism may influence the hypothalamic–pituitary–ovarian axis, follicular development, ovulation, and pregnancy establishment (Butler, 2003; Schneider, 2004; Cardoso et al. 2020). In the present study, however, yeast-based supplementation did not affect serum IGF-1 during the experimental period, IGF-1 concentrations at pregnancy diagnosis, pregnancy probability, or final pregnancy probability. Thus, although yeast products may influence metabolic pathways related to reproduction, the absence of treatment effects indicates that these additives did not markedly modify reproductive-related metabolic signaling under the grazing conditions evaluated.

Follicle diameter changed over time, increasing from day 1 to day 14 and decreasing on day 21. This pattern is consistent with the physiological dynamics of follicular waves, in which follicular recruitment and selection are followed by the establishment of dominance and subsequent regression or emergence of a new follicular wave. Therefore, the day effect observed for follicle diameter appears to reflect the expected temporal progression of ovarian dynamics rather than a direct response to yeast-based supplementation. This interpretation is supported by the absence of treatment effects on follicle diameter and AFC, indicating that neither live yeast nor yeast-derived products consistently altered ovarian follicular development in prepubertal Nellore heifers.

The absence of treatment effects on IGF-1 at the first and second pregnancy diagnoses further supports this interpretation. IGF-1 is an important metabolic mediator involved in follicular growth, steroidogenesis, oocyte competence, and early embryonic development, and may contribute to conceptus development and pregnancy establishment through effects on the uterine environment and embryonic signaling (Moreira et al. 2002; Bilby et al. 2006). However, in the present study, the increase in glucose over time was not accompanied by an increase in IGF-1, suggesting that changes in energy metabolism were not translated into greater anabolic or reproductive-related endocrine signaling. This may help explain why yeast supplementation did not affect follicular development or pregnancy probability.

Pregnancy probability at the first AI and final pregnancy probability were not affected by treatment. Numerically, heifers supplemented with live yeast products showed greater final pregnancy probability than those supplemented with YCW. However, this response was not supported by differences in IGF-1 at pregnancy diagnosis or by consistent changes in ovarian variables. Previous studies have reported variable reproductive responses to yeast supplementation, which may be related to differences in animal category, nutritional conditions, yeast product, and supplementation strategy (Bruno et al. 2008; Kalmus et al. 2009; Nasiri et al. 2018; Garnsworthy et al. 2024; Baker et al. 2022). Therefore, the numerical pattern observed for live yeast products may suggest a modest biological response, but it does not indicate a consistent improvement in reproductive performance under the conditions of the present study.

Together, these results indicate that yeast-based supplementation did not promote consistent changes in the metabolic–reproductive axis of grazing Nellore heifers. The temporal changes observed in follicle diameter and blood metabolites appear to be more closely associated with physiological ovarian dynamics and changes in the nutritional environment than with the yeast products themselves. Thus, although live yeast supplementation showed a numerical advantage in final pregnancy probability, this response was not accompanied by clear changes in IGF-1, AFC, or follicular development, indicating that the reproductive impact of yeast-based supplementation was limited under the grazing conditions evaluated.

Conclusion

The development of grazing *Bos indicus* beef heifers during the prepubertal phase was primarily determined by overall nutrient availability and forage quality rather than by the type of yeast-based additive supplied. Although numerical or isolated differences in BW and pregnancy probability were observed, these responses were not accompanied by consistent improvements in metabolic or reproductive-related indicators, indicating limited additional short-term biological impact of yeast supplementation under the conditions of this study.

Author contributions: CRediT

Letícia Zamberlan Pistillo: Data curation, formal analysis, project administration, writing – original draft, review and editing. **Edenio Detmann:** Conceptualization, data curation, methodology, writing – original draft. **Cláudia Batista Sampaio:** Conceptualization, funding acquisition, investigation, methodology, project administration, resources, writing – original draft, review and editing.

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Table 1

Chemical composition of the supplements offered to grazing Nellore heifers heifers supplemented with live yeast (*Saccharomyces cerevisiae* or *Saccharomyces boulardii*) or a yeast-derived product (yeast cell wall) during the prepubertal period.

	Concentration (g/kg)		
Item[1]	YCW	SC	SB
OM (g/kg DM)	879.6	871.8	873.7
CP (g/kg DM)	201.5	213.8	213.3
EE (g/kg DM)	28.4	35.0	28.8
apNDF (g/kg DM)	31.0	44.5	34.9

[1]OM = organic matter; CP = crude protein; EE = ether extract; apNDF = neutral detergent fiber corrected for ash and protein; YCW = yeast cell wall; SC = *Saccharomyces cerevisiae*; SB = *Saccharomyces boulardii.*

Table 2

Chemical composition of the forage obtained by the hand-plucking method during the experimental period.

	Sampling period	
Item[1]	Day 60	Day 120
DM (g/kg DM)	292.8	260.9
OM (g/kg DM)	915.3	903.8
CP (g/kg DM)	124.0	84.1
apNDF (g/kg DM)	482.0	527.3
iNDF (g/kg DM)	161.9	196.8

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

Forage availability was sampled using a metal square, whereas all other variables were obtained using the hand-plucking method.

Table 3

Performance and *in vivo* carcass measurements of grazing Nellore heifers supplemented with live yeast (*Saccharomyces cerevisiae* or *Saccharomyces boulardii*) or a yeast-derived product (yeast cell wall) during the prepubertal period.

	Treatments								
	YCW		SC		SB		*P-value*		
Item[1]	d60	d120	d60	d120	d60	d120	Treatment (T)	Day (D)	T × D
ADG, g/day	526.5±70.6	808.2±64.8	545.7±70.6	623.6±64.8	712.3±70.6	742.3±64.8	0.21	0.016	0.12
Ribeye area (cm^2)	43.2±1.87	44.2±1.67	45.6±1.87	42.6±1.67	43.1±1.89	43.2±1.67	0.89	0.41	0.11
Rib fat (mm)	3.1±0.21	3.83±0.30	3.3±0.21	3.85±0.30	3.6±0.21	3.8±0.30	0.83	<0.001	0.37
Rump fat (mm)	3.1±0.26	5.79±0.60	3.5±0.26	5.1±0.60	3.7±0.26	6.18±0.60	0.50	<0.001	0.32

[1] ADG: average daily gain; YCW = yeast cell wall; SC = *Saccharomyces cerevisiae*; SB = *Saccharomyces boulardii.*

Table 4

Blood parameters of grazing Nellore heifers supplemented with live yeast (*Saccharomyces cerevisiae* or *Saccharomyces boulardii*) or a yeast-derived product (yeast cell wall) during the prepubertal period.

	Treatments								
	YCW		SC		SB		*P-value*		
Item[1]	d60	d120	d60	d120	d60	d120	Treatment (T)	Day (D)	T × D
Glucose (mg/dL)	56.5±2.32	57.3±1.97	52.7±2.32	56.2±1.97	48.1±2.32	53.8±1.97	0.13	0.024	0.39
SUN (mg/dL)	13.8±0.97	14.6±0.88	14.3±0.97	14.5±0.88	12.3±0.97	13.0±0.88	0.37	0.043	0.67
IGF-1 (ng/mL)	234.4±18.91	229.7±15.32	232.1±18.91	226.2±15.32	230.5±18.91	196.6±15.32	0.69	0.046	0.19

[1] SUN = serum urea nitrogen; IGF-1 = insulin-like growth factor 1; YCW = yeast cell wall; SC = *Saccharomyces cerevisiae*; SB = *Saccharomyces boulardii.*

Table 5

Hormonal concentrations in grazing Nellore heifers supplemented with live yeast (*Saccharomyces cerevisiae* or *Saccharomyces boulardii*) or a yeast-derived product (yeast cell wall) during the prepubertal period.

Item[1]	YCW	SC	SB	*P-value*
IGF-1(ng/mL) at 1st PD	255.7±22.75	211.3±16.23	200.9±18.49	0.24
IGF-1(ng/mL) at 2nd PD	190.8±23.44	198.8±26.45	178.1±24.4	0.84
PP (%) at 1st AI	32	59	41	0.65
fPP (%)	39	50	60	0.87

[1]IGF-1 = insulin-like growth factor 1; PD = pregnancy diagnosis; PP = pregnancy probability; fPP = final pregnancy probability; YCW = yeast cell wall; SC = *Saccharomyces cerevisiae*; SB = *Saccharomyces boulardii.*

IGF-1 concentrations at the 1st PD correspond to heifers inseminated at the 1st AI, whereas IGF-1 concentrations at the 2nd PD correspond to heifers inseminated at the 2nd AI.

Pregnancy probability was assessed 30 days after each AI.

Probability outcomes are presented as probabilities (%). Least squares mean were back-transformed from the logit scale using the inverse logit function, and standard errors were obtained using the delta method.

Table 6

Antral follicle count of Nellore grazing heifers supplemented with live yeast (*Saccharomyces cerevisiae* or *Saccharomyces boulardii*) or a yeast-derived product (yeast cell wall) during the prepubertal period.

	Days				*P-value*
AFC[1]	1	7	14	21	Day
Left ovary	13.3±0.85	13.7±0.87	12.4±0.82	11.1±0.77	0.070
Right ovary	14.5±0.86	14.6±0.85	13.9±0.83	12.0±0.76	0.056

[1] AFC = antral follicle count.

Least squares means were back-transformed from the log scale using the exponential function, and standard errors were calculated using the delta method. No effects of plan or plan × day interaction was detected ($P > 0.05$).

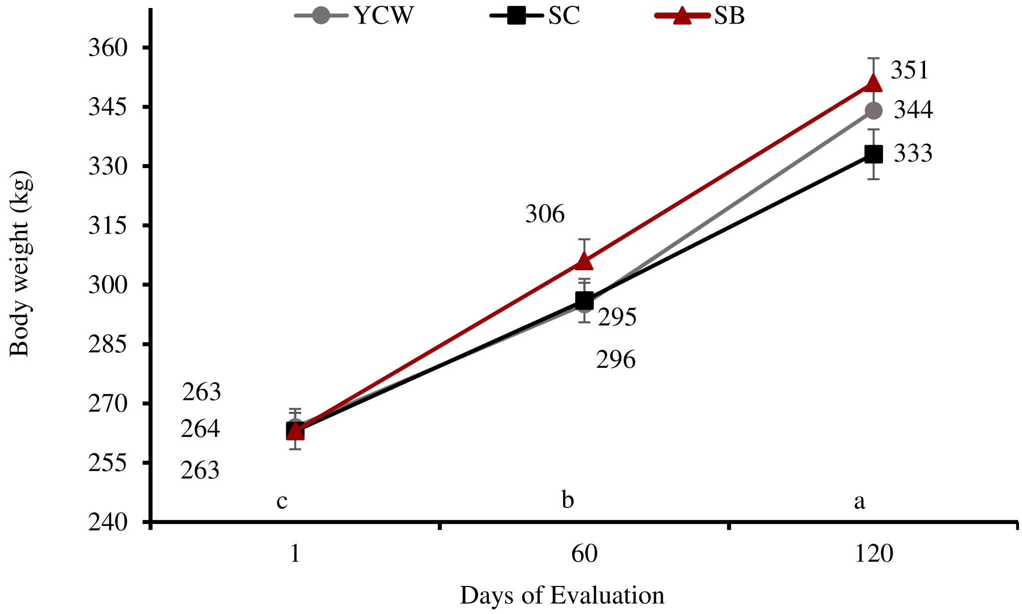

Fig. 1. Body weight in grazing Nellore heifers supplemented with live yeast (*Saccharomyces cerevisiae* or *Saccharomyces boulardii*) or a yeast-derived product (yeast cell wall) during the prepubertal period. Different letters on the x-axis indicate differences across days of evaluation within each supplementation treatment ($P < 0.05$). Effects: Treatments (T) = 0.46; Day (D) < 0.001; T × D = 0.018.

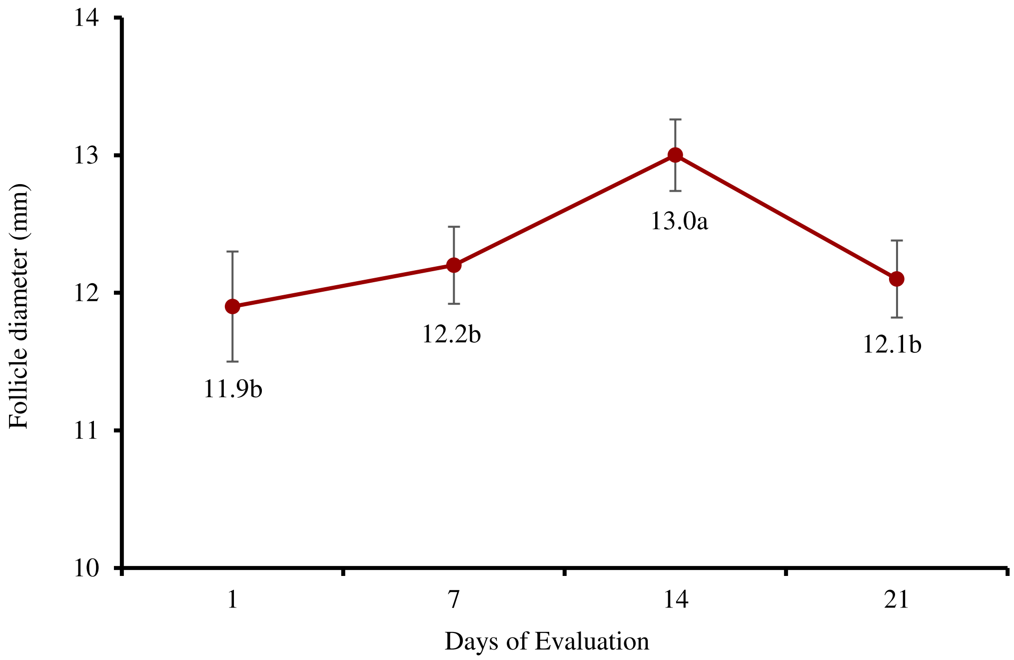

Fig. 2. Follicle diameter in grazing Nellore heifers supplemented with live yeast (*Saccharomyces cerevisiae* or *Saccharomyces boulardii*) or a yeast-derived product (yeast cell wall) during the prepubertal period. Means followed by different letters differ within each day of evaluation ($P < 0.05$). Effects: Treatments (T) = 0.45; Day (D) = 0.045; T × D = 0.20.