ANTÔNIO PAULO DE OLIVEIRA NETO

DIFFERENTIAL AVERAGE DAILY GAIN OF PREGNANT HOLSTEIN × GYR HEIFERS: EFFECTS ON FUTURE MILK PRODUCTION AND PERFORMANCE OF THEIR CALVES

Thesis presented to the Universidade Federal de Viçosa, as part of the requirements of the Postgraduate Program in Animal Science, to obtain the title of Doctor Scientiae.

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Co-supervisors: Alex Lopes da Silva
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Advisor

“Our greatest glory is not that we never fall, but that we always get up after every fall..”

(Oliver Goldsmith)

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BIOGRAPHY

Antônio Paulo de Oliveira Neto was born on August 13, 1992, in Barão de Monte Alto, Minas Gerais, Brazil. He is the son of Sergio Murilo de Oliveira and Rosa Maria Nunes Oliveira. Antônio began his academic journey in animal science at the Universidade Estadual do Norte Fluminense in 2012, where he completed his undergraduate studies and received his bachelor's degree in 2017.

Following his undergraduate education, Antônio commenced his Master of Science (M.S.) program at the Universidade Federal de Viçosa, specializing in nutrition and ruminant production. He successfully earned his M.S. degree in Animal Science in December 2019. Continuing his academic pursuits in the same year, he began his Doctor of Science (DS) program in Animal Science, with a focus on ruminant dairy cattle nutrition and production.

From February 2020 to March 2024, Antônio conducted his doctoral research at the Unidade de Ensino, Pesquisa e Extensão em Gado de Leite, affiliated with the Universidade Federal de Viçosa. He submitted his dissertation to the thesis committee on February 14, 2024, aiming to obtain the degree of Doctor Scientiae in Animal Science.

ABSTRACT

OLIVEIRA NETO, Antônio Paulo, D.Sc., Universidade Federal de Viçosa, March 2024. **Differential average daily gain of pregnant Holstein × Gyr heifers: Effects on future milk production and performance of their calves**. Advisor: Polyana Pizzi Rotta. Co-supervisors: Alex Lopes da Silva and Simone E. F. Guimarães

This study assessed the effects of different average daily gains (ADG) during pregnancy on the growth, nutrient digestibility, milk production, composition, and metabolism of dairy heifers, as well as the performance of their offspring. Twenty Holstein × Gir heifers, averaging 420 ± 15.0 kg in body weight and 18 ± 3.9 months in age, were randomly assigned to two treatment groups: a moderate ADG of 0.37 kg (MOD, n = 10) and a high ADG of 0.72 kg (HIG, n = 10). These heifers subsequently produced sixteen 3/4 Holstein × 1/4 Gyr calves. The heifers were fed a total mixed ration twice daily from the 70th day of pregnancy until calving. Calves were provided with colostrum constituting 15% of body weight with a minimum Brix of 25%, 6 L of transition milk for the first two days, followed by the same volume of raw milk daily until weaning. From day three onwards, calves had ad libitum access to a starter (DM 87.8%, CP 19.6%, starch 44.8%) and were introduced to hay (Cynodon dactylon) at 40 days. Data collection occurred over five periods (feed, feces, urine, and blood), each lasting five days starting from 100 days of gestation and occurring at 30-day intervals. Calves were monitored from birth to weaning with morphometric measurements, body weight, and blood collections on days 1, 31, 61, and 91, alongside daily collections of concentrate and hay and their leftovers. Results indicated that the MOD and HIG heifers had increasing intakes of dry matter, organic matter, crude protein, starch, and neutral detergent fiber throughout pregnancy. Nutrient digestibility did not differ between the MOD and HIG groups. However, there was a significant interaction for nitrogen of microbial origin (N-mic), which was higher in MOD heifers during the 4th and 5th months of pregnancy. Milk production over 305 days and milk corrected for 4% fat showed no differences between the ADG groups. The MOD heifers produced milk with a higher fat percentage, although protein percentages were similar between groups. Total solids were higher in the MOD group. Hormone and metabolite levels showed no interactions between treatments and lactation time, except for triiodothyronine, which was higher in the HIG group during the 4th and 9th months. No differences were observed in the ADG of calves from both groups at any evaluated times (31, 61, and 91 days). Calves from the MOD group exhibited compensatory growth, performing well from birth to weaning despite the lower nutritional intake of their mothers during gestation. In conclusion, a moderate ADG during pregnancy in 5/8 Holstein × Gyr heifers did not affect nutrient digestibility or milk production but resulted in higher milk fat content.

Key words: fetal programming, Holstein × Gyr, milk production, nutrition, performance.

RESUMO

OLIVEIRA NETO, Antônio Paul, D.Sc., Universidade Federal de Viçosa, março de 2024. **Diferente ganho médio diário em novilhas prenhas 5/8 Holandesas × 3/8 Gir: Efeitos na produção futura de leite e no desempenho de suas bezerras**. Orientadora: Polyana Pizzi Rotta. Coorientadores: Alex Lopes da Silva e Simone E. F. Guimarães.

Este estudo teve como objetivo avaliar o impacto do ganho médio diário (GMD) em novilhas leiteiras durante a gestação sobre o crescimento, digestibilidade dos nutrientes, produção, composição do leite, além dos impactos dessas diferentes estratégias nutricionais no desempenho de suas bezerras. Vinte novilhas Holandesas × Gir, com peso corporal médio de 420 ± 15,0 kg e idade média de 18 ± 3,9 meses, foram distribuídas aleatoriamente em dois grupos: GMD de 0,37 kg (moderado - MOD, n = 10) e GMD de 0,72 kg (alto - HIG, n = 10). Estas novilhas geraram dezesseis bezerras 3/4 Holandesas × 1/4 Gir, incluídas no estudo. As novilhas receberam ração total misturada duas vezes ao dia, do 70º dia de gestação até o parto. As bezerras receberam colostro, constituindo 15% do peso corporal com um mínimo de 25% de Brix, recebendo 6 L de leite de transição até 2 dias de vida, seguido por igual quantidade de leite cru diariamente até o desmame. A partir do terceiro dia, as bezerras tiveram acesso ad libitum a concentrado (MS 87.8%, PB 19.6% e amido 44.8%) e aos 40 dias foi introduzido feno (Cynodon dactylon). Foram realizados cinco períodos de coleta (ração, fezes, urina e sangue), cada um com duração de cinco dias, iniciando aos 100 dias de gestação, com intervalos de 30 dias entre cada período de coleta. As bezerras foram monitoradas durante 3 meses, desde o nascimento até o desmame, com realização de avaliações morfométricas, pesagem corporal e coletas de sangue realizadas nos dias 1, 31, 61 e 91 pós nascimento para avaliar o desempenho, concentrações de hormônios e metabólitos, além de coletas diárias do concentrado e feno oferecidos, bem como suas ocasionais sobras. Após o parto da novilha, o leite foi pesado semanalmente e sua composição foi analisada mensalmente. Ao parto, as novilhas do grupo HIG apresentaram um peso corporal de 628 kg, enquanto as novilhas do grupo MOD apresentaram 563 kg. Embora as dietas tenham sido formuladas para 0,35 e 0,70 kg/d para MOD e HIG, respectivamente, o GMD observado foi de 0,38 e 0,72 kg/d, sugerindo que as recomendações da NASEM para essas novilhas Holandesas × Gir podem estar subestimadas, resultando em um GMD maior que o esperado. Não foi observada diferença na digestibilidade dos nutrientes ao comparar as estratégias MOD e HIG para as novilhas durante a gestação. No entanto, foi observada uma interação para o nitrogênio de origem microbiana (N-mic) produzido diariamente, sendo maior nas novilhas que receberam a dieta MOD durante os meses 4 e 5 de gestação. Não houve diferença na eficiência da síntese de N microbiano entre as novilhas HIG e MOD. Em termos de produção de leite para um período de lactação de 305 dias e produção de leite corrigida para 4% de gordura, não foram observadas diferenças entre os dois GMD avaliados. Em termos de composição do leite, as novilhas MOD apresentaram um maior percentual de gordura, sem efeito no percentual de proteína. Os sólidos totais tendiam a ser maiores no grupo MOD. Não foi observada interação entre os tratamentos e o tempo de lactação para globulina, albumina, proteínas totais, glicose, tiroxina e IGF-1. No entanto, a triiodotironina apresentou uma interação significativa entre o tratamento durante a gestação e o período de lactação, com valores maiores observados para o grupo HIG nos meses 4 e 9. Um GMD moderado durante a gestação das novilhas 5/8 Holandesas × Gir não influenciou a digestibilidade, a síntese de N microbiano e a produção de leite, mas resultou em maior produção de gordura (%). Quando avaliamos as bezerras, observou-se uma tendência para maior peso corporal ao nascer nas bezerras HIG em comparação com as bezerras MOD. Em relação ao desempenho das bezerras, o GMD das bezerras de ambos os tratamentos permaneceu semelhante em todos os momentos avaliados (31, 61 e 91 dias). Bezerras oriundas de mães do grupo MOD demonstraram capacidade compensatória, apresentando bom desempenho desde o nascimento até o desmame.

Palavras-chave: desempenho, Holandesas × Gir, nutrição, programação fetal, produção de leite

SUMÁRIO

GENERAL INTRODUCTION

Raising dairy cattle, especially in tropical climates where pasture is the main source of feed, presents unique challenges and opportunities for the development of mammary glands, or mammogenesis, which is critical for milk production (Villanova et al., 2022). The process begins in the fetal stage and is governed primarily by genetic factors that orchestrate the formation of a system composed of milk ducts, surrounded by connective and adipose tissue. The foundational structure of this system is the mammary bud, which is regulated by the surrounding mammary mesenchyme, a specialized type of connective tissue.

During the embryonic development of dairy cattle, a distinct feature known as the milk line appears along each side of the abdominal wall, parallel to the ventral midline. This line marks the future sites of the mammary glands and is a critical indicator of the initial stages of mammogenesis (Reece, 2008). The regulation of this complex process involves a variety of hormones that control and regulate the growth, development, and functional acquisition of the mammary tissues.

Growth and Development Dynamics:

Growth typically involves an increase in physical mass which can occur through hyperplasia (increase in the number of cells) or hypertrophy (increase in cell size). Development, on the other hand, refers to the process by which cells acquire specific functions, differentiate into various tissue types, and begin to express distinct proteins that define their roles within an organism. These developmental processes are particularly critical during the embryonic phase and are heavily influenced by hormonal actions. Hormonal Influences in Mammogenesis and Embryonic Development:

Estrogen: This hormone is pivotal in the development of the mammary ducts during the pubertal phases and plays a crucial role during fetal development. Estrogen is synthesized and secreted by the ovaries and the placenta, contributing to the duct elongation and lobulo-alveolar formation. Moreover, it helps protect the uterine environment from exogenous contaminants by stimulating the production of cervical mucus and enhancing the contractility of the myometrium (Heidari et al., 2016; Ulbrich et al., 2009).

Progesterone: Derived from cholesterol, this fat-soluble steroid hormone is essential for the growth and development of alveolar structures within the mammary gland, particularly during the final trimester of pregnancy. It also plays a significant role in maintaining pregnancy by enhancing metabolic activity to support fetal growth and stimulating the production of key antiviral proteins such as Interferon-Tau (Mattos et al., 2000; Spencer et al., 2006).

Prolactin: Produced in the anterior pituitary gland, prolactin is responsible for the differentiation of mammary gland cells. Its levels increase significantly during the last third of pregnancy, playing a crucial role in the development of breast tissue and influencing fetal growth and adipose tissue development (Islam et al., 2016; Aires et al., 2018).

Placental Lactogen (hPL): This hormone, synthesized in the placenta, is part of the growth hormone/prolactin gene family. It functions similarly to prolactin and growth hormone, supporting mammary growth and fetal development by enhancing cell proliferation in the mammary gland and stimulating the synthesis of IGF-1 and insulin in the fetus. This results in increased amino acid uptake and enhanced protein synthesis, particularly in muscle cells and fetal fibroblasts (Aires et al., 2018).

Growth Hormone (GH): GH is a peptide hormone from the anterior pituitary gland, instrumental in increasing gluconeogenesis, which ensures a steady glucose supply to the mammary tissues. It also plays a critical role in lipid metabolism by promoting lipolysis and the release of free fatty acids into the systemic circulation (Islam et al., 2016).

Thyroid Hormones (T3 and T4): These hormones are crucial for enhancing metabolic activity and oxygen consumption, facilitating the growth and development of the central nervous system, and inducing the expression of IGF-1. They are essential for neuronal proliferation, synaptogenesis, dendrite development, and the differentiation of oligodendrocytes, significantly impacting the overall growth and development of the fetus (Reece, 2008; Aires et al., 2018).

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CHAPTER I - DIFFERENTIAL AVERAGE DAILY GAIN OF PREGNANT HOLSTEIN × GYR HEIFERS: EFFECTS ON FUTURE MILK YIELD

Short title: Different ADGs and their consequences in dairy heifers

Chapter formatted according to the scientific journal: Journal of Dairy Science, except for the arrangement of tables and figures

ABSTRACT

This study evaluated the effects of average daily gain (ADG) during pregnancy on growth, digestibility, milk yield and composition, and metabolism in dairy heifers. Twenty 5/8 Holstein × 3/8 Gyr heifers, with an initial average body weight of 420 ± 15.0 kg and age of 18 ± 3.9 mo, were randomly assigned to two treatment groups targeting an ADG of 0.35 kg (moderate - MOD, n = 10) and 0.70 kg (high - HIG, n = 10). The heifers were fed a total mixed ration twice daily from day 70 of gestation until calving. Five collection periods, each lasting five days, were conducted at 100 d of gestation, with 30-d intervals between each period, collecting feed, feces, urine, and blood samples. The observed ADG was 0.37 kg for the MOD group and 0.72 kg for the HIG group. Post-calving, milk yield was recorded weekly, and milk composition was analyzed monthly. At calving, HIG heifers had a body weight of 628 kg, while MOD heifers weighed 563 kg. Among growth measurements, only final withers height differed significantly, being greater in HIG heifers. Nutrient digestibility did not differ between MOD and HIG groups along gestation. However, an interaction for microbial nitrogen (N-mic) production was observed, with higher N-mic in the MOD group during months 4 and 5 of pregnancy. Milk yield and milk yield corrected for 4.0% fat and 3.3% protein did not differ between ADG levels. The MOD heifers produced milk with higher fat and total solids content. No effect was observed between ADG levels for globulin, albumin, total proteins, glucose, thyroxine, and IGF-1 concentrations during pregnancy. However, triiodothyronine levels showed significant interaction, with higher values in the HIG group during months 4 and 9. Heifers with high ADG calved heavier and had greater body condition scores, but ADG levels did not affect future milk yield. Key words: fetal programming, heifer, milk composition, milk yield.

KEY WORDS

fetal programming, heifer, milk composition, milk yield

1. INTRODUCTION

In Brazil and many tropical countries worldwide, a significant number of dairy farmers depend on tropical pasture systems as the primary nutrient source for growing heifers (Villanova et al., 2022). Typically, heifers in these systems face conditions that can result in suboptimal performance, evidenced by low ADG and an age at first calving exceeding 36 mo (Machado et al., 2019). This suboptimal performance is often due to the use of pastures with limited forage availability and quality, frequently lacking proper supplementation (Machado et al., 2019; Noya et al., 2019).

Based on NASEM (2021) recommendations, Holstein heifers should ideally reach 91% of their pre-calving maturity BW. However, similar data for Holstein × Gyr heifers is lacking. To better understand this, a simulation with heifers exhibiting moderate and high ADG until pre-calving was performed, with moderate ADG at 0.35 kg/d and high ADG at 0.70 kg/d. In our simulation, the heifers became pregnant at a BW of 450 kg. We considered that a mature BW of Holstein × Gyr cow would be 610 kg. Heifers with an ADG of 0.35 kg/d reached a BW of 520 kg at pre-calving, which corresponds to approximately 85% of their mature BW. Heifers with an ADG of 0.70 kg/d reached a BW of 590 kg at pre-calving, which corresponds to approximately 97% of their mature BW.

Studies have demonstrated the impact of nutrition on the pregnancy and growth of dairy heifers (Pereira et al., 2020; Han et al., 2021; Oliveira et al., 2023). However, a critical aspect to consider is the influence of diet on the supply, absorption, and availability of nutrients to the heifer. This is crucial because the concentration of a nutrient can affect its availability, absorption, or interaction with the production of other nutrients (Lean et al., 2014; Rodney et al., 2015; Castro et al., 2020).

Pregnant heifers exhibit distinctive nutritional requirements compared to other cattle categories. Beyond the needs for maintenance and gestation, heifers are concurrently undergoing growth and development. Any nutritional deficit during this period can result in setbacks that hinder the expression of their maximum genetic potential, creating challenges in reversing such damage throughout their productive life (Micke et al., 2010).

The main objective of this study was to evaluate the impacts of moderate (0.35 kg/d) and high (0.70 kg/d) ADG on 5/8 Holstein × 3/8 Gyr heifers during pregnancy. Our assessment covered multiple facets, including growth, nutrient digestibility, milk yield and composition, as well as several metabolic parameters. Our hypothesis is that heifers fed to attain an appropriate BW at calving would transition into more productive cows.

2. MATERIALS AND METHODS

The experiment was conducted at the Dairy Research Facility of the Department of Animal Science at the Universidade Federal de Viçosa, located in Viçosa, Minas Gerais, Brazil. All procedures were pre-approved by the Animal Use Ethics Committee of the Department of Animal Science at the Universidade Federal de Viçosa, under protocol number 015/2022.

2.1 Experimental Design, Animals and Diet

The experiment, conducted over a period of 2 yr and 4 mo, initially included 35 heifers (5/8 Holstein × 3/8 Gyr). These heifers, averaging 15 mo old and 420 ± 15.0 kg, underwent three synchronization protocols for embryo transfer to achieve the necessary number of animals for the study. The hormonal estrus synchronization protocol was structured as follows: Day 1: insertion of a slow-release progesterone intravaginal device (controlled drug release - CDR); Day 7: administration of 2.5 mL of Dinoprost Tromethamine and 1 mL of Equine Chorionic Gonadotropin (ECG); Day 9: removal of the CDR and administration of 2.5 mL of Dinoprost Tromethamine and 0.3 mL of Estradiol Cypionate (ECP); Day 18: embryo implantation and 1 mL of Lecirelin.

The embryos, all sexed females 3/4 Holstein × 1/4 Gyr from the same bull (Mosaic-ET, No. 003141559764), were from a same dairy farm. Post-transfer, all heifers were allocated to Mombasa (Megathyrsus maximus) pasture, supplemented with a TMR diet based on corn silage, corn grain, soybean meal and a mixture of mineral and vitamin. Pregnancy was initially diagnosed via Doppler ultrasound at 32 d post-transfer and confirmed at 120 d through rectal palpation.

Upon confirmation of pregnancy at 70 d of gestation, the heifers were moved to individual stalls (9 m^2) in a Free Stall system. The heifers were randomized distributed in one of two treatments: MOD: received 1.05% of their BW in a TMR diet, targeting an ADG of 0.35 kg/d; and HIG: received 1.5% of their BW in TMR, targeting an ADG of 0.70 kg/d.

During the experiment, no orts were observed as the treatments were specifically designed to restrict the ADG, aligning with the objectives of the study. The

TMR was consistently offered to all heifers twice daily, at 08:00 and 16:00 h (Table 1). Additionally, samples of the corn silage and concentrate were collected daily for analysis to ensure consistency and quality of the feed components throughout the duration of the study.

Table 1. Ingredients and chemical composition of experimental diets.

Item	
Ingredient, %	
Corn silage	71.9
Soybean meal	24.6
Dicalcium phosphate	2.3
Mineral mix	1.2
Chemical composition, % DM	
Dry matter	38.1
Neutral detergent fiber	43.0
Crude protein	17.2
Rumen degradable protein	12.0
Rumen undegradable protein	5.2
Starch	25.8
Ether extract	2.7
Metabolizable protein:metabolizable energy	45.1

From the first day that the heifers were allocated to the Free Stall system, they were weighed every 15 d. This frequent weighing was essential to monitor and control the ADG and to adjust the amount of TMR offered to each heifer daily, ensuring that the feeding regime was tailored to meet the specific growth targets set for the study.

2.2 *Animal Measurements and Sampling*

Body morphometry assessments were conducted at the start of the experiment and pre-partum (at 100 and 270 d of gestation), focusing on key morphological metrics including withers height (WH), rump height (RH), body length (BL), and thoracic perimeter (TP). All measurements were taken with the heifers positioned on a flat surface to ensure accuracy and consistency, as recommended by Menezes et al. (2023). The heifers were at rest during these measurements, and an anthropometric ruler was used to measure their heights. Additionally, the BCS of each heifer was assessed by the same individual throughout the study to maintain consistency. The BCS was evaluated using a 5-point scale, where a score of 1 indicates an extremely thin heifer and a score of 5 indicates an obese heifer.

Once the heifers reached 100 d of gestation, the collection period commenced, structured into five periods, each lasting five days with 30-d intervals. Fecal and urine sample collections were scheduled at 06:00, 09:00, 12:00, 15:00, and 18:00 h. Daily fecal was performed by rectal sampling. The fecal samples were carefully placed in labeled aluminum trays and dried in a forced ventilation oven at 55°C for 72 h. Following the pre-drying step, the samples were ground in a knife mill using 2 mm and 1 mm sieves, and then stored in sealed, labeled containers.

Urine samples were collected from the animals by stimulating excretion through vulva massage. Two types of samples were taken: one of pure urine and one diluted in sulfuric acid at a concentration of 0.036 N (Silva et al., 2018). These samples were then stored in a freezer at -20°C until analysis. A composite sample was created for each cow per period and analyzed for creatinine and purine derivative levels.

Blood sampling was performed on the last day of each collection period, 4 h after feeding, using the coccygeal vein. Sterile vacuum tubes equipped with a clot activator and separation gel were used for this process. After the collection, the blood samples were centrifuged to separate the serum, which was then transferred into duly labeled 2 mL Eppendorf tubes. These samples were stored at -20°C to ensure that the biological samples were preserved under optimal conditions for subsequent analysis.

After calving, primiparous were transferred to a compost barn system for the lactation phase. Throughout lactation, the diet provided to these heifers remained consistent extending until the end of their lactation period (Table 2). Milk yield for each heifer was monitored weekly. To accurately assess the milk production of each heifer in each experimental group throughout lactation.

Table 2. Ingredients and chemical composition of experimental diets.

Item	
Ingredient, %	
Corn silage	72.4
Corne grain dry – fine grind	13.0
Soybean meal	7.8
Vitamin Mineral Core – TN Milk12	1.8
Urea	1.7
Sodium Bicarbonate	1.2
Protected fat	1.1
Magnesium oxide	0.6
Dicalcium phosphate	0.3
Sulfur flower	0.1
Virginiamycin	0.02
Chemical composition, % DM	
Dry matter	42.1
Neutral detergent fiber	43.9
Crude protein	16.0
Rumen degradable protein	12.5
Rumen undegradable protein	3.5
Starch	32.2
Ether extract	3.9
Metabolizable protein:metabolizable energy	56.1

Once a month, milk samples were collected from each cow (350 mL) during each milking session using a mechanical milking electronic flow meter (GEA Westfalia Surge of Brazil, GEA Farm Technologies of Brazil, Jaguariuna, São Paulo, Brazil). This process was conducted for three consecutive days, and the samples were then analyzed for fat, protein, and lactose content using an ultrasonic milk analyzer Lactoscan S LP (Milkotronic LTD, Nova Zagora, Bulgaria). Each milking time was analyzed separately for all parameters and then were polled. The milk production was correct for energy (ECM) through the following equation: 0.25 × kg Milk + 12.2 × kg Fat + 7.7 × kg Crude Protein (Sjaunja et al., 1991).

2.3 *Laboratory Analysis*

The feces and feed samples (corn silage and concentrate) were analyzed for DM content using method 934.01, OM using method 972.43, ether extract (EE) using method 920.39, CP calculated as N multiplied by 6.25 by method 984.13, and OM using method 942.05, all according to AOAC (1990) guidelines. Neutral detergent fiber analysis was conducted following the protocol outlined by Mertens et al. (2002), which involves the use of thermostable α-amylase without the addition of sodium sulfite during the detergent phase and includes autoclaving as part of the sample preparation (Pell and Schofield, 1993). Starch analysis was conducted using the acetate buffer method as described by Hall (2009).

Indigestible NDF was analyzed in triplicate for samples of the feed ingredients (concentrate, corn silage) and feces ground to 2 mm (Valente et al., 2011). For this analysis, samples were incubated for 288 h in cannulated cows (with a roughage to concentrate ratio of 50:50), using polyester bags with a pore size of 12 µm that were pre-weighed.

Urine samples were analyzed for concentrations of purine derivatives, specifically creatinine and uric acid. A Mindray automated biochemical analyzer (Mindray, Shenzhen, China) was employed to measure allantoin concentrations using the method described by Fujihara et al. (1987) and Chen and Gomes (1992). The efficiency of microbial synthesis was estimated following the approach by Barbosa et al. (2011), based on the daily excretion of purine derivatives, calculated as the sum of the excretions of allantoin and uric acid in the urine. Urinary excretion of purine derivatives was calculated by summing the total excretions of allantoin, creatinine, and uric acid, and these values were multiplied by the daily urinary volume to obtain total excretion levels.

Albumin and total protein levels were determined using the bromocresol green method, according to the methodology outlined by Doumas et al. (1971). Globulin levels were subsequently calculated by subtracting the albumin values from the total protein levels. Glycemic levels were assessed using the enzymatic method detailed by Raabo and Terkildsen (1960). Furthermore, levels of thyroid hormones T3 (triiodothyronine) and T4 (thyroxine), as well as insulin-like growth factor 1 (IGF-1), were measured using the chemiluminescence method, as described by Ferreira and Rossi (2002).

2.4 Statistical Analyses

Data concerning performance, blood metabolites, digestibility, milk yield, and milk composition were analyzed using the MIXED procedure in SAS (SAS Institute Inc., 2008). The basic statistical model employed was:

$$Y_{ij}=\mu+ D_i+B_j+\varepsilon_{ij},$$

where Yij represents the response variable, μ is the overall mean, Di is the effect of diet "D" on experimental unit "i", Bj is the random effect of blocking (formed into 3 blocks using the date of calving of each heifer as the blocking criterion), and ϵij represents the associated unobserved random error. Statistical significance was again determined at $P < 0.05$ and trends were considered at $0.05 < P \leq 0.10$.

For repeated measures data (blood metabolites, digestibility, milk yield, and milk composition), the model was extended as follows:

$$Y_{ijk}=\mu+ D_i + T_j + (D\times T)_{ij} + B_k + \delta_{ijk} + \varepsilon_{ijk},$$

where Yijk represents the obtained response, Di the effect of treatment "D" on experimental unit "i", Tj the effect of time (repeated measure) on experimental unit "j", (D×T)ij was the interaction between diet and time, Bk was the random effect of blocking (using calving dates divided into 3 blocks: born at 1st from October to November 2020, 2nd in January 2021, and 3rd from September to October 2021), δijk was the random error where variance between animals within diet equals covariance between repeated measures within animals, and εijk was the associated unobserved random error.

Statistical significance was again determined at $P < 0.05$ and trends were considered at $0.05 < P \leq 0.10$.

The tested covariance structures included compound symmetry, heterogeneous compound symmetry, heterogeneous first-order autoregressive, and unstructured matrices. The selection of the appropriate structure was based on the corrected Akaike information criterion, ultimately favoring the heterogeneous compound symmetry.

Outlier detection was implemented using internal student residuals greater than |2.5||2.5||2.5|. When necessary, means comparisons were performed using the Tukey test at a significance level of $P < 0.05$, and trends were acknowledged at $0.05 < P \leq 0.10$.

Power analysis was performed considering a CV of 10%, for a significance level of 0.05 and a power of 80%, which determined that a minimum of 7 animals per treatment was required.

3. RESULTS

3.1 Nutrient Digestibility

As gestation progressed, the intake of DM, OM, CP, NDF, and starch significantly increased (Table 3). This increase was directly related to the rising BW of the heifers in both groups. Consequently, the diet offered was proportionally increased to ensure that the nutritional needs of the heifers were adequately met, supporting their growing physiological demands throughout gestation. The adjustments in the offered diet were done according to the methodological aim of this study; therefore, no statistical analyses were performed for these parameters.

Table 3. Intake and total tract apparent digestibility of pregnant 5/8 Holstein × 3/8 Gyr heifers fed with different ADGs.

Item	MOD[1] day of gestation						HIG[2] day of gestation						SEM	*P*-value		
	122	153	184	213	246	277	122	153	184	213	246	277		ADG	day	ADG × day
Intake, kg/d																
Dry matter	4.93	5.22	5.34	5.49	5.57	5.65	6.82	7.23	7.56	7.91	8.07	8.33	--	--	--	--
Organic matter	4.68	4.95	5.06	5.20	5.28	5.36	6.47	6.85	7.17	7.50	7.66	7.90	--	--	--	--
Crude protein	0.85	0.89	0.92	0.94	0.96	0.97	1.17	1.24	1.30	1.36	1.39	1.43	--	--	--	--
NDF[3]	2.12	2.46	2.30	2.26	2.40	2.43	2.93	3.11	3.25	3.40	3.47	3.58	--	--	--	--
Starch	1.27	1.35	1.38	1.42	1.44	1.46	1.76	1.87	1.95	2.04	2.08	2.15	--	--	--	--
Digestibility, %																
Dry matter	65.2	66.2	68.0	65.5	67.7	67.4	65.3	66.7	66.6	64.9	66.0	66.0	0.80	0.34	0.28	0.82
Organic matter	67.5	68.4	70.3	67.6	69.6	71.0	65.8	69.0	69.1	67.1	67.7	67.8	0.74	0.25	0.17	0.60
Crude protein	76.4	78.8	78.4	77.0	77.4	77.6	67.8	69.0	68	67.2	67.7	67.9	0.58	0.19	0.26	0.94
NDF[3]	47.5	52.1	57.6	46.1	54.1	51.8	46.2	48.1	56.1	48.0	54.5	51.9	1.03	0.64	<0.01	0.81
Starch	97.7	96.0	96.8	98.4	96.9	96.4	97.6	96.9	96.0	96.2	95.4	96.4	0.67	0.14	0.11	0.90
Feed efficiency	0.06	0.19	0.07	0.07	0.06	0.04	0.09	0.12	0.10	0.10	0.05	0.09	0.021	0.32	<0.01	0.08
N-MIC[4], g/d	47.0	43.8	44.0	39.5	41.3	45.1	72.4	82.3	85.2	91.9	89.9	93.6	0.93	<0.01	<0.01	<0.01
EffMIC[5], g MP/kg OMDR	27.6	28.1	28.6	27.2	27.1	27.1	23.1	20.3	19.2	17.1	17.2	18.9	0.76	<0.01	0.06	0.32

[1]MOD = average daily gain 0.37 kg; [2]HIG = average daily gain 0.72 kg; [3]Neutral detergent fiber; [4]Microbial nitrogen; [5]Efficiency of rumen microorganisms in N production as a function of the amount of organic matter digested in the rumen (assuming that rumen digestion was 65% organic matter of digestion in total tract).

No interaction (P > 0.10) was observed between ADG and days of gestation regarding nutrient digestibility. Similarly, no differences (P > 0.10) in nutrient digestibility were noted for different ADG levels during gestation. However, NDF digestibility was higher (P < 0.01) for longer gestation periods. Feed efficiency was negatively affected (P < 0.01) by the length of gestation, with the lowest values observed during longer gestation periods.

A significant interaction was found for the N of microbial origin (P < 0.01; Table 3). Similar values were observed for MOD and HIG at 122, 153, and 184 d. However, at 215, 246 and 277 d of gestation, HIG presented greater values than MOD (Figure 1). Additionally, a pronounced effect of ADG on the efficiency of protein synthesis of microbial origin was observed (P < 0.01; Table 3). Heifers in the MOD group were more efficient in synthesizing microbial N compared to those in the HIG group throughout their pregnancy.

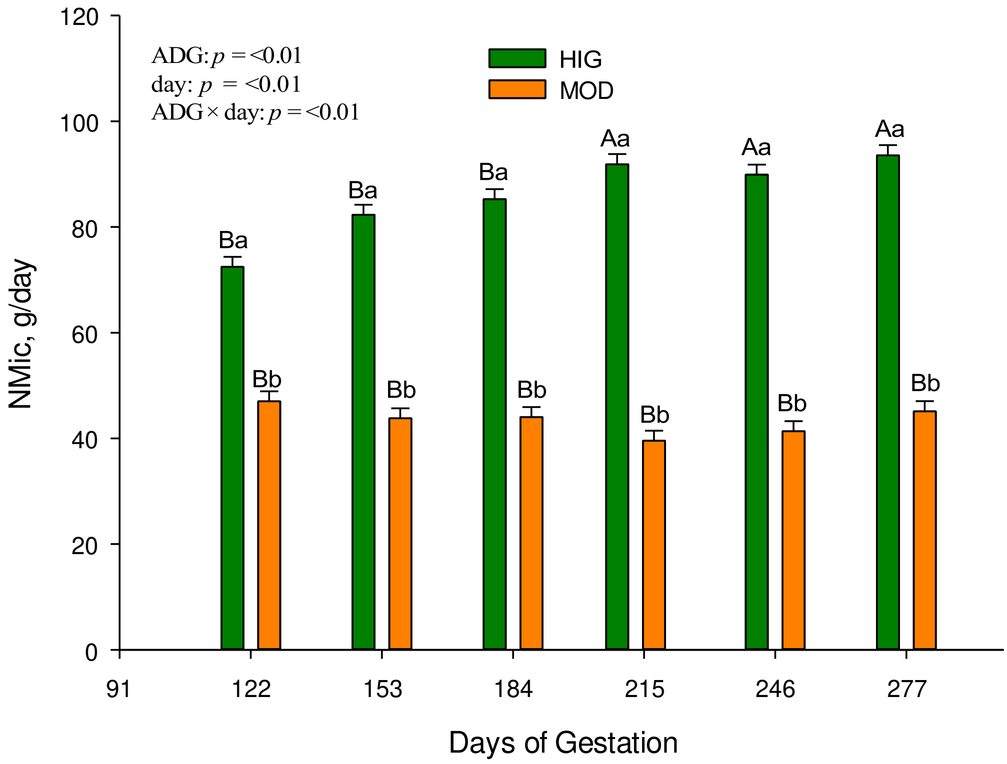


Figure 1. Mean and standard errors of the mean for microbial N in 5/8 Holstein × 3/8 Gyr heifers subjected to either moderate gain (MOD; 0.37 kg/d) or high gain (HIG; 0.72 kg/d) throughout pregnancy. Significance was declared at $P \leq 0.05$, with trends noted

between 0.10 ≤ P ≥ 0.05. The green bar represents heifers fed HIG, while the orange bar represents heifers fed MOD. * Indicates a significant of $P < 0.05$.

3.2 Growth

At 70 d of gestation, initial BW were similar between the treatments, showing no significant difference (P = 0.48; Table 4). However, heifers in the HIG group demonstrated higher ADG of 0.72 kg/d, in contrast to 0.37 kg/d in MOD group (P < 0.01), as proposed by the study. This resulted in a substantial difference in BW at 270 d of gestation, with HIG heifers weighing 602.8 kg versus 543.4 kg for MOD heifers (P < 0.01; Table 4), illustrating the profound impact of higher ADG on the growth trajectory of heifers during gestation.

Table 4. Animal performance of 5/8 Holstein × 3/8 Gyr heifers during gestation fed different ADGs.

Item	MOD[1]	HIG[2]	SEM	*P*-value
Initial BW[3], kg	476.4	457.8	25.93	0.48
Final BW[4], kg	543.4	602.8	7.60	<0.01
ADG[5], kg	0.37	0.72	0.044	<0.01
WH[6], m	1.51	1.50	0.001	0.50
RH[7], m	1.48	1.52	0.023	0.28
HG[8], m	2.33	2.40	0.029	0.02
BL[9], m	1.47	1.46	0.003	0.15
Initial BCS[10]	2.89	2.84	0.127	0.67
Final BCS[11]	3.23	3.70	0.070	<0.01

[1]MOD = average daily gain 0.37 kg; [2]HIG = average daily gain 0.72 kg; [3] Initial body weight (weight when the heifers entered the experiment); [4] Final body weight (pre-calving weight of heifers - 270 days of gestation); [5]Average daily gain; [6]Withers height; [7]Rump height; [8]Heart girth; [9]Body length; [10]Initial body condition score; [11]Final body condition score.

Morphometric analysis revealed no differences in WH (P = 0.50), RH (P = 0.28), and BL (P = 0.15). However, the HG of the heifers had a significant variation; those in the HIG group had greater measurements (P = 0.02) compared to the MOD group heifers (Table 4).

Body condition scores at the start of the experiment were similar across treatments (P = 0.67). However, a significant difference in BCS was observed at the end of pregnancy (270 d of gestation), considering BCS at the beginning of the experiment used as a covariate. Heifers in the HIG group showed higher BCS (3.70) compared to those in the MOD group (3.23; P < 0.01), mirroring the differences observed in BW.

3.3 Metabolites

No interaction (P > 0.10) was observed between ADG and days of gestation for blood concentrations of insulin-like growth factor 1 (IGF-1), thyroxine (T4), glucose, albumin, globulin, total proteins, and urea (Table 5). However, an interaction (P < 0.01) between ADG and days of gestation was observed for T3 (Figure 2). Notably, heifers in the MOD group presented higher T3 values at 91 and 246 d of gestation. Regarding ADG effect on metabolites, we observed difference for urea (P = 0.03), where heifers in the HIG group exhibited higher concentrations of urea throughout pregnancy compared to those in the MOD group.

Table 5. Blood concentrations of Insulin-like Growth Factor (IGF-1), T3 (triiodothyronine), T4 (thyroxine), Glucose, Albumin, Globulin, Total proteins and Urea in 5/8 Holstein × 3/8 Gyr heifers.

Item	MOD[1]						HIG[2]						SEM	*P*-value		
	day of gestation						day of gestation									
	122	153	184	213	246	277	122	153	184	213	246	277		ADG	day	ADG × day
IGF-1[3], ng/mL	182	224	207	169	163	121	174	189	194	165	149	133	12.8	0.16	<0.01	0.68
T3[4], ng/mL	1.03	0.89	0.91	0.78	0.71	0.72	0.84	0.88	0.83	0.83	0.81	0.91	0.064	0.79	<0.01	<0.01
T4[5], mcg/mL	5.44	5.41	5.55	4.43	4.26	4.15	5.2	5.09	5.34	5.18	5.1	5.12	0.304	0.11	0.07	0.18
Glucose, mg/mL	63.3	61.6	60.7	57.3	56.5	58.6	62.5	60.2	59.8	56.8	55.5	54.6	2.439	0.15	<0.01	0.94
Albumin, g/dL	4.67	4.5	4.34	4.04	3.86	3.38	4.54	4.48	4.54	4.26	4.14	3.44	0.277	0.36	<0.01	0.86
Globulin, g/dL	3.42	2.46	3.62	3.5	3.6	3.52	3.36	3.32	3.53	3.47	3.61	3.47	0.134	0.32	0.13	0.98
TP[6], g/dL	8.09	7.97	7.96	7.53	7.46	6.9	7.89	7.74	7.95	7.6	7.74	6.91	0.216	0.91	<0.01	0.77
Urea, mg/dL	125	152	154	146	124	115	134	159	174	175	150	130	13.1	0.03	<0.01	0.84

[1]MOD = average daily gain 0.37 kg; [2]HIG = average daily gain 0.72 kg; [3]Insulin-like Growth Factor.

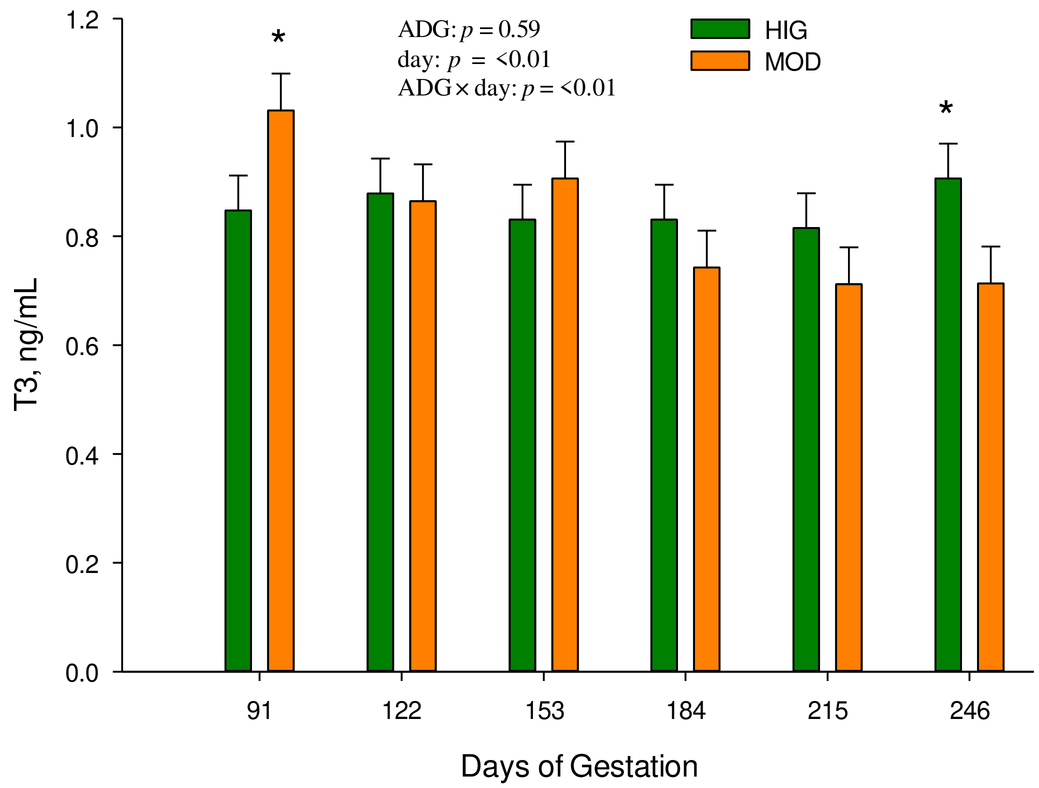


Figure 2. Means and standard errors of the mean for blood T3 concentration in 5/8 Holstein × 3/8 Gyr heifers fed moderate gain (MOD; 0.37 kg/d) or high gain (HIG; 0.72 kg/d) during pregnancy. Significance was declared $P \leq 0.05$ and trend $0.1 \leq P \geq 0.05$. The green bar represents heifers fed HIG, and the orange bar heifers fed MOD. * = Months where interaction was found $P < 0.05$.

3.4 Milk Yield and Composition

No interaction (P > 0.10) was observed between ADG and week of lactation for MY, ECM, MY 305 d, ECM 305d, and milk components, except for trends in fat percentage (P = 0.05), total solids (P = 0.07; Table 6), and MUN (P = 0.06). Fat percentage tended to be greater in the MOD group, showing higher values in 6 of the 9 mo evaluated. In contrast, the HIG group showed a higher fat percentage in only one month (Figure 3A). Regarding total solids, the MOD group presented greater values in 2 of the 9 mo evaluated, while the HIG group showed higher values in only one month (Figure 3B). Milk urea nitrogen was higher for the HIG group during the first two months of lactation (Figure 4).

Table 6. Milk yield and composition of Holstein × Gyr primiparous fed with different ADGs during gestation.

Item	MOD[1]	HIG[2]	SEM	*P-value*		
				ADG	week	ADG × week
Milk yield, kg/d	22.5	23.3	2.61	0.72	<0.01	0.96
Milk yield corrected protein and fat, kg/d	21.4	21.0	2.23	0.69	<0.01	0.99
Total milk yield 305-d, kg/d	6823.5	6834.1	689.6	0.99	--	--
Total milk yield 305-d corrected protein and fat, kg	6333.0	5952.2	620.1	0.55	--	--
Fat, %	3.32	3.06	0.166	0.27	0.24	0.05
Fat, kg	0.73	0.74	0.095	0.94	<0.01	0.22
Protein, %	3.23	3.26	0.036	0.54	0.24	0.43
Protein, kg	0.72	0.80	0.079	0.26	<0.01	0.55
Lactose, %	4.92	4.87	0.039	0.29	<0.01	0.40
Lactose, kg	1.11	1.20	0.124	0.48	<0.01	0.22
Total solids, %	12.3	11.9	0.204	0.12	<0.01	0.07
Total solids, kg	2.80	2.90	0.333	0.73	<0.01	0.32
SCC[3], 1000 cells/mL	37.4	37.7	0.152	0.86	0.70	0.46
MUN[4], mg/dL	15.4	16.2	0.458	0.17	<0.01	0.06

[1]MOD = average daily gain 0.37 kg; [2]HIG = average daily gain 0.72 kg; [3]Somatic Cell Count (the data were statistically analyzed in a Log (10) base, and the means were converted to table presentation). [4]Milk urea nitrogen.

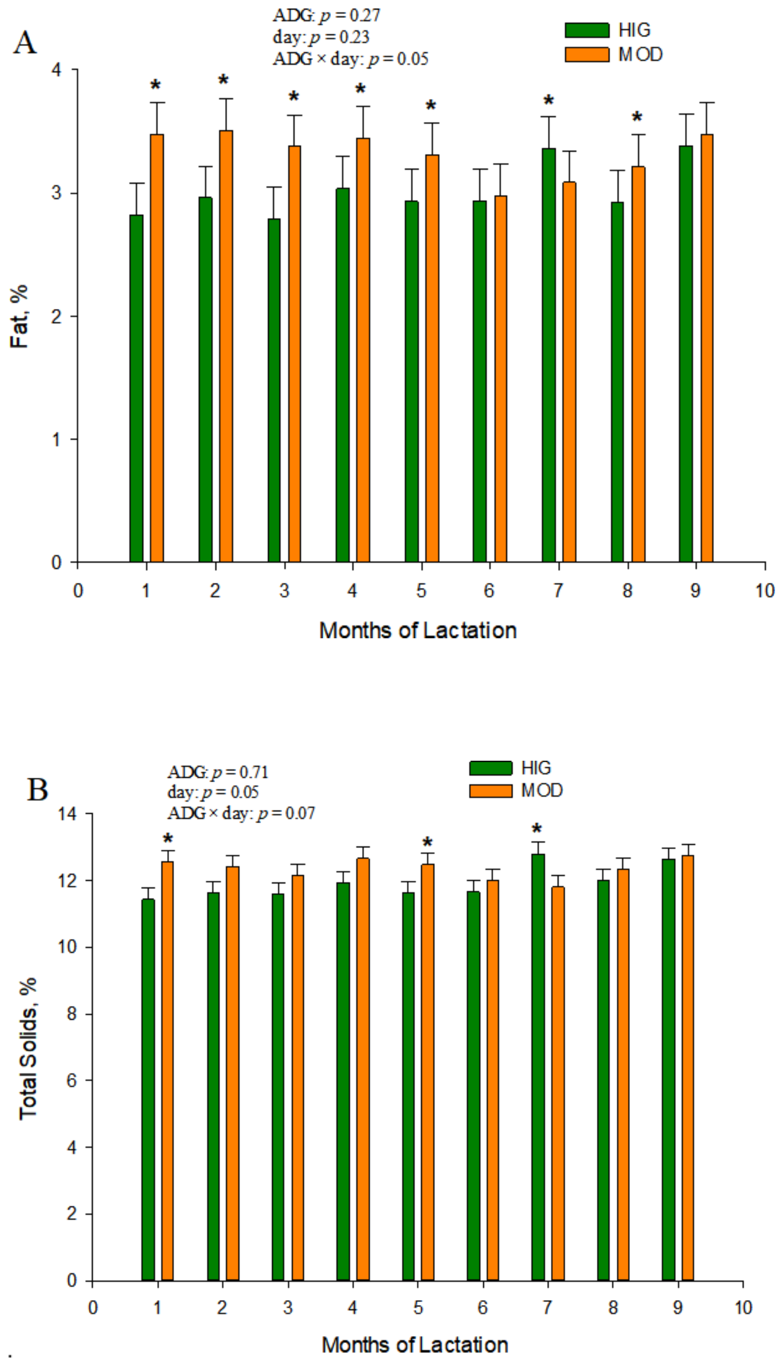


Figure 3. Mean and standard errors of the mean for percentage of fat (A) and total solids (B) in the milk of 5/8 Holstein × 3/8 Gyr heifers subjected to moderate gain (MOD; 0.37 kg/d) or high gain (HIG; 0.72 kg/d) throughout pregnancy. Significance was declared at $P \leq 0.05$, with trends observed between $0.10 \leq P \geq 0.05$. The green bar represents heifers fed HIG, while the orange bar represents heifers fed MOD. *Indicates significant at $P < 0.05$.

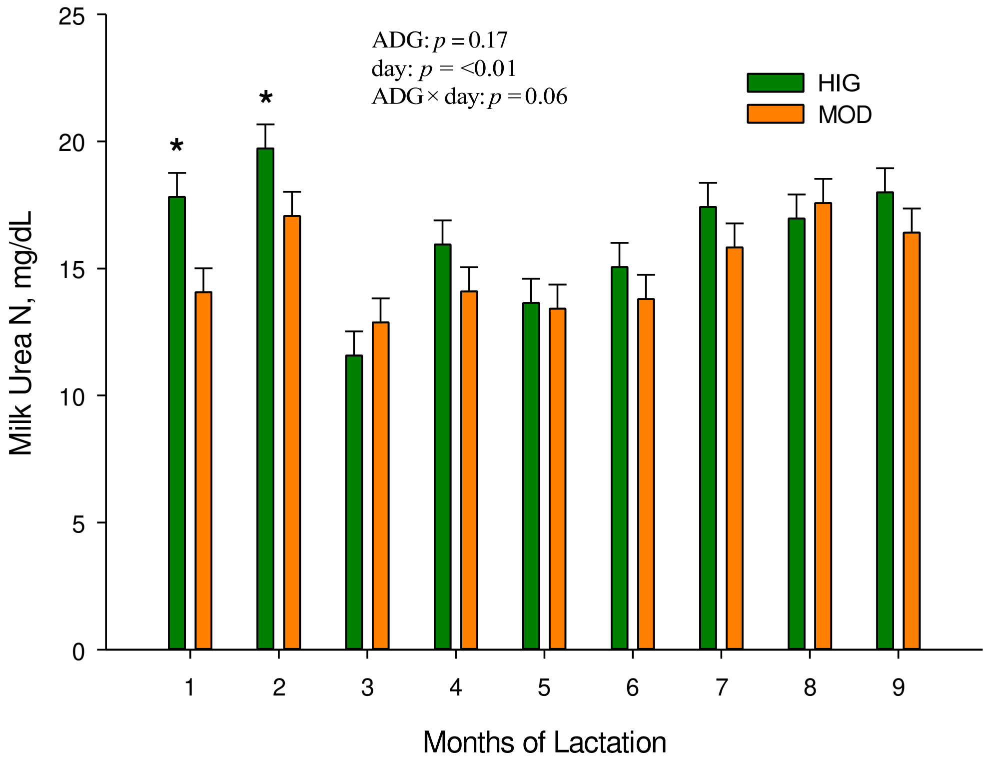
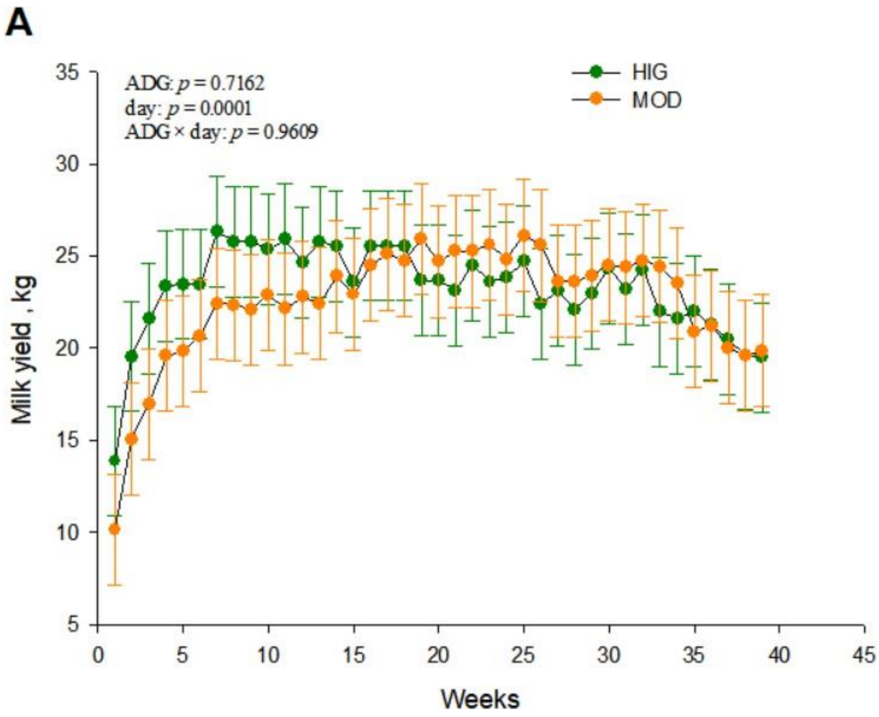


Figure 4. Means and standard errors of the mean for the MUN in Holstein × Gyr cows fed to achieve moderate (MOD; 0.37 kg/d) or adequate (HIG; 0.72 kg/d) during gestation.

This can be observed graphically (Fig. 4), where we can observe the behavior of the lactation curves of the nipples of both experimental groups. Analyzing the lactation curve of the heifers from both groups, we see that the HIG heifers presented their peak production between weeks 5 - 10, while the MOD heifers between weeks 15 - 20 of lactation.



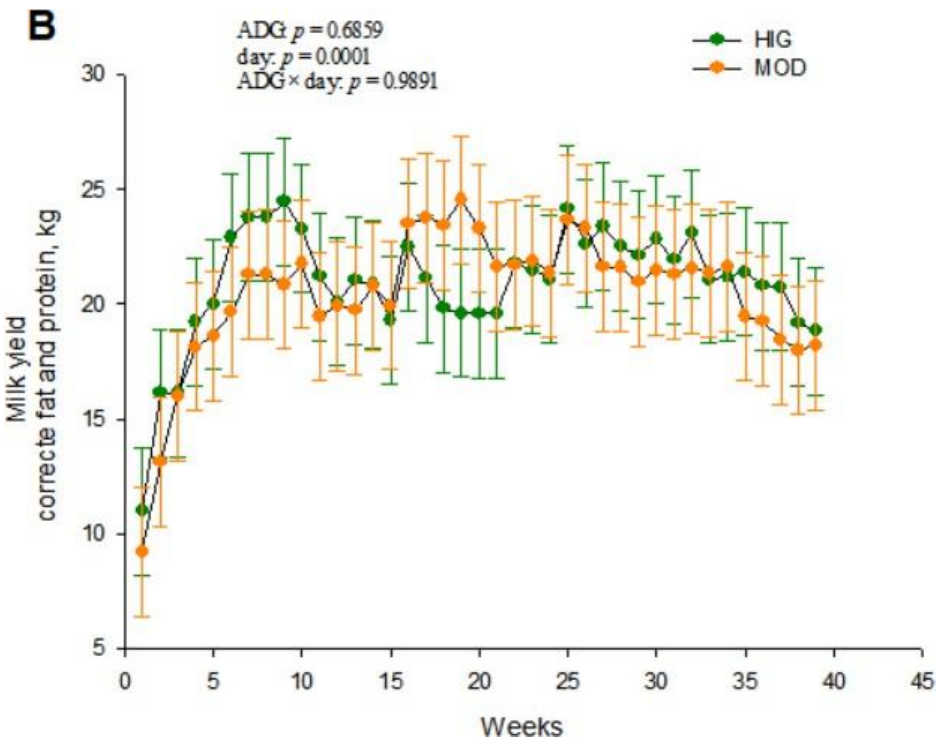


Figure 5. Means and standard errors of the mean for milk yield (A) and milk yield produced for 4% fat and 3.3% protein (B) in the milk of 5/8 Holstein × 3/8 Gyr heifers subjected to gain moderate (MOD; 0.37 kg/d) or high gain (HIG; 0.72 kg/d) throughout pregnancy. Significance was declared at $P \leq 0.05$, with trends observed between $0.10 \leq P \geq 0.05$. The green bar represents heifers fed HIG, while the orange bar represents heifers fed MOD.

The different ADG during the gestation did not affect (P > 0.10) the milk production and milk composition since using the same diet after calving. However, the week of lactation influenced most of those parameters, with exception protein percentage and SCC (Table 6).

4. DISCUSSION

The observed proportional increases in the intake of DM, OM, CP, NDF, and starch were a necessary adaptation to fulfill the heightened nutritional needs prompted by increased BW and physiological changes during gestation. While these dietary adjustments were not subjected to statistical analysis, given their integration into the study's methodological framework, the findings underscore the critical role of tailored feeding strategies in maintaining the health and productivity of pregnant heifers. Such strategies ensure that nutritional support adapts dynamically to the changing physiological demands throughout pregnancy, thus optimizing outcomes.

The reduced ingestive capacity in the final trimester of pregnancy, as documented in prior research (Poland et al., 2005; Rotta et al., 2015; Pereira et al., 2020), is attributed to the substantial enlargement of the uterus—by up to 50% in the last two months—which compresses the digestive compartments (rumen, reticulum, omasum, and abomasum). This physiological modification necessitates meticulous dietary management to prevent undernutrition during this critical period. In this study, the feeding regime was specifically designed to meet predetermined ADG, which precluded the possibility of offering ad libitum diets. This approach highlights the intricate balance between achieving targeted growth rates and addressing the physical limitations imposed by late gestation, illustrating the complex interplay between nutritional strategy and physiological capacity in pregnant heifers.

The variations in NDF digestibility and feed efficiency noted over the course of gestation, particularly the decline in feed efficiency as gestation progresses, mirror the physiological stress and nutritional challenges inherent in later stages of pregnancy. Enhanced NDF digestibility during these advanced stages may serve as a compensatory response by the animals, aimed at maximizing nutrient absorption amid decreasing gut motility. This physiological adjustment is crucial as it supports optimal nutrient utilization when the physical expansion of the uterus imposes constraints on the digestive organs.

Such adaptations are vital to prevent nutritional deficits that could adversely affect both the cow and the developing fetus (Rotta et al., 2015; Pereira et al., 2020). Despite these challenges, heifers from both study groups exhibited consistent ingestive behaviors throughout the pregnancy. This observation underscores their resilience and the effectiveness of carefully managed feeding regimes that consider the unique nutritional needs imposed by pregnancy, even under conditions of feed restriction.

The notable interaction observed for NMIc between different gestation periods and ADG with HIG exhibiting greater values in later stages, underscores a more efficient microbial protein synthesis linked to higher nutritional intake. This enhanced microbial activity in the HIG group likely stems from an improved rumen environment conducive to microbial proliferation, thereby optimizing nutrient utilization. The relationship between NMIc production and DM intake, as evidenced by prior research (Bateman et al., 2005; Broderick et al., 2010; Roman-Garcia et al., 2016), is primarily driven by the increased availability of carbohydrates, which serves as a key substrate for ruminal microorganisms.

An increase in DM intake typically alters the passage rate through the digestive system. While an increased passage rate can enhance nutrient availability and intake capacity, it may paradoxically reduce DM digestibility by shortening the time available for microbial action (Firkins et al., 2007). The distinct difference in microbial N synthesis efficiency observed between the HIG and MOD groups can be attributed to the differences in DM intake. Specifically, the MOD group, receiving a lower quantity of DM, may experience less efficient microbial protein synthesis due to reduced substrate availability. This nuanced understanding highlights the delicate balance required in feed management strategies to optimize both nutrient intake and microbial efficiency, thereby ensuring that dietary provisions are aligned with the physiological demands of pregnancy in dairy heifers.

The growth trajectory differences between the HIG and MOD groups, with HIG heifers demonstrating significantly higher BW and ADG by the end of gestation, distinctly illustrate the profound impact of increased ADG on overall growth performance. This effect is manifested not only in the elevated BW but also in the superior HG measurements and BCS noted in the HIG group. These physical growth parameters are vital as they are directly associated with enhanced future reproductive and lactational performance.

During the gestational phase of this study, the HIG group achieved a daily gain of 0.72 kg/d, a value considered elevated for Holstein × Gyr crossbreed heifers in the rearing phase. It is essential to acknowledge that pregnant heifers have specific nutritional needs encompassing maintenance, gestation, and growth, as they have not yet reached their mature BW. Achieving an appropriate growth rate is crucial as it ensures that both the heifer and her calf can express their maximum genetic potential, thereby optimizing productivity (Han et al., 2021).

Conversely, heifers in the MOD group, which achieved lower gains of 0.37 kg/d, may encounter difficulties in allowing their calves to reach their full productive potential during the first lactation (Kertz et al., 2017). This underscores the significance of adequate fetal nutrition, which, as suggested by Sguizzato et al. (2020), should commence from the 70th day of gestation to ensure satisfactory growth and development. Nutritional deficiencies during this critical developmental phase can profoundly impact the future productivity and reproductive performance of the offspring. Although compensatory growth mechanisms exist, they may not fully offset the detriments caused by nutritional shortcomings during this pivotal period (van Niekerk et al., 2021).

Moreover, Barcelos et al. (2022) highlighted the adverse effects of nutritional deficits during mid to late gestation on fetal development in sheep, specifically impairing muscle growth by reducing myonuclei and muscle DNA. These effects are attributed to the disruption of satellite cell proliferation and incorporation—critical processes since myogenesis occurs exclusively in the prenatal period. Although similar detailed studies on Holstein × Gyr heifers are lacking, the implications of these findings warrant careful consideration. It is crucial to investigate whether MOD heifers, with their lower ADG, are similarly at risk of these developmental issues. Understanding and addressing these potential nutritional challenges during gestation is paramount to ensuring the health and productivity of the next generation of livestock.

The recommendations for heifers at first calving, as outlined by NASEM (2021), suggest a target of 91% of mature BW. In our study, heifers from HIG group notably exceeded this recommendation, reaching 98% of their expected mature BW for the Holstein × Gyr breed at pre-partum, whereas those from MOD group were slightly below the guideline at 89% of their mature BW. This disparity in pre-partum weights between the two groups echoes findings in other breeds, such as Holstein, where higher calving weights have been linked to increased milk production in subsequent lactations. Specifically, research by Abeni et al. (2000) indicates that Holstein heifers with higher BW at calving can produce an additional 550 kg of milk per lactation, and each kilogram less in BW at calving could equate to approximately 10 kg less milk yield, though this effect is less pronounced in the first lactation. However, we did not observe this effect in the present study.

The applicability of these findings to Holstein × Gyr heifers is particularly significant given the genetic differences and potential for variations in growth and lactation dynamics between breeds. The higher pre-partum weights observed in the HIG group may therefore suggest a potential for enhanced milk production and improved reproductive performance, aligning with trends observed in purebred studies. However, this hypothesis warrants more studies to determine if the increased BW at calving directly correlates with similar benefits in MY and reproductive outcomes for Holstein × Gyr heifers. Additionally, another part of this study assessed other reproductive parameters such as the duration of gestation, time elapsed from calving, time for expulsion of the placenta, and placenta weight, with no observed differences between the HIG and MOD groups (Oliveira et al., 2023).

The implications of these findings are substantial, underscoring the importance of optimal nutritional management and growth strategies during gestation to achieve desirable outcomes in both lactation and reproduction. As such, continuous monitoring and adjustment of growth trajectories in heifers, particularly in breeds like Holstein × Gyr with less extensive research, are crucial for maximizing productivity and enhancing the overall efficiency of dairy operations. Further studies tailored to these crossbred populations are needed to fully elucidate the relationships between BW at calving, milk production, and reproductive performance, thereby informing more precise and breed-specific management practices.

5. CONCLUSIONS

Despite the differences in BW and BCS, milk yield did not vary between heifers in the HIG and MOD groups. This suggests that while targeted nutritional strategies to enhance growth during gestation can positively impact physical development, they may not directly correlate with differences in milk production. Further investigation is needed to explore the long-term lactational responses and reproductive performance following different gestational growth rates.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DECLARATION OF DATA AVAILABILITY

The authors have access to all data obtained in this study and assume responsibility for the integrity and accuracy of the analyses. The data supporting the conclusions of this study are available via the corresponding author upon request.

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CHAPTER II - DIFFERENTIAL AVERAGE DAILY GAIN OF PREGNANT HOLSTEIN × GYR HEIFERS: CALF PERFORMANCE

Short title: Different nutritional strategies in dairy heifers and the effects on their calves

Chapter formatted according to the scientific journal: Journal of Dairy Science, except for the arrangement of tables and figures

Abstract

This study aimed to evaluate the impact of different average daily gains (ADG) in 5/8 Holstein × 3/8 Gir dairy heifers during pregnancy on the subsequent performance of their calves. Sixteen female 3/4 Holstein × 1/4 Gyr calves were included in the study. Their dams were randomly assigned to one of two treatments: 1) moderate feeding during pregnancy to achieve an ADG of 0.37 kg/d (MOD; n = 8) and 2) high feeding during pregnancy to achieve an ADG of 0.72 kg/d (HIG; n = 8). Colostrum, supplied at 15% of body weight, with a minimum Brix of 25% (via colostrum bank - UEPE dairy cattle). It was provided in the first two hours of life. Calves were closely monitored for 3 months from calving to weaning. Receiving 6 L of transition milk until 2 days of age, and 6 L of milk from the tank until weaning (the 6 L of milk offered was divided into two feedings of 3 L each - 8 in the morning and 16 in the afternoon). From the third day onwards, the calves received ad libitum starter (87% DM, 19.6% CP and 50.8% starch) and at 40 days hay (Cynodon dactylon) was introduced (collecting samples of both food - offered and leftovers). Morphometric measurements, body weight and blood collections were carried out on days 1, 31, 61 and 91. These collections aimed to evaluate the performance of the animals, concentrations of hormones and plasma metabolites. Calves in the HIG group tended to have greater body length at calving, as well as being born heavier compared to those in the MOD group. However, calves in the MOD group weaned heavier, due to having greater ADG. Added to this, there was no difference in the consumption of dry matter from concentrate or hay. In conclusion, calves born to HIG heifers tended to be born larger and heavier. However, they presented lower ADG (due to greater nutritional requirements) when purchased with calves from MOD heifers.

Key words: fetal programming, nutrition, performance, weaning

1. INTRODUCTION

In tropical regions, it is common practice to keep heifers in extensive grazing systems. Producers often maintain pregnant heifers on pasture until shortly before calving, which can lead to suboptimal nutritional conditions (Endecott et al., 2013). This approach aims to reduce production costs during the growth phase, resulting in low or moderate ADG during this period. Consequently, heifers frequently calve with BW below the recommended level of 91% of their mature BW (NASEM, 2021). To reach the recommended BW at calving, Holstein heifers should achieve an ADG of approximately 0.70 kg (Villanova et al., 2022).

However, in Brazil, it is an indicative that 70% of the dairy herd comprises Holstein × Gyr breed animals. Understanding the characteristics of this breed is vital for dairy farming in tropical countries. It is essential to recognize the breed's specific needs during the lactation phase and determine optimal strategies for nourishing dairy calves.

Studies suggest that calves born to heifers with low ADG during gestation may face several challenges, some irreversible, which can hinder their genetic potential and result in financial losses (Wilson et al., 2020; Magan et al., 2021). Regardless of the production system, adequate maternal nutrition is crucial for producing healthy newborns (Zhu et al., 2014). However, the residual effects of maternal nutrition during pregnancy remain unclear, primarily due to the lack of research evaluating different ADG on the future performance of calves, especially Holstein × Gyr animals.

A lower nutritional supply to the fetus may result from metabolic factors associated with maternal nutrition, leading to disturbances in placental metabolism, reduced blood flow, and compromised nutritional transport capacity (Rotta et al., 2015; Edwards et al., 2020; Oliveira et al., 2023). Such factors can result in lower BW at calving, as this parameter is influenced during the fetal phase (Vonnahme et al., 2007). Holstein cows or heifers that conceive with greater BW and BCS give birth to heavier calves (Bahashwan et al., 2015).

In dairy farming, calves represent the future of the productive herd; therefore, ensuring proper rearing, even during the fetal phase, contributes to the sustainability and profitability of the dairy farm (Heinrichs and Heinrichs, 2011). Providing sufficient fodder and starter to calves until weaning helps maintain ruminal pH, prevents hyperkeratinization, and improves the health status of these dairy calves (Khan et al., 2011; Beiranvand et al., 2014; Xiao et al., 2020).

The objective of this study was to evaluate the impact of two different ADG levels, 0.37 kg or 0.72 kg, in pregnant heifers on the future performance and development of their calves. The hypothesis of this study is that calves born from dams with ADG of 0.72 kg will exhibit better performance and overall health compared to calves born from dams with low ADG of 0.37 kg.

2. MATERIALS AND METHODS

The experiment was conducted at the Dairy Research Facility within the Animal Science Department of the Federal University of Viçosa, located in Viçosa, Minas Gerais, Brazil. All procedures were meticulously approved by the Animal Use Ethics Committee of the Department of Animal Science at the Federal University of Viçosa, Minas Gerais, Brazil (protocol 015/2022).

2.1 Experimental Design, Animals, and Diet

In this study, 16 female calves (3/4 Holstein × Gyr – from embryo transfer from the same farm and the same bull, Mosaic-ET, No. 003141559764) were used. These calves were born from crossbred 5/8 Holstein × 3/8 Gyr heifers. During gestation, these heifers were subjected to two different ADG – moderate (MOD) ADG of 0.37 kg and high (HIG) ADG of 0.72 kg. Detailed information on diets and management of pregnant heifers can be found in Oliveira Neto et al. (2025). The treatments were separated in MOD: animals received 1.08% of their BW in the form of TMR, targeting a moderate ADG of 0.37 kg/d; HIG: animals received 1.42% of their BW in the form of TMR, targeting a high ADG of 0.72 kg/d.

At 70 d of gestation, the heifers were housed individually in 9 m² (3 × 3 m) pens equipped with mattresses, feeders, and drinkers with free access to water. The individualized amount of feed required to achieve the specified ADG was provided based on each heifer's BW. The TMR was fed twice daily at 0800 and 1600 h. The diet formulation followed the guidelines described by NASEM (2021) for Holstein heifers.

Immediately after birth, the vitality of the calves was assessed using the criteria of Murray et al. (2015), modified by von Konigslow (2022). The assessment covered several parameters, each scored on a scale from 0 to 3 (with 3 indicating excellent and 0 indicating poor), except for eye reflex to touch, heart rate, and respiratory rate, which were scored on a scale from 0 to 2. Parameters assessed included meconium staining, head and tongue condition, calf movement, thumb sucking reflex, head bobbing in response to nasal cavity movement, tongue movement, ocular touch reflex, mucous membrane color, heart rate, and respiratory rate.

Subsequently, the umbilical cord of each calf was treated with 10% iodine, and the calves were weighed. The calves were fed colostrum amounting to 15% of their BW at birth, divided into two feedings: the first providing 10% of the BW within the first 2 h of life, and the second offering 5% of the BW between 2 and 4 h post-birth. Colostrum feeding was standardized to a concentration of 25% Brix, ensuring all colostrum was supplied within the first 2 h postpartum from a colostrum bank. The transfer of passive immunity via colostrum was assessed using a protein refractometer, with blood serum samples collected on the 5th day post-colostrum feeding, following the methodology proposed by Lopez and Heinrichs (2022).

From the 1st to 2nd day of life, the calves were fed transitional milk, with a daily total of 6 L divided into two feedings (3 L each at 0900 and 1600 h). From the 3rd day onwards, they were fed raw milk, maintaining the same total daily intake throughout the pre-weaning phase (6 L/day, divided into two feedings at 0900 and 1600 h), with no milk left at any point during the experimental period. The milk's composition included total solids of 12.2%, lactose 4.9%, fat 3.4%, and protein 3.3%.

The starter feed, comprising corn meal, soybean bran, wheat bran, mineral premix, calcium carbonate, dicalcium phosphate, vitamin premix, and salt (Table 1), was provided ad libitum. The feeding protocol began with 50 g on the 1st day, with daily increases based on the calf's ingestive behavior. From the 40th day of the calf's life, Tifton hay was introduced into the diet and offered ad libitum. The initial daily amount was 50 g, with subsequent daily adjustments to maintain the ad libitum feeding system based on consumption.

Table 1. Ingredients and chemical composition of the starter offered to calves during the lactation period.

Item	
Ingredient, %	
Corn grain dry, fine grind	70.60
Soybean meal	24.70
Wheat bran	3.10
Calcium carbonate	1.00
Dicalcium phosphate	0.30
Sodium chloride (salt)	0.20
VitTM Premix	0.10
Micro minerals	0.02
Chemical composition, % DM	
Dry matter	87.8
Neutral detergent fiber	10.9
Crude protein	19.6
Starch	50.8
Ether extract	3.1
Metabolizable protein:metabolizable energy	24.71

Stool scoring was performed every morning at 0800 h, using the following scale: 0 = normal; 1 = semi-formed and pasty; 2 = loose, but remaining on the mat; 3 = watery, sieved on the conveyor belt. These assessments aimed to monitor the calves' fecal consistency and the incidence of diarrhea. Diarrhea was considered present when the fecal score exceeded 2 (Lesmeister et al., 2004). Stool scoring was conducted using a standardized health scoring system by a single individual throughout the experimental period, following the criteria outlined by Renaud et al. (2018). Upon detection of diarrhea, the following treatment protocol was applied: Enrofloxacin at a dosage of 2.5 mg/kg body weight for three days + Hydration therapy, involving 2 liters administered twice a day for three days, with the hydration solution comprising 10 g of NaCl, 2 g of KCl, 8 g of $NaHCO_3$, 40 g of maltodextrin, and 2 liters of water.

2.2 *Animal Measurements, Sampling, and Laboratory Analysis*

Throughout the experimental period, daily practices included retaining and analyzing samples of the initially offered starter and hay. Any orts feed was collected and weighed to estimate the DM intake of the calves at the end of the experimental period. To obtain this data, samples of the starter and hay (both offered and orts) were dried in a forced-air oven at 55ºC for 72 h, ground to 1 mm using a knife mill, and stored in jars. The DM content of these samples was then determined using method 934.01 (AOAC, 1990), allowing us to calculate the daily DM consumption of the calves.

The calves remained in the study from birth until weaning (1-91 d of life). During this period, four collection phases (d- 1, 31, 61, and 91) were implemented, involving the following procedures: 1) Body morphometry: measurements of BW, withers height (WH), rump height (RH), body length (BL), and thoracic circumference (TP) were taken. All measurements were conducted on a flat surface (Menezes et al., 2023) with calves at rest, using an anthropometric ruler. 2) Blood collection: blood samples were collected at 0800 h (always before the morning milk feeding) via jugular vein puncture using sterile vacuum tubes with a clot activator and separator gel (Vacutainer®). The tubes were centrifuged to obtain serum. Albumin levels were determined using the bromocresol green method (Doumas et al., 1971). Globulin values were calculated as the difference between total proteins and albumin. Glucose levels were measured using the enzymatic method described by Raabo and Terkildsen (1960). Blood IGF-1 levels were obtained using the chemiluminescence method (Ferreira and Rossi, 2002). Beta-Hydroxybutyrate was measured using the enzymatic kinetic method, which involves the oxidation of D-3-hydroxybutyrate to acetoacetate (Ref. Numbers FA115 and RB1007, SYNLAB, Minas Gerais - Brazil); Triglycerides and Total Cholesterol analyses were performed using the Triglycerides FS reagent (DiaSys Diagnostic Systems GmbH & Co. KG, Holzheim, Germany) and the Cholesterol reagent (BioSystems S.A., Barcelona, Spain), respectively, on a Spectrum CCX II equipment (Abbott Diagnostics, Abbott Park, IL, USA). Tests were calibrated with the CCX Multicalibrator Set (Abbott) using three-point curves. Urea was determined using the Berthelot enzymatic method (Labtest, Lagoa Santa-MG, Brazil).

2.3 Statistical Analyses

The performance data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., 2008), based on the following statistical model:

$$Y_{ij}=\mu+ D_i + B_j + \varepsilon_{ij},$$

where Yij represents the obtained response, μ represents the overall mean, Di is the effect of diet "D" in experimental unit "i"; Bj random effect of blocking (3 blocks were formed, using the date of calving of each heifer as a blocking criterion) is the effect of blocking "B" in experimental unit "j", and εij which represents the associated unobserved random error. P values < 0.05 were considered significant.

For blood metabolites, body morphometry, dry matter intake, feed efficiency, ADG, and the incidence of diarrhea, data were included as repeated measures in the model, specified as follows:

$$Y_{ijk}=\mu+ D_i + T_j + (D\times T)_{ij} + B_k + \delta_{ijk} + \varepsilon_{ijk},$$

where Yijk represents the obtained response, μ represents the overall mean, Di is the effect of treatment "D" in experimental unit "i"; Tj is the effect of time (repeated measure) in experimental unit "j", (D×T)ij diet × time interaction, Bk random effect of blocking (3 blocks were formed based on the date of calving: 1st from October to November 2020, 2nd in January 2021, and 3rd from September to October 2021) is the random error when the variance between animals within a diet equals the covariance between repeated measures within animals, and represents the associated unobserved random error. P-values less than 0.05 were deemed significant.

The covariance structures evaluated were compound symmetry, heterogeneous compound symmetry, heterogeneous first-order autoregressive, and unstructured matrices. The selection of the appropriate covariance structure was based on the lowest corrected Akaike information criterion (AICc), with the heterogeneous compound symmetry structure being selected.

Outlier detection was conducted, considering internal student residuals greater than |2.5|. When necessary, means were compared using the Tukey test at a significance level of 0.05, and trends were acknowledged for 0.05 < P ≤ 0.10.

Power analysis indicated that, considering a coefficient of variation (CV) of 12%, for a significance level of 0.05 and a power of 80%, a minimum of 7 animals per treatment was required.

3. RESULTS

3.1 Animal Performance and Morphometry

An interaction (P < 0.01) was observed between ADG and days of life concerning BL, with the greatest value for BL noted for calves from the HIG group at 1-day post-calving (Figure 1). Additionally, trends for interactions between ADG and days of life were observed for BW (P = 0.09) and HG (P = 0.07; Table 1), with the greatest values again observed for calves from the HIG group at 1-day post-calving for both BW (Figure 2) and HG (Figure 3).

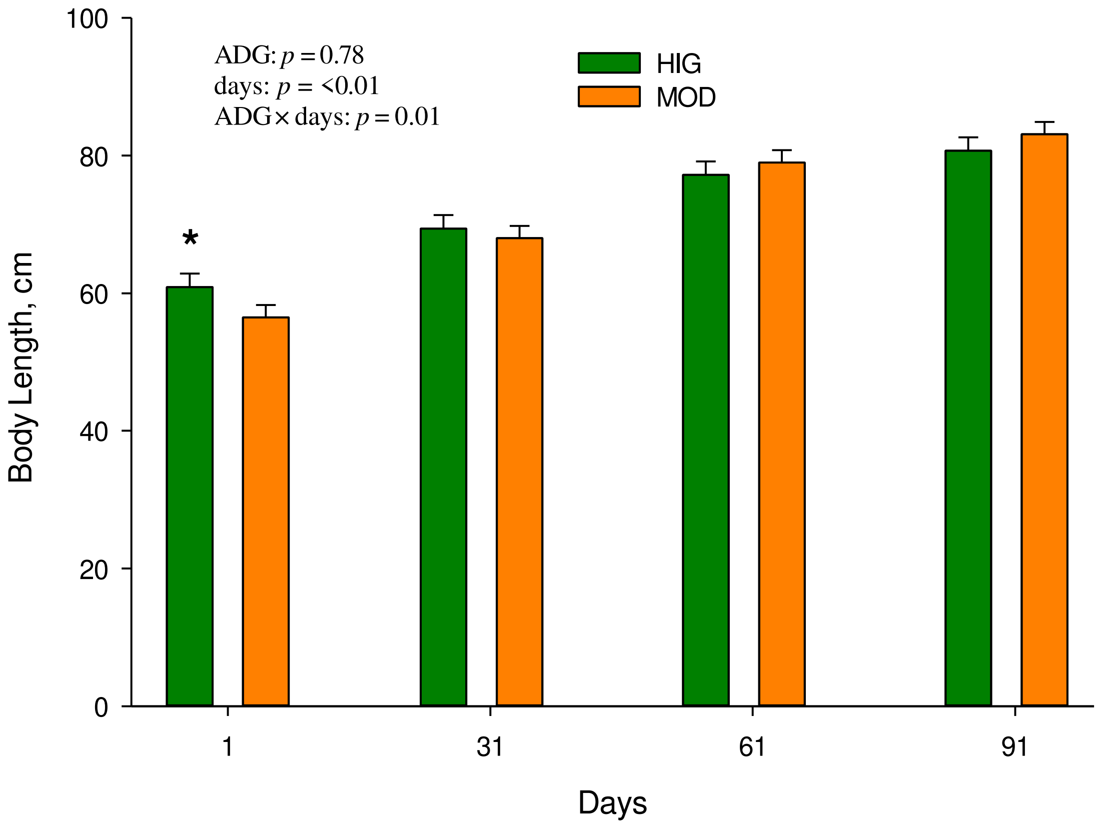


Figure 1. Mean and standard error of mean for length (cm) of calves from calving to weaning at 1, 31, 61 and 91 days for 5/8 Holstein × 3/8 Gyr heifers subjected to either moderate gain (MOD - 0.37 kg/d) or high gain (HIG - 0.72 kg/d) throughout pregnancy. Significance was declared $P \leq 0.05$, with trends noted between $0.10 \leq P \geq 0.05$. The

green bar represents daughters of mothers fed HIG, while the orange bar represents daughters of mothers fed MOD. * Indicates a significant of *P* = 0.01.

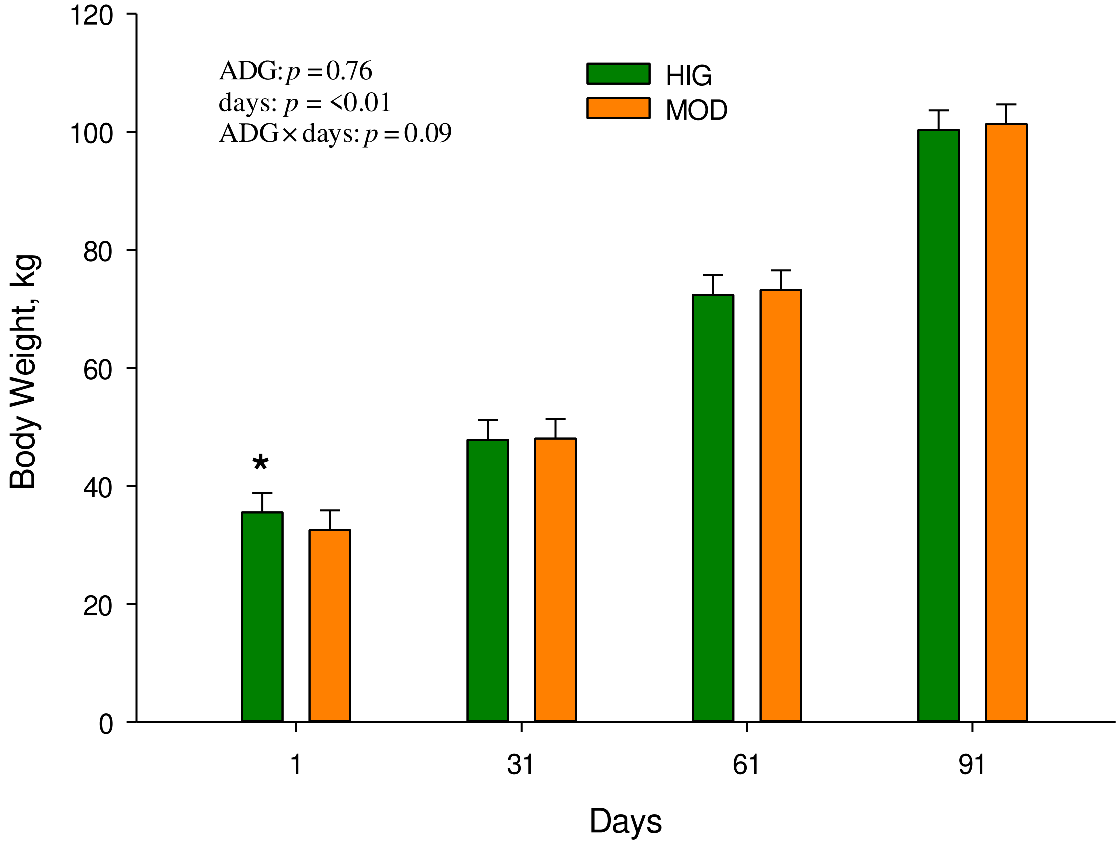


Figure 2. Mean and standard error of mean for body weight (kg) of calves from calving to weaning at 1, 31, 61 and 91 days for 5/8 Holstein × 3/8 Gyr heifers subjected to either moderate gain (MOD - 0.37 kg/d) or high gain (HIG - 0.72 kg/d) throughout pregnancy. Significance was declared at $P \leq 0.05$, with trends noted between $0.10 \leq P \geq 0.05$. The green bar represents daughters of mothers fed HIG, while the orange bar represents daughters of mothers fed MOD. * Indicates a significant of *P* = 0.10.

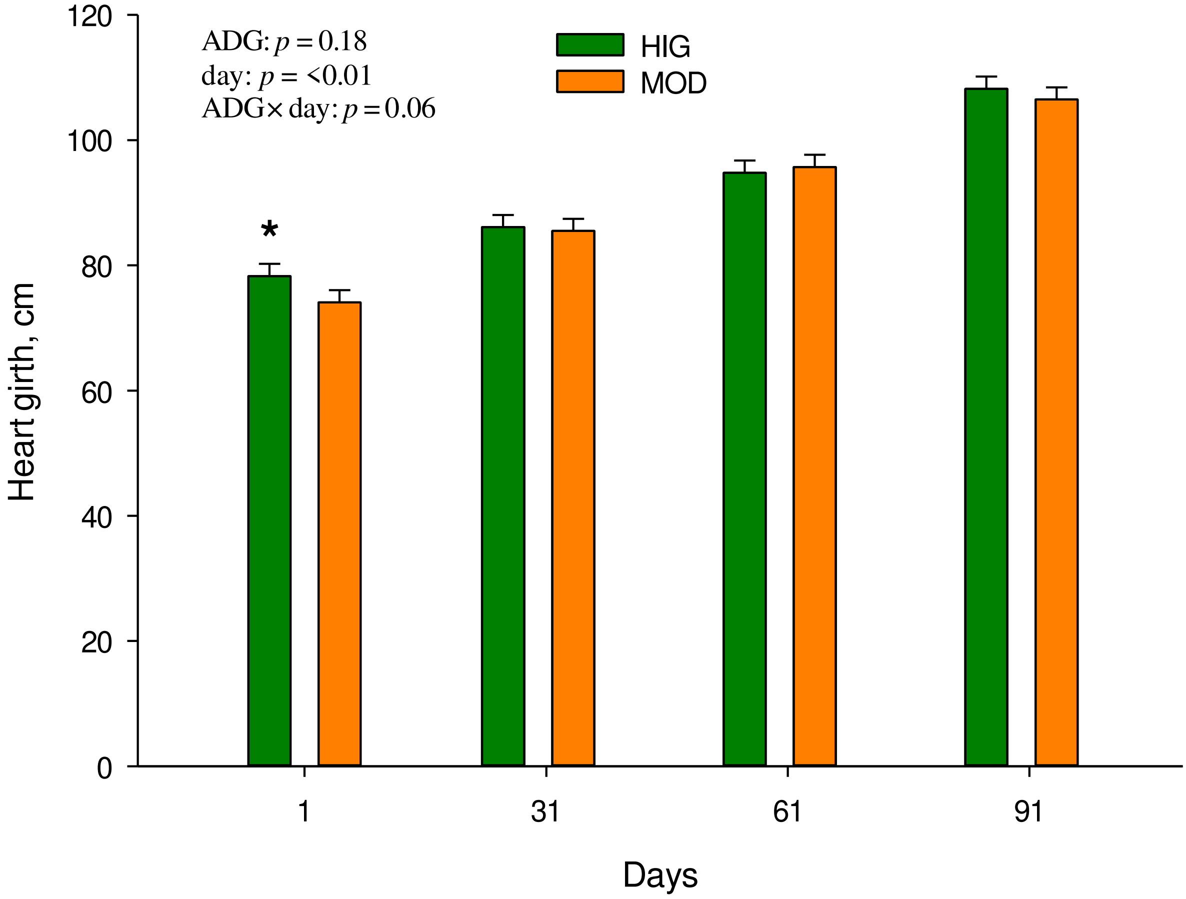


Figure 3. Mean and standard error of mean for thoracic perimeter (cm) of calves from calving to weaning at 1, 31, 61 and 91 days of Holstein × Gyr heifers subjected to either moderate gain (MOD; 0.37 kg/d) or high gain (HIG; 0.72 kg/d) throughout pregnancy. Significance was declared $P \leq 0.05$ and trends $0.10 \leq P \geq 0.05$. The green bar represents daughters of mothers fed HIG, while the orange bar represents daughters of mothers fed MOD. * Indicates a significant of $P = 0.07$.

For all parameters regarding performance and morphometry, significant differences ($P < 0.01$) were observed across days of life, with greater values noted for increasing days of life (Table 2). In terms of the different ADG to which the dams were subjected and their impact on calf performance, a significant effect ($P < 0.01$) was noted for calves' ADG, with MOD calves showing greater ADG. Conversely, WH values tended ($P = 0.08$) to be greater for calves from the HIG group. Feed efficiency was greater ($P = 0.02$) for calves from MOD than HIG.

Table 2. Performance and morphometry of calves, whose dams were subjected to different ADGs during the gestational period.

Item	MOD[1] days of life				HIG[2] days of life				SEM	*P-value*		
	1	31	61	91	1	31	61	91		ADG	days	ADG × days
Body weight, kg	32.5	48.0	73.2	101.3	35.5	47.8	72.4	100.3	3.34	0.76	< 0.01	0.10
ADG[3], kg	- -	0.52	0.68	0.76	- -	0.31	0.55	0.66	0.038	< 0.01	< 0.01	0.49
Feed efficiency	- -	0.54	0.07	0.07	- -	0.32	0.09	0.02	0.077	0.29	< 0.01	0.18
WH[4], cm	70.8	77.6	84.4	90.8	73.4	79.9	85.8	90.3	1.53	0.08	< 0.01	0.17
RH[5], cm	73.1	81.2	89.1	95.2	76.4	83.3	90.2	95.5	1.43	0.04	< 0.01	0.25
BL[6], cm	56.4	68.0	78.9	83.1	60.9	69.4	77.2	80.7	1.68	0.78	< 0.01	< 0.01
HG[7], cm	74.1	85.5	95.7	106.5	78.3	86.1	94.8	108.2	1.95	0.18	< 0.01	0.07
TPIC[8], g/dL	11.4	- -	- -	- -	10.7	- -	- -	- -	0.59	0.10	- -	- -
Vitality score	24.8	- -	- -	- -	25.4	- -	- -	- -	1.45	0.09	- -	- -
Diarrhea[9], d	- -	5.5	1.1	0.5	- -	6.8	0.0	0.0	1.38	0.91	< 0.01	0.62

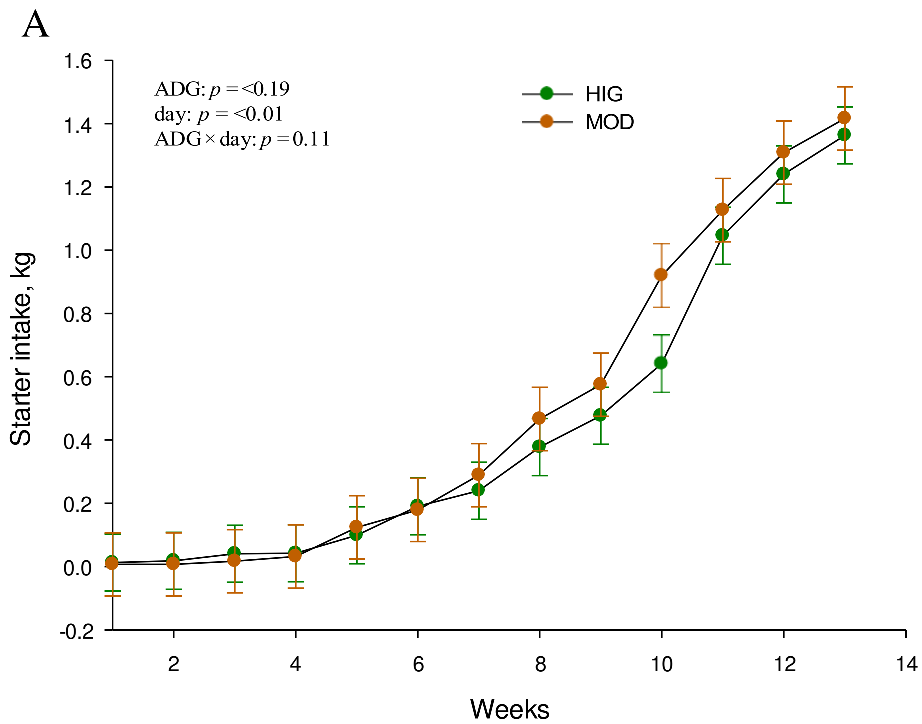
[1]MOD = calves born to heifers placed on a nutritional plan moderate body weight gain (0.37 kg/d); [2]HIG = calves born to heifers placed on a nutritional plan high body weight gain (0.72 kg/d); [3]Average daily gain; [4]Withers height; [5]Rump height; [6]Body length; [7]Heart girth; [8]Transfer of passive immunity through colostrum; [9]Duration of diarrhea treatment in days, for each calf.

Additionally, a trend was detected (P = 0.10) in the efficiency of passive absorption of immunoglobulins from colostrum, with calves in the MOD group exhibiting higher absorption efficiencies than those in the HIG group (Table 2).

In assessing the vitality score, a noticeable trend was observed (P = 0.09), where calves in the HIG group tended to present higher vitality scores compared to those in the MOD group (Table 2). The vitality score reflects the immediate health status of calves post-calving.

3.2 Intake

When evaluating the consumption of starter, hay, and total DM by the calves, no significant interactions (P > 0.10) were observed between the dams' ADG and the calves' age (Figures 4 A, B, C). In the same way, no effect was (P > 0.10) observed for ADG for these parameters. We only observed differences for weeks of live and at the final milking phase, calves had a similar starter intake about 1.4 kg/d.



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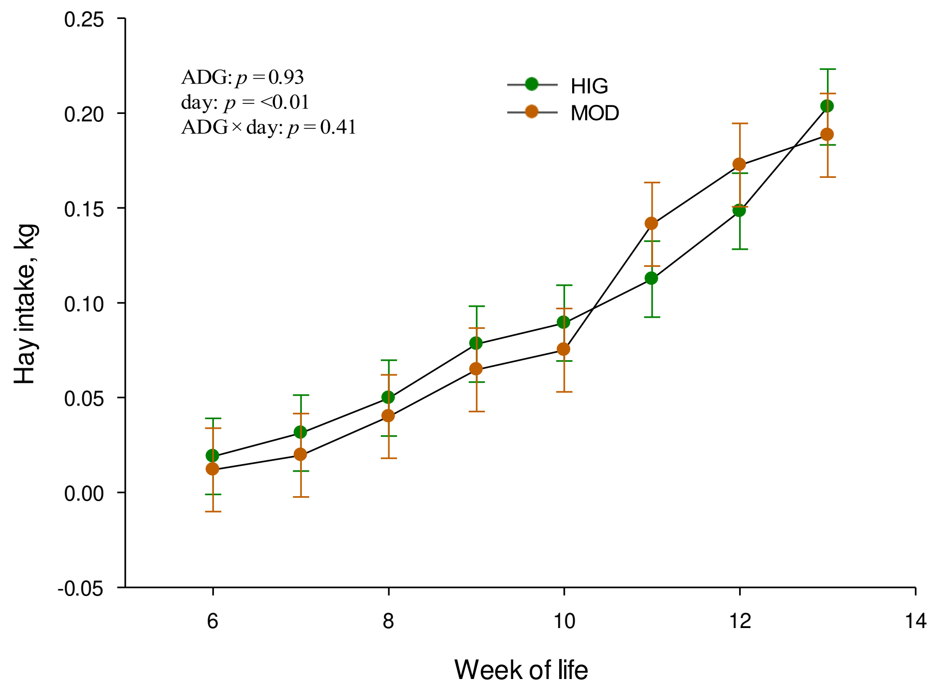

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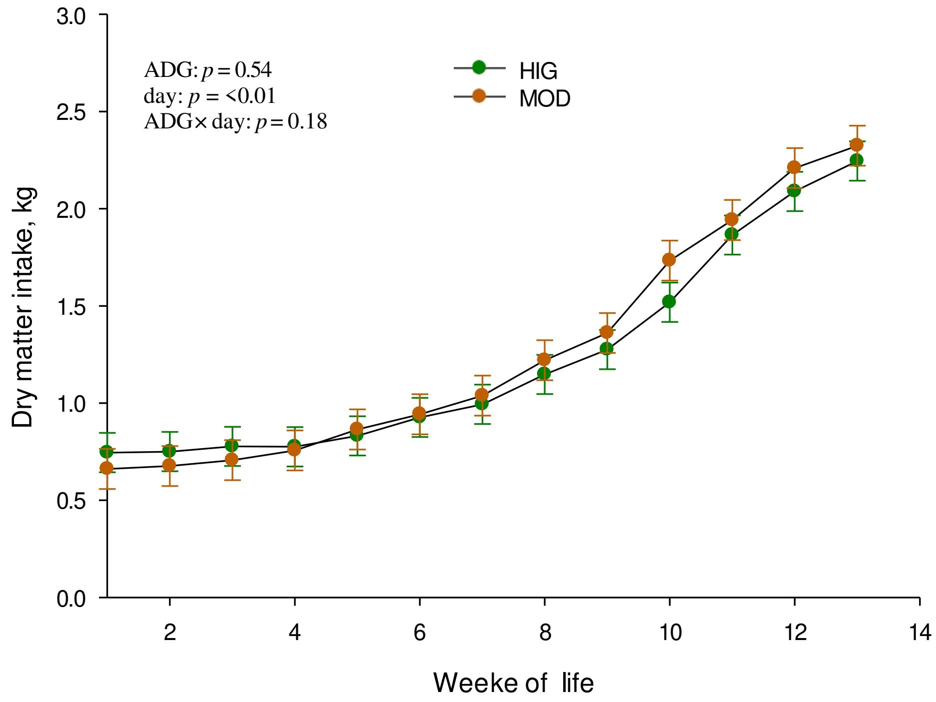
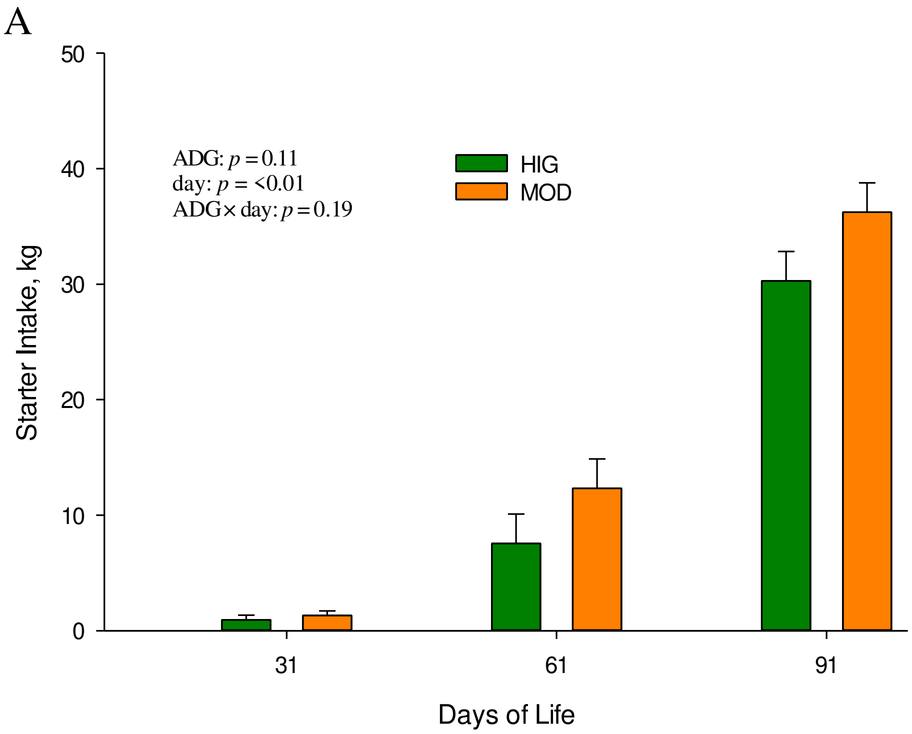
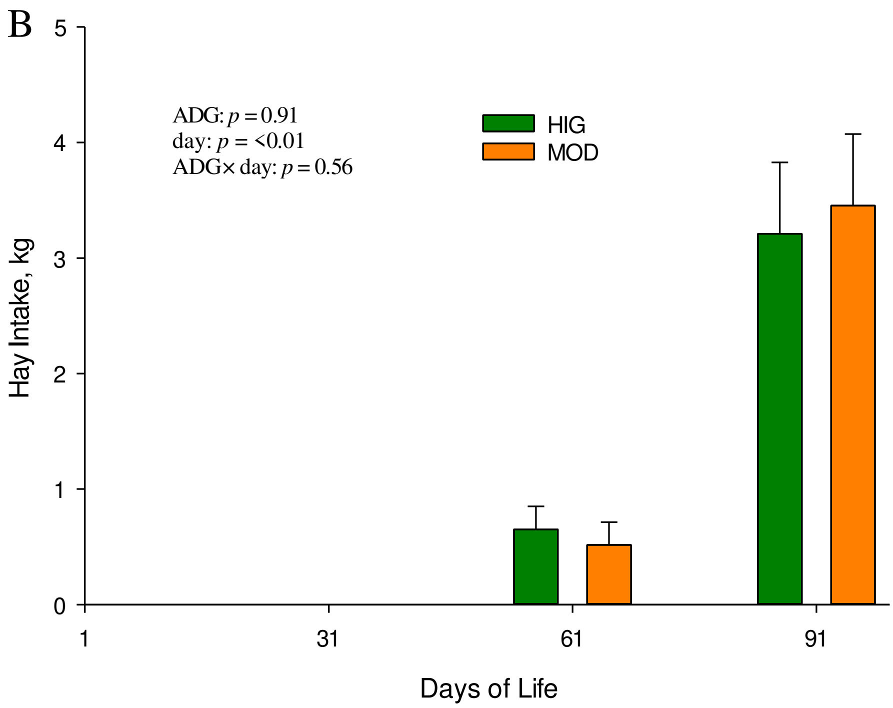


Figure 4. Mean and standard error of the average daily intake of starter feed (A), hay (B) and total dry matter (C) for calves from calving to weaning of 5/8 Holstein × 3/8 Gyr daughters of mothers, fed to achieve gain moderate (MOD - 0.37 kg/d) or high gain (HIG - 0.72 kg/d) during the gestational period. Significance was declared $P \leq 0.05$ and trend $0.10 \leq P \geq 0.05$.

The accumulative intake of starter, hay, and total DM were not altered by dams´ ADG. (Figures 5 A, B, C). At 91 d of life, the total starter intake of HIG and MOD calves were 30.5 ± 2.40 and 36.0 ± 2.40 kg DM, respectively.





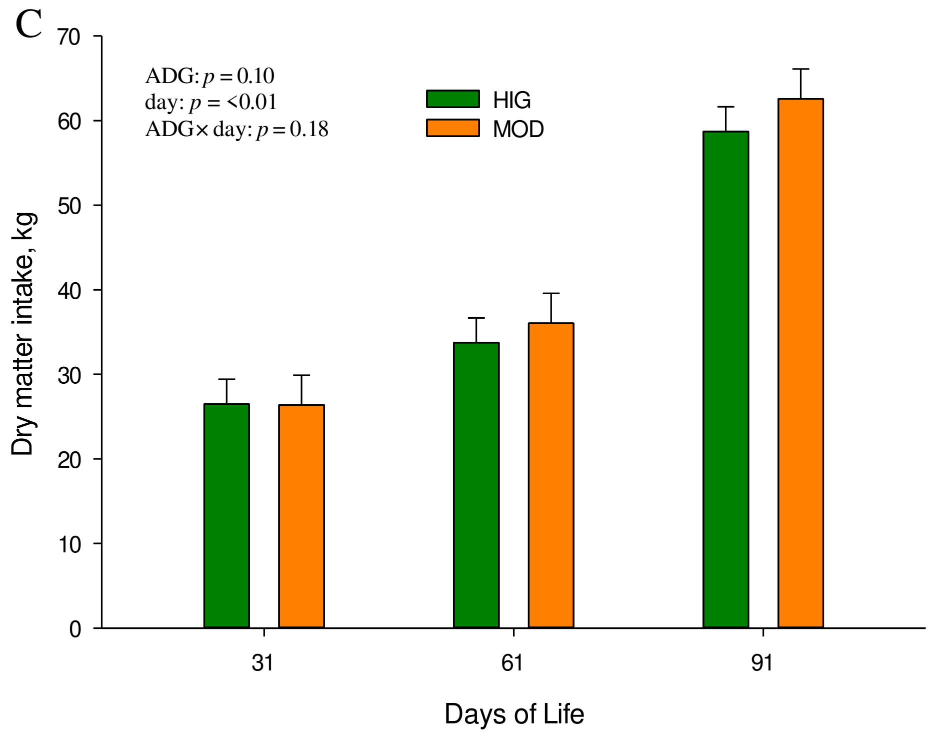


Figure 5. Mean and standard error of the mean cumulative intake of starter (A), hay (B) and total dry matter (C) for calves in the periods of 31, 61 and 91 years of daughters of mothers 5/8 Holstein × 3/8 Gyr fed to achieve moderate gain (MOD - 0.37 kg/d) or high gain (HIG - 0.72 kg/d) during the gestational period. Significance was declared $P \leq 0.05$ and trend $0.10 \leq P \geq 0.05$. The green bar represents heifers fed HIG, while the orange bar represents heifers fed MOD.

3.3 Metabolites

No interactions ($P > 0.10$) were observed between ADG and days of life when evaluating hormones and metabolites (Table 3). Similarly, no differences were found in hormone and metabolite levels across different dams' ADG. However, a significant effect of days of life was noted for BHB, IGF-1, and glucose ($P = 0.01$). The concentrations of these specific metabolites demonstrated a marked increase over time, with levels of BHB, IGF-1, and glucose being substantially higher at the time of weaning compared to at calving (Table 3).

Table 3. Animal performance and morphometric data of calves subjected to different gestational nutrition plans.

Item	MOD[1]				HIG[2]				SEM	P-value		
	days of life				days of life							
	1	31	61	91	1	31	61	91		ADG	days	ADG × days
BHB[3], µmol/mL	70.5	141.0	136.5	139.8	75.5	122.0	127.0	126.3	11.76	0.82	< 0.01	0.77
TG[4], mmol/mL	27.5	22.0	23.5	23.5	37.0	17.5	28.1	27.5	5.37	0.71	0.29	0.62
TC[5], mmol/mL	70.0	45.5	38.1	36.4	69.7	42.7	30.1	29.2	11.63	0.64	0.25	0.98
Albumin, g/dL	2.9	2.1	2.2	2.5	5.7	3.3	3.0	2.5	0.97	0.15	0.12	0.27
Globulin, g/dL	6.4	6.0	6.5	7.0	9.9	7.0	7.1	7.4	0.95	0.14	0.25	0.25
Total protein, g/dL	6.4	6.0	6.6	7.0	9.9	7.0	7.1	7.3	0.95	0.14	0.25	0.25
Urea, mmol/mL	3.6	4.0	4.1	4.5	4.3	3.8	4.5	4.9	0.37	0.41	0.13	0.54
IGF-1, ng/mL	65.0	73.0	96.0	178.0	96.0	75.0	111.0	184.0	17.68	0.71	< 0.01	0.38
Glucose, md/mL	98.0	85.0	88.0	90.0	119.0	82.0	89.0	89.0	16.64	0.31	0.01	0.26

[1]MOD = calves born to heifers placed on a nutritional plan moderate body weight gain (0.37 kg/d); [2]HIG = calves born to heifers placed on a nutritional plan high body weight gain (0.72 kg/d); [3]β-hydroxybutyrate; [4]Total triglycerides; [5]Total cholesterol

4. DISCUSSION

The observed trend for calves in the HIG group to exhibit higher vitality scores can be attributed to the relatively easier calving experienced by HIG heifers compared to those in the MOD group (Feitosa et al., 2012; Oliveira et al., 2024). It is well documented that easier calving, whether assisted or unassisted, significantly enhances the vitality of newborn calves (Kovács et al., 2016). Dystocia during calving is a major contributor to calf mortality during and shortly after birth (Pettersin et al., 1987). Among heifers, the predominant cause of dystocic calvings is the mismatch between the dam's BW and the calf's size (Anderson, 1993).

Regarding the transfer of passive immunity, calves from both groups demonstrated effective absorption of immunoglobulins via colostrum, with levels exceeding 9.4 g/dL, which is equivalent to 25 g/L of serum IgG (Lombard et al., 2020). Notably, calves in the MOD group showed an impressive ability to compensate for their mothers' lower ADG during the gestational period. The early absorption of immunoglobulins is critical for calf health, with the first 2 h post-birth being especially crucial (Weaver et al., 2000; Godden et al., 2019). Thus, the quantity and quality of colostrum provided are paramount in ensuring optimal calf health, as further evidenced by Fischer-Tlustos et al. (2021).

Research on fetal programming in calves clearly indicates that while there is an increased nutritional demand and development in the final third of pregnancy, a lower nutritional intake (low ADG) at the beginning of pregnancy can significantly compromise placental morphology and the efficiency of nutrient transport (Rotta et al., 2005; Long et al., 2021; Oliveira et al. 2023). This suboptimal condition can be somewhat mitigated by achieving a higher ADG for pregnant heifers, which can counterbalance the inevitable decrease in DMI observed in the final third of pregnancy.

The BL at birth was different in our study, indicating that calves in the HIG group exhibited greater values, while calves in the MOD group achieved similar BL at weaning. The increased BL at birth for the HIG group can be attributed to the higher ADG achieved by the HIG heifers throughout their gestational period (Oliveira Neto et al., 2025). The overall nutritional contribution provided to the heifer during the entire gestational period plays a crucial role in the growth and organogenesis of the fetus, as demonstrated by studies such as those by Chmurzynska (2010) and Bach (2012).

Calves in the HIG group were observed to be born heavier, a factor that could significantly influence their overall performance outcomes. Alongside being heavier, these calves also demonstrated greater RH, indicative of enhanced fetal growth and development due to the higher ADG of their dams during pregnancy (McMillen and Robinson, 2005).

The BW of calves at birth is a crucial determinant in understanding various performance metrics. For instance, a notable treatment effect during gestation was observed in ADG for calves, where calves in the MOD group, although born lighter, exhibited higher growth rates. This phenomenon is partly explained by the nutritional requirements—such as protein and energy—which are known to scale with the animal’s BW (Silva et al., 2002; Zervoudakis et al., 2002).

The incidence of diarrhea in both calf groups also showed a decreasing trend over time. This decline is likely due to the maturation of the immune system and intestinal development, which are influenced by the quality of colostrum consumed and the calves’ environmental conditions. Such factors are integral in combating the effects of pathogens, thereby reducing the incidence of diarrhea (Yang et al., 2015). Thus, the observed patterns in diarrhea incidence across the groups were more likely impacted by environmental variables rather than the differing ADG experienced by the dams during pregnancy.

Metabolically, the animals performed similarly, particularly in relation to IGF-1 concentration levels. Both experimental groups exhibited comparable concentrations of this hormone, which increased steadily from calving to weaning. Despite the differing ADG experienced by the dams during gestation, some studies indicate that pregnant animals with low ADG may activate physiological mechanisms to compensate and ensure the satisfactory growth and development of their fetus (Oliveira et al., 2023). These compensatory mechanisms include increased blood flow to the fetus, enhancing the nutritional supply, thus ensuring that the calves' nutritional needs are fully met by their mothers (Oliveira et al., 2023). Additionally, the standardization of the colostrum provided, in terms of quality, ensured optimal transfer of passive immunity (immunoglobulins).

Blood levels of BHB in calves showed a positive correlation with the onset of solid feed intake (starter and hay). An increase in solid feed intake stimulates greater activity and metabolic and epithelial development of the rumen, which in turn raises the concentration of BHB in the calves' plasma. This pattern was observed in our study across both experimental groups and aligns with findings by Suarez-Mena et al. (2017). The concentration of BHB exhibited a similar pattern in calves from both groups, suggesting that both experimental groups received satisfactory nutrition during the fetal period, resulting in consistent performance throughout the rearing phase up to weaning. This finding underscores the efficacy of the nutritional strategies employed, ensuring uniform outcomes despite variations in maternal ADG.

5. CONCLUSIONS

We concluded that calves in the HIG group tended to be born heavier and larger, which resulted in them becoming more nutritionally demanding throughout the breastfeeding period. Despite this, calves in the MOD group were able to perform similarly and satisfactorily in comparison to their HIG counterparts. This suggests that the MOD group managed to effectively compensate for any initial disadvantages in birth weight and size through efficient management and nutritional strategies during the breastfeeding period.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DECLARATION OF DATA AVAILABILITY

The authors have access to all data obtained in this study and assume responsibility for the integrity and accuracy of the analyses. The data supporting the conclusions of this study are available via the corresponding author upon request.

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