




# Impact of using Angus in Charolais × Nellore rotational crossbreeding on calf performance

Rodrigo Soares Volpatto<sup>1\*</sup> , Dari Celestino Alves Filho<sup>1</sup> , Henrique da Costa Mendes Muniz<sup>2</sup> , Luiz Angelo Damian Pizzuti<sup>1</sup> , Sander Martinho Adams<sup>3</sup> , John Lenon Klein<sup>4</sup> , Joziane Michelon Cocco<sup>1</sup> , Raíssa Lixinski de Souza<sup>1</sup> 

<sup>1</sup> Universidade Federal de Santa Maria, Departamento de Zootecnia, Santa Maria, RS, Brasil.

<sup>2</sup> Universidade Federal do Pampa, Uruguaiana, RS, Brasil.

<sup>3</sup> Instituto Federal Farroupilha, Santo Augusto, RS, Brasil.

<sup>4</sup> Faculdade de Santo Ângelo, Santo Ângelo, RS, Brasil.

\*Corresponding author:  
[rodrigovolpatto97@gmail.com](mailto:rodrigovolpatto97@gmail.com)

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**ABSTRACT** - This study aimed to evaluate the effects of incorporating the Angus breed into a rotational crossbreeding system involving the Charolais and Nellore breeds. The experiment included 67 calves born in 2021, which were allocated to four treatments according to their genetic composition: calves with Charolais predominance (PCH), Nellore predominance (PNE), and F1 Angus-cross calves with Charolais (AACH) or Nellore (AANE) maternal predominance. Calves were monitored from birth to seven months of age, and dams were evaluated from calving to weaning. Differences in birth weight were observed among the treatments ( $P < 0.05$ ), with mean weights of 35.8, 38.0, 31.7, and 33.2 kg for PCH, PNE, AACH, and AANE, respectively. Post-weaning average daily gain also differed among treatments ( $P < 0.05$ ), with higher gains for AACH (0.716 kg/day) and AANE (0.655 kg/day) compared to PCH (0.518 kg/day) and PNE (0.474 kg/day). No significant differences were found in milk yield among treatments ( $P > 0.05$ ), with a mean daily production of 5.60 L and a total production of 432 L. Mating rotationally crossbred (Charolais × Nellore) cows with Angus bulls improved calf performance, particularly after weaning, due to increased individual heterozygosity. Additionally, the reduced birth weight of these calves may decrease the risk of dystocia without impairing growth. This strategy appears to be effective for enhancing productivity in cow-calf systems.

**Keywords:** average daily gain, birth weight, heterozygosity, milk yield

## 1. Introduction

In 2021, Brazil produced 9.71 million tons of carcass-equivalent beef, of which 25.51% were exported (ABIEC, 2022). Considering the growing population and the consequent trend toward increasing domestic consumption and exports, the adoption of technologies capable of optimizing production without expanding land use becomes essential. Among the available alternatives, crossbreeding stands out because it enables the combination of favorable traits from different breeds, resulting in animals with superior productive performance relative to their parents or the pure breeds involved in the cross (Menezes et al., 2016).

Trait improvements through crossbreeding represent a viable strategy for intensifying production and supporting the beef cattle industry in meeting the demands of the entire production chain. The main advantage of crossbreeding lies in the mating of animals from distinct breeds, which increases heterozygosity and, consequently, enhances heterosis as known as hybrid vigor (Roso and Fries, 2000). Thus, the adoption of crossbreeding enables the exploitation of the positive effects of heterozygosity on both maternal and calf performance, contributing to increased herd productivity.

To achieve the benefits of both maternal and individual heterozygosity, alternating rotational crossbreeding systems are recommended. These systems reach stabilization of genetic composition and heterozygosity levels from the fifth generation onward. However, rotational systems may be enhanced by association with terminal crossbreeding, such as two-breed crosses involving a third breed, which can increase individual heterozygosity from 67 to 100%, thereby maximizing animal performance. It is important to note that all offspring resulting from this genetic composition are destined for slaughter.

Although crossbreeding systems such as Charolais × Nellore and Angus × Nellore have been extensively used in tropical beef production, most available studies have focused on growth performance, carcass composition, or meat quality in feedlot-finished animals (Oliveira et al., 2021). However, few studies in the scientific literature have evaluated the early performance of calves born from rotational Charolais–Nellore cows mated with Angus sires under pasture conditions. This knowledge gap is relevant because the combination of British and continental breeds with Zebu genetics may offer complementary advantages in adaptability, fertility, and pre-weaning performance (Favero et al., 2019).

Therefore, the objective of the present study was to evaluate the early performance of calves born to cows derived from an alternating rotational crossbreeding system between the Charolais and Nellore breeds, mated with Angus sires, thus providing new evidence regarding the productive potential of composite crossbreeding systems in Brazilian beef production.

## 2. Material and methods

The experiment was conducted on an experimental farm in Santa Maria, Rio Grande do Sul, Brazil (29°43'26"S and 53°44'06"W). Animal research was conducted according to the institutional committee on animal use (case number 3781220520).

### 2.1. Animals, treatments and experimental design

A total of 67 calves were used, originating from a rotational crossbreeding system involving Charolais and Nellore breeds, as well as from a commercial cross involving Charolais, Nellore, and Angus. The animals were assigned to four treatments (Table 1).

The calving period occurred from September 10 to November 16, 2021. At birth, calves and their dams were taken to the handling facility, where morphometric measurements were taken from the calves, including withers height, hip height, body length, rump length, rump width, chest width, thoracic girth,

**Table 1** - Relationship between treatment and genetic composition of calves used in the study

Treatments <sup>1</sup>	Calf genetic composition	Cow genetic composition	BB
PCH	2/3 Charolais 1/3 Nellore	2/3 Nellore 1/3 Charolais	Charolais
PNE	2/3 Nellore 1/3 Charolais	2/3 Charolais 1/3 Nellore	Nellore
AACH	3/6 Angus 2/6 Charolais 1/6 Nellore	2/3 Charolais 1/3 Nellore	Angus
AANE	3/6 Angus 2/6 Nellore 1/6 Charolais	2/3 Nellore 1/3 Charolais	Angus

BB - breed of bull used.

<sup>1</sup> PCH - calves from predominantly Charolais cows; PNE - calves from predominantly Nellore cows; AACH - calves from breed bulls Aberdeen Angus and predominantly Charolais cow; AANE - calves from breed bulls Aberdeen Angus and predominantly Nellore cow.

and forearm circumference. In addition, calves were weighed, had their umbilical cord treated, and received a subcutaneous application of 1 mL of 1% Ivermectin®.

Dams were weighed and assigned a body condition score (BCS) on a scale of 1 to 5, where 1 = very thin and 5 = very fat. Calves remained with their dams on native pasture typical of the state of Rio Grande do Sul, with free access to water and mineral salt (80P).

## 2.2. Milk intake and weaning

Milk intake was estimated using the weigh-suckle-weigh technique (Espasandin et al., 2001), which involves an overnight fasting period (12 hours) without access to solid or liquid feed. After the fasting period, calves were weighed, allowed to suckle from their respective dams for 30 minutes, and then weighed again. The difference between the second and the first weights, multiplied by two, was considered the estimated daily milk intake of each calf. This procedure was repeated three times for each experimental unit (calf), at approximately 25, 50, and 75 days of age.

Calves were weaned at around 75 days of age, depending on their birth dates, which resulted in different weaning groups. After weaning, calves remained at the handling facility for a 10-day adaptation period to concentrate feed intake. During the first four days, dams were allowed to approach the calves without physical contact, as a strategy to reduce stress associated with weaning for both dams and calves.

To facilitate the transition to the new feeding routine, the calves' concentrate intake was monitored and gradually increased throughout the weaning period (Table 2). After the adaptation period, calves were transferred to a pasture area composed of *Cynodon* spp. (Tifton-85) and received supplementation twice daily. The concentrate was composed of ground corn, soybean meal, mineral salt (80P), and plain white salt, with an average intake of 1.5% of body weight. After 45 days post-weaning, the concentrate composition was changed to ground corn, white oats, soybean meal, mineral salt (80P), and plain white salt, with an average intake of 1.2% of body weight.

**Table 2 - Adaptation of concentrate intake during the first days after weaning**

Days	kg of concentrate <sup>1</sup> per meal	kg of feed per day
First	0.075	0.150
Second	0.150	0.300
Third	0.225	0.450
Fourth	0.300	0.600
Fifth	0.375	0.750
Sixth	0.450	0.900
Seventh - Tenth	0.525	1.050

<sup>1</sup> Centesimal composition of the supplement 0-45 days after weaning (23.5% crude protein; 83.5% total digestible nutrients); after 45 days (19.8% crude protein and 81% total digestible nutrients).

## 2.3. Statistical analysis

The experimental design was completely randomized, with a variable number of replicates per treatment. Statistical analyses were performed using the following mathematical model:

$$Y_i = \mu + T_i + \varepsilon_i$$

in which  $Y_i$  = dependent variable,  $\mu$  = overall mean,  $T_i$  = fixed effect of the  $i$ -th treatment, and  $\varepsilon_i$  = random residual error.

Residual normality was assessed using the Shapiro–Wilk test, and data were transformed when necessary. The data were subjected to analysis of variance (ANOVA) using the F-test, under the general linear model procedure. When significant treatment effects were detected, least squares means (LS means) were compared using Tukey’s test at a 5% significance level.

### 3. Results

#### 3.1. Morphometric evaluation

Differences were observed among genetic groups of calves for withers height, hip height, and thoracic circumference ( $P < 0.05$ ). Conversely, no differences were detected for body length, hip length, hip width, chest width, or arm perimeter ( $P > 0.05$ ; Table 3).

**Table 3 - Morphometric measurements of calves at birth according to their respective genetic groups**

Morphometric measurement	Genetic group of calves <sup>1</sup>				P-value
	PCH	PNE	AACH	AANE	
Height at withers (cm)	69.9ab	73.8a	65.5c	68.4bc	<0.01
Height at croup (cm)	75.1b	78.8a	70.8c	72.5bc	<0.01
Body length (cm)	64.3	64.3	60.6	61.7	0.08
Length at croup (cm)	23.0	22.8	21.3	22.1	0.14
Width at croup (cm)	16.0	16.3	15.9	17.0	0.48
Width at chest (cm)	18.1	18.2	18.3	18.5	0.97
Chest circumference (cm)	74.0ab	75.5a	71.5b	74.5ab	0.06
Chest depth (cm)	24.7	24.5	24.4	25.0	0.43
Arm circumference (cm)	17.4	16.9	16.6	16.4	0.23

<sup>1</sup> PCH - calves from predominantly Charolais cows; PNE - calves from predominantly Nellore cows; AACH - calves from breed bulls Aberdeen Angus and predominantly Charolais cow; AANE - calves from breed bulls Aberdeen Angus and predominantly Nellore cow. Different letters within a row indicate statistical differences at  $P < 0.05$ .

#### 3.2. Calf performance from birth to weaning

Birth weight differed among the evaluated genetic groups ( $P < 0.05$ ). Calves from rotational crossbreeding presented higher birth weights, with means of 35.84 kg for those with Charolais predominance and 37.95 kg for those with Nellore predominance. In contrast, calves from terminal crossbreeding, specifically the  $\frac{1}{2}$  Angus × Charolais group, exhibited lower birth weights, averaging 31.70 kg (Table 4).

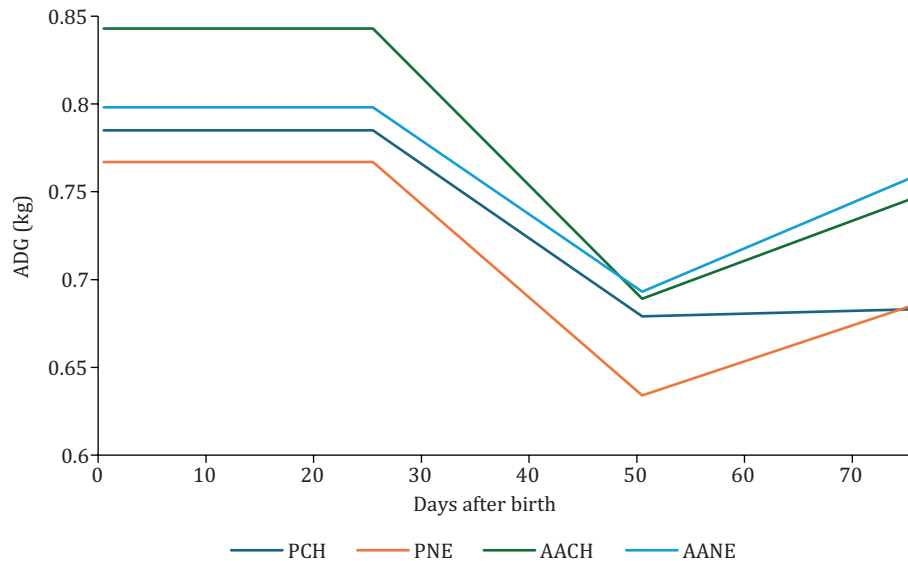
**Table 4 - Performance variables between genetic groups**

Variable <sup>1</sup>	Genetic group of calves <sup>2</sup>				P-value
	PCH	PNE	AACH	AANE	
BW	35.8±2.9a	38.0±1.9a	31.7±3.1b	33.2±4.0ab	<0.01
WW	94.7±7.2	95.0±5.7	90.9±8.9	92.4±11.6	0.87
Aw205	162.4±8.5ab	158.3±6.7b	183.8±9.6a	176.9±13.6a	<0.01
ADG BW	0.76	0.74	0.79	0.78	0.70
ADG W7	0.52±0.05b	0.47±0.04b	0.72±0.06a	0.66±0.08a	<0.01

<sup>1</sup> BW - birth weight; WW - weaning weight; Aw205 - adjusted weight at 205 days of age; ADG BW - average daily gain from birth to weaning; ADG W7 - average daily gain from weaning to 7 months.

<sup>2</sup> PCH - calves from predominantly Charolais cows; PNE - calves from predominantly Nellore cows; AACH - calves from breed bulls Aberdeen Angus and predominantly Charolais cow; AANE - calves from breed bulls Aberdeen Angus and predominantly Nellore cow. Different letters within a row indicate statistical differences at  $P < 0.05$ .

Regarding performance up to weaning, average daily gain (ADG) did not differ between treatments ( $P>0.05$ ), with an overall average of 0.766 kg/day. However, when evaluating specific age intervals such as from 0 to 25 days, 25 to 50 days, and from 50 days to weaning, variations in growth patterns were observed (Figure 1). During the first 25 days, ADG was 0.798 kg/day. Between 25 and 50 days, it dropped to 0.673 kg/day, followed by a recovery in the final stage, with an average of 0.718 kg/day. ADG differed significantly across these periods ( $P<0.05$ ), with the highest performance in the first 25 days of life (Table 5).



ADG - average daily gain; PCH - calves from predominantly Charolais cows; PNE - calves from predominantly Nellore cows; AACH - calves from breed bulls Aberdeen Angus and predominantly Charolais cow; AANE - calves from breed bulls Aberdeen Angus and predominantly Nellore cow.

**Figure 1** - Calf performance from birth to weaning.

**Table 5** - Productive parameters of cows and calves according to days after birth

Variable	Days after birth				P-value
	0	25	50	75	
ADG calves ( $\text{kg}\cdot\text{day}^{-1}$ )	-	0.79±0.04a	0.67±0.03b	0.71±0.04b	<0.01
Weight cow (kg)	432.3±12.5a	406.2±12.2b	410.8±12.2ab	406.40±12.19b	0.01
ADG cow ( $\text{kg}\cdot\text{day}^{-1}$ )	-	-1.00±0.2c	0.21±0.2a	-0.18±0.21b	<0.01
Milk production ( $\text{kg}\cdot\text{day}^{-1}$ )	-	6.03±0.5a	6.26±0.51a	4.79±0.51b	<0.01

ADG - average daily gain.

Different letters within a row indicate statistical differences at  $P<0.05$ .

### 3.3. Cow performance

Milk yield did not differ between treatments ( $P>0.05$ ; Table 6). Total milk production was also not influenced by the calf's genetic group. Daily milk yield varied over the lactation period, peaking between days 25 and 50 postpartum at 6.23 L/day. In the first 25 days, the average was 5.92 L/day, while between days 50 and 75, it dropped to 4.94 L/day. Regarding the cows' genetic group, those

with 2/3 Charolais ancestry produced an average of 5.66 L/day, while cows with 2/3 Nellore produced 5.54 L/day. The overall average milk yield was 5.60 L/day, totaling 433.23 liters over the evaluation period.

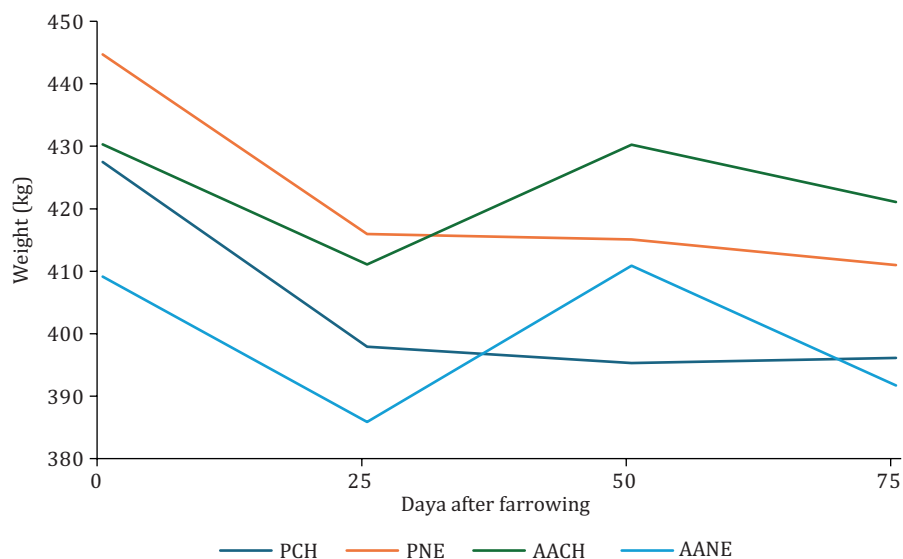
Body weight variation throughout lactation (Figure 2) showed distinct patterns between treatments. Dams of ½ Angus calves lost weight in the first 25 days postpartum, regained weight between days 25 and 50, and lost weight again in the final stage. Cows from the rotational crossbreeding group (alternating Charolais and Nellore) experienced a sharper weight decline in the first 25 days, followed by a more moderate reduction thereafter. Body condition score (BCS) did not differ between treatments ( $P < 0.05$ ; Table 7). Although fluctuations were observed throughout the evaluation period, no association with the calf's genetic group was found.

**Table 6** - Milk yield of cows at different stages of lactation according to the calves' genetic group

Variable	Genetic group of calves <sup>1</sup>				P-value
	PCH	PNE	AACH	AANE	
AP at 25 days	5.48	6.48	5.63	6.11	0.38
AP at 50 days	6.38	6.18	6.40	5.97	0.97
AP at 75 days	4.97	5.04	4.28	4.42	0.71
AP	5.59	5.90	5.43	5.50	0.83
TP at 25 days	150.7±29.7	174.1±22.4	138.9±35.4	154.4±46.3	0.34
TP day 25-50	171.8±30.1	161.5±23.3	166.5± 36.9	152.4±48.3	0.90
TP day 50-75	126.4±24.7	131.2±19.4	108.0±30.2	109.9±39.6	0.53
TP	440.5±67.0	462.4±51.9	413.4±82.1	416.7±107.5	0.72

AP - average production; TP - total production.

<sup>1</sup> PCH - calves from predominantly Charolais cows; PNE - calves from predominantly Nellore cows; AACH - calves from breed bulls Aberdeen Angus and predominantly Charolais cow; AANE - calves from breed bulls Aberdeen Angus and predominantly Nellore cow.



PCH - calves from predominantly Charolais cows; PNE - calves from predominantly Nellore cows; AACH - calves from breed bulls Aberdeen Angus and predominantly Charolais cow; AANE - calves from breed bulls Aberdeen Angus and predominantly Nellore cow.

**Figure 2** - Evolution of cow weight according to the genetic group of calves, from farrowing to weaning.

**Table 7** - Body condition score of cows and its variation during the suckling phase, according to the calves' genetic group

Variable	Genetic group of calves <sup>1</sup>				P-value
	PCH	PNE	AACH	AANE	
BCS at calving	2.42±0.07	2.46±0.06	2.51±0.09	2.45±0.09	0.43
Variation from calving to 25th day	-0.09±0.08	-0.08±0.06	-0.04±0.09	-0.11±0.09	0.78
Variation 25th at 50th day	0.06±0.05	0.04±0.04	0.02±0.07	0.12±0.09	0.28
Variation 50th at 75th day	0.00±0.05	-0.03±0.04	-0.04±0.07	0.01±0.09	0.70
Variation from calving to weaning	-0.03±0.06	-0.07±0.06	-0.05±0.04	0.02±0.09	0.48
BCS at weaning	2.38±0.06	2.38±0.05	2.45±0.08	2.48±0.09	0.33

BCS - body condition score.

<sup>1</sup> PCH - calves from predominantly Charolais cows; PNE - calves from predominantly Nellore cows; AACH - calves from breed bulls Aberdeen Angus and predominantly Charolais cow; AANE - calves from breed bulls Aberdeen Angus and predominantly Nellore cow.

### 3.4. Post-weaning calf performance

Adjusted weaning weight (205 days) differed among genetic groups ( $P < 0.05$ ; Table 3). Calves with 100% individual heterozygosity had higher weights than those with 66%, highlighting the positive impact of genetic composition on post-weaning performance. A similar trend was observed for ADG after weaning. Calves from dams with greater Charolais ancestry and sired by Angus bulls (i.e., 100% heterozygosity) showed a 33% higher ADG compared to those sired by Nellore bulls (66% heterozygosity). This difference emphasizes the importance of individual heterozygosity for performance during later growth stages.

## 4. Discussion

### 4.1. Morphometric evaluation

The lower birth weight observed in  $\frac{1}{2}$  Angus calves born to Charolais-predominant cows can be associated with their lower withers and hip heights, as well as smaller thoracic circumference. According to Brito et al. (2015), there is a strong correlation between body weight and thoracic circumference ( $r = 0.96$ ), which supports the use of this measurement to estimate body weight. Given that calves from rotational crossbreeding with Nellore predominance share maternal genetic background with the  $\frac{1}{2}$  Angus × Charolais calves, an important morphometric trait becomes evident.

### 4.2. Calf performance from birth to weaning

The lower birth weight observed in terminally crossbred calves, particularly those with Angus genetics, may be considered advantageous in terms of reproductive management. Lighter calves at birth are associated with a lower dystocia risk, as noted by Gunn et al. (2014). Thus, using Angus sires on Charolais-predominant females may reduce calving difficulty without compromising pre-weaning performance. In contrast to our findings, Perotto et al. (1999) did not observe differences in birth weight among calves from Nellore × Red Angus crosses. Differences in breed combinations, crossbreeding strategy (rotational vs. terminal), or environmental conditions may account for this discrepancy.

Regarding ADG, the similarity across treatments suggests a similar growth potential regardless of genetic group. However, the fluctuations observed across age intervals deserve attention. The decline in ADG between days 25 and 50, despite increased milk yield (Table 5), may reflect physiological adaptations or changes in calf feeding behavior. Cow weight dynamics during lactation provide additional insight. The initial weight loss (0–25 days postpartum) may have limited milk production,

thereby reducing nutrient availability for the calves. Recovery in cow body weight between days 25 and 50 (0.211 kg/day) may have supported increased milk yield during this period.

Environmental conditions also influence milk production. Cows on native pastures show a linear decline in milk yield, whereas those on cultivated pastures tend to exhibit a quadratic pattern (Restle et al., 2003). These findings underline the importance of environment, nutrition, and management in shaping calf performance during lactation.

#### 4.3. Cow performance

The absence of significant differences in milk yield among treatments likely reflects the individual variability typical of extensive production systems. Similarly, no effect of calf genetic group on milk production was reported when evaluating Nellore cows crossed with Angus, Canchim, and Simmental sires (Espasandin et al., 2001).

Despite statistical similarity, milk yield fluctuated over the lactation period, peaking between 25 and 50 days postpartum, likely reflecting the physiological peak of lactation. The subsequent decline may indicate the beginning of the natural decline in milk production, especially in native pasture systems. The values observed in the present study are similar to those reported by Vaz et al. (2020). Although a different technique was used (manual milking), an average milk yield of 6.00 L/day was reported, with collections at 21, 42, and 63 days after calving. This indicates that similar values can be obtained even with different measurement techniques when animals are in the same physiological stage and under similar management conditions.

Cow weight loss patterns varied depending on the genetic group of the calf. Nursing  $\frac{1}{2}$  Angus calves lost weight early postpartum, likely due to higher nutritional demands from faster-growing calves. Cows in the rotational crossbreeding group also lost weight, but to a lesser extent. As noted by Cerdótes et al. (2004), postpartum weight is expected when nutrient intake does not meet the animal's nutritional requirements, a condition known as negative energy balance.

This is supported by body condition score (BCS) patterns, which, although not statistically different among treatments, followed trends expected for lactating cows in pasture-based systems. According to Adams (2021) and Kenyon et al. (2014), BCS is considered a reliable indicator of the animal's nutritional status. The lack of BCS recovery during lactation, in contrast to what has been reported under better nutritional conditions (Silveira et al., 2014), suggests limitations in the nutritional supply of native pastures during periods of adverse climatic conditions.

#### 4.4. Post-weaning calf performance

The superior performance of calves with 100% individual heterozygosity highlights the benefits of heterosis (hybrid vigor) in livestock production. The introduction of a third breed, such as Angus, into a Charolais–Nellore rotational crossbreeding system can enhance hybrid vigor, especially in traits related to growth and precocity.

According to Cardoso et al. (2004), the post-weaning is a critical phase for evaluating genetic merit, as maternal influence is reduced. Therefore, the weight gains observed likely reflect the calves' genetic potential, with Angus sires providing notable advantages. The ADG of calves with 100% individual heterozygosity exceeded that reported by Ítavo et al. (2007), who found an ADG of 0.520 kg/day in supplemented calves, a value similar to that observed for our 66% heterozygous group. These results support the role of heterozygosity in improving growth efficiency, even in extensive or early-weaning systems.

Eifert et al. (2004) reported that ADG may be strongly influenced by the level of dietary concentrate, ranging from 0.270 to 0.784 kg/day. Menezes and Restle (2005) observed an ADG of 0.986 kg/day in early-weaned calves under confinement, which is expected to be higher than that observed in our pasture-based system. Collectively, these findings suggest that mating rotational crossbred cows

with sires of a third breed (Angus) may be an effective strategy for enhancing calf performance in short-cycle or early-market systems. They also show that even under low-input conditions, significant genetic gains can be achieved through proper reproductive planning.

This study has some limitations that should be acknowledged. Although the present work provides consistent evidence regarding the effects of using Angus sires in Charolais × Nellore rotational crossbreeding systems, the experimental conditions imposed inherent constraints. Conducting the trial under field conditions, with native pasture as the main forage source, may have introduced variability in forage availability and quality, potentially influencing productive responses during lactation and post-weaning. While this represents a limitation, it may also be considered a strength, as it allowed the generation of results that more closely reflect the production reality of many beef cattle operations that rely on native pasture.

In addition, further research is needed to assess the impacts of this crossbreeding strategy at later stages of the production cycle, particularly after seven months of age. Evaluations of animal performance under more intensive production systems, as well as studies focusing on post-slaughter characteristics, may provide broader insights. Such studies would help determine the feasibility and practicality of this crossbreeding approach as a strategy to improve animal performance without requiring substantial changes in the production environment.

## 5. Conclusions

Mating rotationally crossbred (Charolais × Nellore) cows with Angus bulls improved calf performance, during the post-weaning period, due to increased individual heterozygosity. Additionally, the reduced birth weight of these calves may decrease the risk of dystocia without impairing growth. This strategy appears to be effective for enhancing productivity in cow-calf systems.

## Data availability

All content (raw and analyzed data and software codes) underlying the manuscript text is available upon request to the corresponding author.

## Author contributions

**Conceptualization:** Volpatto, R. S.; Pizzuti, L. A. D.; Adams, S. M.; Klein, J. L. and Cocco, J. M. **Data curation:** Volpatto, R. S.; Alves Filho, D. C.; Muniz, H. C. M. and Souza, R. L. **Formal analysis:** Volpatto, R. S.; Muniz, H. C. M.; Pizzuti, L. A. D.; Adams, S. M.; Klein, J. L. and Cocco, J. M. **Funding acquisition:** Alves Filho, D. C. and Pizzuti, L. A. D. **Investigation:** Volpatto, R. S.; Alves Filho, D. C.; Adams, S. M.; Klein, J. L.; Cocco, J. M. and Souza, R. L. **Methodology:** Alves Filho, D. C.; Muniz, H. C. M.; Adams, S. M.; Klein, J. L.; Cocco, J. M. and Souza, R. L. **Project administration:** Volpatto, R. S.; Alves Filho, D. C.; Pizzuti, L. A. D.; Adams, S. M. and Cocco, J. M. **Resources:** Alves Filho, D. C. **Supervision:** Alves Filho, D. C. **Validation:** Alves Filho, D. C. **Visualization:** Souza, R. L. **Writing – original draft:** Volpatto, R. S. **Writing – review & editing:** Muniz, H. C. M.; Adams, S. M. and Klein, J. L.

## Conflict of interest

The authors declare no conflict of interest.

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