Performance and feeding behavior of Holstein and Holstein × Gyr crossbred heifers grazing temperate forages

Marcelo B. Abreu · Camila S. Cunha · João H. C. Costa · Emily K. Miller-Cushon · Polyana P. Rotta · Andreia F. Machado · Valber C. L. Morais · Fernanda H. M. Chizzotti · Marcos I. Marcondes

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Abstract
This study aimed to investigate the difference between Holstein and Holstein × Gyr breeds on feeding behavior and performance of heifers grazing temperate pasture. The experiment was carried out in 89 days, split into 14 days of adaptation, and 3 periods of 25 days. Two treatments were used: Holstein (HOL; n = 7) and Holstein × Gyr (HG; n = 7). Heifers grazed a consortium of ryegrass and bristle oats and were supplemented individually daily with cornmeal at 0.33% of body weight plus 5 kg/day of corn silage. For 3 consecutive days, feeding behavior was observed for individual animals from direct visual observation recording at 10-min intervals. The digestibility trial was performed on day (d) 16 to d24 of each period. Body measurements and weight were taken at d0 and at d23, 24, and 25 of each period. Grazing duration, grazing frequency, and bite rate were greater for HOL than those for HG. Rumination characteristics, intake, digestibility, and body measurements were not affected by breed. Breeds had differences in grazing characteristics, but they did not influence performance or intake parameters. Therefore, HOL and HG heifers managed under temperate pasture in tropical countries have similar performances.

Keywords Girolando · Tropical dairy production · Replacement heifers · Rotational grazing · Ryegrass

Introduction
In most tropical countries, milk production is based predominantly on grazing systems for dairy cattle because of the greater productivity of tropical forages and the low costs (Aguirre-Villegas et al. 2017). The increase of dairy production under grazing is due to the expression of natural behavior improving animal welfare (Arnott et al. 2017).

However, during the dry season, tropical pasture production in many areas is limited by low rainfall and sunlight availability (García and Fulkerson 2005). Nonetheless, temperature and luminosity during winter are generally adequate for pasture growth (Jaramillo et al. 2021), as long as water availability is not a limiting factor. Therefore, there is an unexplored opportunity for using temperate forages in tropical countries during the dry season in combination with irrigation systems.

In tropical countries, Holstein × Gyr crossbreed (HG) has become the most important breed in grazing systems (Mellado et al. 2010) because of its great ability to graze in high temperatures and low ectoparasite incidence (Otto et al. 2018). When grazing in tropical conditions, HG cows had better performance and fertility when compared with Holstein–Friesian (HOL) cows (Guimarães et al. 2002). Furthermore, Morais et al. (2018) showed that HG heifers had better performance than HOL heifers in grazing tropical pastures during the summer. The findings of Morais et al. (2018) are in agreement with Machado et al. (2019), which demonstrated that HOL heifers were found to have decreased performance when grazing an intensively managed pasture.
during the summer. Nonetheless, we found no studies comparing the performance of these two breeds grazing temperate forage simultaneously during wintertime. Although temperate forages are widely used for HOL heifers and lactating cows, there is limited knowledge about HG performance grazing temperate grass.

Therefore, we hypothesized that HG heifers would have greater feed intake, dry matter (DM) digestibility, and performance compared with HOL when grazing intensively managed temperate forages during winter in a tropical area. We also hypothesized that this better performance might be explained by a different grazing behavior of HG animals, with more grazing hours and ruminating time compared to HOL. Thus, we aimed to evaluate intake, digestibility, performance, and feeding behavior of HG and HOL replacement heifers grazing temperate forages during winter in Brazil.

Materials and methods

Site and weather conditions

The experiment was carried out at the Universidade Federal de Viçosa (UFV), following the standard procedures for Animal Care and Handling stated in the UFV guidelines. The protocol was approved by the Committee on Ethics under protocol number CEUA 48/2018. Geographical coordinates of UEPE-GL are 20° 47' 18” S, and 42° 51' 45” W, and it is 728 m above sea level. Data from temperature and humidity through the experimental period are presented in Supplementary Material (SM; Fig. 1).

Treatments and experimental design

The experiment was conducted in the winter season from June to September 2017, and it was divided into three periods of 25 days, preceded by one 14-day period of adaptation. All heifers were treated against endo- and ectoparasites before the beginning of the trial.

Heifers aged 12 to 14 months were split into two treatments: Holstein (HOL, n = 7), averaging 235 ± 31.7 kg BW and F1 Holstein × Gyr (HG, n = 7) averaging 247 ± 34.0 kg BW. The number of animals per treatment was defined by a power analysis performed as suggested by Ryan (2013), aiming 90% of power and considering 41% (CV = 26%) as the difference in the performance of grazing HG and HOL heifers (Silva et al. 2017). Heifers from both treatments were managed on the same paddocks of ryegrass (Lolium multifolium Scherb.) and bristle oats (Avena strigose Lam.) consortium (Table 1) for a 1-day grazing period. The area was divided into 25 paddocks of 580 m² (SM; Fig. 2). All paddocks had free access to a resting area with shade (3 m²/animal), water, and mineral supplement ad libitum (Table 2).

At the beginning of the experimental period, pasture DM availability was estimated using the method of Carvalho et al. (2010). We expected enough DM production to feed 14 heifers. The expected herbage allowance was 0.44 kg DM of pasture/kg BW; however, the actual herbage allowance was, on average, 0.27 kg DM of pasture/kg BW (Table 1). Therefore, aiming to meet nutritional requirements for 0.75 kg/day weight gain (NRC 2001), 5 kg of corn silage (as fed) per day was supplied to the heifers in addition to the pasture. Additionally, heifers were fed 0.33% BW of finely ground corn as a supplement (Table 2). Every day at 12:00 h, heifers were fed individually in a tie stall structure with corn silage and the finely ground corn as a partial mixed ration. All heifers consumed the partial mixed ration within a maximum of 1 h without orts being detected for any heifers during the experimental period. During the first period, four heifers (2 in each treatment) were removed from the experiment due to health problems with babesiosis, remaining 5 animals per treatment. We decided to move on with the experiment because the power analyses still indicate 80% power when 5 animals per treatment were used.

Table 1  Characteristics of herbage during experimental periods

| Item                              | Period    |
|-----------------------------------|-----------|
|                                   | 1         | 2         | 3         |
| Accumulated herbage (kg DM/ha/cycle) | 3013.23  | 615.69  | 1013.10  |
| Accumulated herbage (kg DM/paddock/cycle) | 52.69  | 35.52  | 58.45  |
| Herbage allowance (kg DM/animal/day) | 5.26  | 3.55  | 5.84  |
| Forage allowance (kg DM of pasture/kg BW) | 0.35  | 0.21  | 0.31  |
| Grazing efficiency1 (%)            | 105  | 114  | 81  |
| Pre-grazing height (cm)            | 21.57  | 35.44  | 32.14  |
| Post-grazing height (cm)           | 10.26  | 13.42  | 15.33  |

1Calculate as total pasture DMI (sum of all animals)/accumulate herbage (kg/paddock/cycle) according to Sollienber et al. (2005)

Pasture measurements

The pasture was managed under intermittent stocking using heights of 30 and 15 cm as pre- and post-grazing targets, respectively, following Carvalho et al. (2010) and Marchesan et al. (2015). Pre-grazing herbage accumulation was determined in each paddock on day (d) 21 to 24 of each experimental period. Samples were collected at the actual post-grazing canopy height. Two 1.0 × 1.5 m (width × length) exclusion cages were placed in representative areas (based on height and morphological structure) immediately before beginning a new grazing cycle in each paddock. Pasture
samples were taken by clipping the area within the exclusion cages in the residual height after animal removal. The pasture pre-grazing height averaged 28 cm, ranging from 21 to 34 cm, respectively. Pasture samples were oven dried at 55°C for 72 h and then ground in a Wiley mill (TECNAL, Piracicaba, São Paulo, Brazil) with 2-mm and 1-mm sieve knives.

**Grazing and ruminating behavior**

Each period, feeding behavior was observed through 24-h live observation for 3 consecutive days (d 13–15) by instantaneous recording at 10-min intervals (Mitlhoner et al. 2001; Fehmi et al. 2002) by a pair of trained observers stationed 50 m from the paddock, which were changed every 6 h. During the study, eight training observers recorded the animal behavior. Heifers were identified with a number using a color spray, and the observers had visual contact with the animals at all times.

On d 13, immediately after initiating a new grazing cycle in a fresh paddock, the bite rate was assessed based on the average between three trained observers. The evaluations were carried out by measuring the time taken by animals to perform twenty bites (Hodgson 1990). Grazing was defined when the animal was ingesting with chewing visible or head lowered, and nose directed towards the grass. Rumination was defined when the food was regurgitated, followed by remastication of bolus by lateral movements of the mandible and no other action in play. Idle time, water intake, socialization (playing with each other), walking, and consuming mineral and partial mixed ration intake were considered other activities.

To assess bout characteristics from data obtained using instantaneous recording, each positive scan in the dataset was assigned a block of time equivalent to the 10-min sampling interval to approximate behavior throughout the day. The quantification of the number of meals was obtained by the minimum interval between feeding to consider the next feeding visit as a new meal, also called the meal criteria (Tolkamp et al. 1998; Yeates et al. 2001). Bout criterion was defined as the longest nonfeeding interval within a meal (Bailey et al. 2012). The bout criteria analysis was performed separately for grazing and ruminating behavior, both being done individually per heifer (totaling 72 h of behavior data per animal and observation period). The intervals between grazing or ruminating were calculated and log10 transformed. To estimate the bout criteria, we used the package mixdist of R software (Macdonald and Du 2018) to fit two normal distributions to the log 10-transformed intervals between meals as per methods described by Horvath and Miller-Cushon (2019). Based on conditional probabilities generated within the mixdist package, the bout criteria were determined as the point at which the two distribution curves intersected, with the first distribution corresponding to the shorter intervals interpreted as “within bout” and the second distribution curve corresponding to the longer intervals, interpreted as “between bout” (Fig. 1). The frequency of bouts (events/day) was calculated from the number of intervals that exceeded the criterion plus adding 1. The bout duration (min/day) was estimated from the time of the first hit to the interval that reached the meal criteria. The sum of the duration of all bouts represented the total daily bout time (min/day).

**Digestibility trial**

Between d 16 and 24 of each period, 15 g of chromium oxide was infused by the esophageal probe in each animal to estimate fecal excretion. On d21 to 24, feces and urine samples were collected at 06:00, 10:00, 12:00, and 18:00 h, respectively. Approximately 300 g of feces was sampled directly from the rectum. The same method of drying and grinding forage samples was applied to fecal samples. Urine samples were taken by vulva stimulation, and 50 mL of urine was sampled and immediately frozen at −20°C. After the fourth collection day, all samples were thawed, and then a
pooled sample was obtained by mixing 15 mL of urine of each sampling day. From the pooled sample, 10 mL was diluted into 40 mL of sulfuric acid (0.036 N) and stored at −20 °C to prevent purine derivatives. A 50-mL sample of the concentrated urine was also stored at −20 °C.

**Performance**

Heifers were weighed at d0 and on d23, 24, and 25 of each experimental period, always at 06:00 h, and the average daily gain (ADG) was calculated for each experimental period.

**Chemical analysis**

Samples of forage, corn silage, finely ground corn, and feces were analyzed for DM, crude protein (CP), neutral detergent fiber (NDF), ether extract (EE), and ash (AOAC 2005). The 2-mm samples were used for the analysis of indigestible neutral detergent fiber (iNDF) as suggested by Valente et al. (2011). Additionally, feces samples were analyzed for chromic oxide according to Detmann et al. (2012). The non-fibrous carbohydrates (NFC) were calculated as proposed by Weiss (1999). Estimates of fecal excretion were determined according to Detmann et al. (2001). Pasture intake was estimated using iNDF as an internal marker (Detmann et al. 2001) (SM; Eq. 1).

**Statistical analysis**

The response variables were analyzed using the GLIMMIX procedure of SAS University Edition, Version 9.4. The data were analyzed following a completely randomized design. For all performance variables, measurements at d0 were tested as covariates and removed from the models as they were all non-significant (P > 0.05). For all response variables, period was included as repeated measurements. Fifteen variance–covariance structures were tested for each response variable. Thus, we used the variance–covariance structure that provided the best fit based on lower AIC. Least square means were considered different when P ≤ 0.05.

**Results**

Intake and digestibility parameters were not affected by breed (Table 3). The CP and EE intakes were greater in the first period and decreased in the second and third periods for all breeds (Table 3). The NFC intake was greater in period 3 when compared to the first and second periods (Table 3). A breed×period interaction was observed for NFC digestibility (Table 3), and Holstein animals had greater digestibility in periods 1 and 2 (SM; Fig. 3). The average daily gain was not affected by breed.

A breed×period interaction was observed for grazing time (Table 4), and HOL heifers had greater grazing time in
Table 3  Intake, digestibility of nutrients, performance, and body measurements of Holstein and Holstein × Gyr heifers grazing temperate forages

| Item                          | Breed | Period | SEM | P-value |
|-------------------------------|-------|--------|-----|---------|
| Intake (kg/d)                |       |        |     |         |
| DM                            | HOL   | 6.98   | 7.43| 7.82    |
| Pasture                       | HG    | 4.57   | 5.01| 5.59    |
| CP                            |       | 1.34   | 1.47| 1.85    |
| NDF                          |       | 2.81   | 2.99| 3.06    |
| EE                            |       | 0.24   | 0.26| 0.31    |
| NFC                          |       | 1.95   | 2.02| 1.81    |
| ME (Mcal/d)                  |       | 20.95  | 20.79| 23.83   |

Intake (g/kg of BW)

| Item                          | Breed | Period | SEM | P-value |
|-------------------------------|-------|--------|-----|---------|
| DM                            | HOL   | 2.36   | 2.42| 2.83    |
| Pasture                       | HG    | 1.53   | 1.64| 2.01    |
| CP                            |       | 0.95   | 0.97| 1.11    |
| NDF                          |       | 646.2  | 635.6| 683.4   |
| NFC                          |       | 698.4  | 676.4| 748.4   |

Digestibility (g/kg)

| Item                          | Breed | Period | SEM | P-value |
|-------------------------------|-------|--------|-----|---------|
| DM                            | HOL   | 646.2  | 635.6| 683.4   |
| Pasture                       | HG    | 698.4  | 676.4| 748.4   |
| CP                            |       | 611.8  | 594.6| 639.7   |
| NDF                          |       | 597.0  | 535.3| 625.9   |
| NFC                          |       | 862.6  | 807.9| 853.3   |

Performance

| Item                          | Breed | Period | SEM | P-value |
|-------------------------------|-------|--------|-----|---------|
| ADG (kg/d)                    | HOL   | 1.03   | 1.17| 1.46    |
| Pasture                       | HG    | 1.53   | 1.64| 2.01    |
| DM                            |       | 8.03   | 7.71| 7.10    |
| Pasture                       |       | 3.77   | 3.76| 4.87    |
| DM                            |       | 3.72   | 59.58| 39.07   |
| Pasture                       |       | 4.90   | 71.25| 66.91   |

Table 4  Behavior activities of Holstein and Holstein x Gyr heifers grazing temperate forages

| Item                          | Breed | Period | SEM | P-value |
|-------------------------------|-------|--------|-----|---------|
| Grazing characteristics       |       |        |     |         |
| Grazing time (min/d)*         | HOL   | 376.44 | 356.44| 412.33  |
| Daily meal time (min/d)       | HG    | 935.78 | 903.00| 1040.42 |
| Grazing frequency (bouts/d)   |       | 8.03   | 7.71| 7.10    |
| Meal duration (min/meal)      |       | 130.73 | 179.70| 137.87  |
| Grazing bout criteria (min/d1)|      | 32.74  | 59.58| 39.07   |
| Grazing time per min of meal time | 49.20 | 71.25| 66.91| 54.73   |

Ruminating characteristics

| Item                          | Breed | Period | SEM | P-value |
|-------------------------------|-------|--------|-----|---------|
| Ruminating time (min/d)*      | HOL   | 372.22 | 405.56| 347.00  |
| Daily ruminating time (min/d) | HG    | 560.96 | 440.24| 678.03  |
| Ruminating frequency (events/d) | 3.77  | 3.76   | 4.87  | 3.83    |
| Ruminating duration (min/d)   |       | 125.75 | 114.87| 134.73  |
| Ruminating criteria (min/d1)  |       | 76.18  | 83.53| 79.77   |
| Ruminating time per min of meal time | 49.20 | 71.25| 66.91| 54.73   |

Other activities (min/d)*

| Item                          | Breed | Period | SEM | P-value |
|-------------------------------|-------|--------|-----|---------|
| Bite rate (bites/min)         | HOL   | 20.01  | 17.30| 19.92   |
| Pasture                       | HG    | 690.89 | 683.78| 688.33  |

1 Meal criteria (min/d) calculated as 10^meal criteria

*P<0.05

Breed by period interactions for NFC digestibility can be found in Supplemental material Fig. 3
period 3 but did not differ from HG in the first and second periods (Fig. 2a). Breed did not influence the daily grazing bout time (min/day, Table 4). The frequency of grazing was greater for HOL heifers (8.03 events/day) when compared with HG (7.71 events/day, Table 4). The grazing meal duration was greater for HG heifers (179.71 min/day) when compared with HOL (130.73 min/day, Table 4). When we evaluated the grazing time per min of meal time, HG heifers had a greater value when compared with HOL heifers (Table 4). Ruminating time, frequency, duration (min/day), and ruminating criteria (min) were not affected by breed (Table 4). HOL heifers had a greater bite rate (20.01 bites/min) than HG heifers (15.3 bites/min; Table 4). A breed × period interaction was observed for other activities, whereas HOL heifers had greater other activities in period 1 (Fig. 2c).

Discussion

Previous literature data suggest that Holstein×Gyr crossbred animals are more adapted to tropical conditions than purebred animals such as Holstein-Friesen (Otto et al. 2018). It is usually related to greater production and reproduction outcomes in grazing systems in tropical areas (Oliveira Júnior et al. 2017). However, all those studies were conducted with tropical forages during warmer months. Nevertheless, due to the greater grazing ability of HG animals (Machado et al. 2019, 2020), we hypothesized that HG heifers would have greater intake and performance than HOL heifers even when offered temperate pastures species in a thermo-neutral environment. However, the breed did not influence the performance, intake, and digestibility of the animals. These results indicate that weather conditions during winter in tropical countries are adequate for HOL heifers grazing temperate pastures. In a recent study, Morais et al. (2018) verified that HOL heifers had lower ADG compared with HG when grazing intensively managed tropical pastures. A reduction in DMI by HOL heifers grazing tropical pastures during the summer is frequently related to a reduced grazing time and increased resting time (Diniz et al. 2018) because animals avoid being directly exposed to sunlight during this period.

The greater the grazing pressure, the lower the forage allowance (Sollenberg et al. 2005). In this sense, the lower forage allowance in period 2 was caused by the greater grazing pressure in period 1, once there was less accumulated herbage per BW. On the other hand, there was an adequate pasture re-growth and herbage accumulation in the third period, contributing to lower grazing pressure and increasing the forage allowance. Thus, a larger residual leaf area due to lower forage removal can be confirmed by the higher post-grazing height.

In addition, the grazing efficiency in the first period was affected by excessive pasture defoliation. Thus, grazing efficiency greater than 100% indicates that the animals consumed pasture beyond the programmed post-grazing height goal. Dale et al. (2018) showed that the greater the herbage allowance, the lower the grazing efficiency. Hence, the decrease in forage allowance in period 2 resulted in the

![Fig. 2](image-url)  
Fig. 2 Grazing (A), ruminating (B), and other activities (C) times of Holstein (black square) and Holstein×Gyr (dashed line, white circle) heifers grazing temperate forages. Periods marked with * indicate the difference between breeds at $P < 0.05$
greater grazing efficiency (114%), which might be due to the lower forage allowance in this period.

The better forage quality likely caused the greater CP intake in period 1, which had higher CP and lesser NDF. Likewise, the greater EE intake observed in the first period was due to greater pasture intake. Lastly, the increase in NFCI in periods 2 and 3 was associated with an increase in forage NFC during these periods. The greater DMI (g/kg BW) in period 1 may be associated with the greater pasture allowance in that period, which is the main driver of pasture intake (Pérez-Prieto and Delagarde 2013). Regardless of breed, heifers had greater ADG in the first period than in the second and third periods. The lower ADG in periods 2 and 3 was likely associated with the lower pasture availability during those periods, which is reflected in a lower pasture intake compared with the first period. Thus, both HG and HOL could have had even greater performance in the case of higher forage allowance. In this sense, future studies should focus on greater forage allowance during winter times in tropical countries.

Holstein heifers had more frequent, shorter grazing bouts, a greater bite rate, and a lower ratio of time spent feeding during grazing bouts. Some of these differences in grazing bout structure may be explained by body size and intake rate; as HOL heifers have a greater bite rate, they may spend less time grazing during each grazing bout. Differences in grazing bout frequency and duration may also depend on heat tolerance. In this sense, the low winter temperatures (SM; Fig. 1) affected some behavior parameters of HOL heifers, but the total daily grazing time was not different from that of HG heifers. Nevertheless, Rutter et al. (2002) observed a grazing time of HOL heifers of 436 h/day in England, which is still 19% greater than the average grazing time of our animals. It is probably due to climate and grass differences, but perhaps, the addition of partial mixed ration in the diet reduced the grazing time of the animals in the present study. Both breeds had the same pattern of grazing distribution throughout the day (Fig. 3a), which is a higher grazing frequency during the day, as observed by De Souza et al. (2019). Although the daily grazing time was not affected by breed, HG heifers spent a greater proportion of their grazing bouts feeding. This provides evidence that, within-meal, pauses during grazing are different between breeds. Thus, these data suggest that HOL had longer pauses when grazing (during meal) when compared with HG heifers. Lastly, the higher bite rate of HOL compared with HG (20.0 vs. 17.3) suggests that animals could be grazing for the same amount of time but have a different bite rate during that period. In this study, the bite rate was lower than those found by previous studies (Barrett et al. 2001; Rutter et al. 2002), where the HOL had a bite rate between 40.4 and 69 min/day. In summary, we believe that differences between grazing bout structures are associated with the rate of intake and heat tolerance differences between HOL and HG heifers being interpreted as the possible causes of differences between grazing behaviors.

Although it has been previously suggested that grazing behavior directly affects the rumination process (Nasrollahi et al. 2016), this effect did not lead to changes in rumination patterns between HOL and HG heifers in the present study. The rumination frequency of animals (Fig. 3b) is consistent with those presented by Zanine et al. (2007), in which the percentage of ruminant events concentrated in the night period. Therefore, the impact of ruminating behavior on intake, digestibility, and performance might be more pronounced when animals are grazing tropical pastures when compared to temperate pastures. We highlight that the results of this study should be cautiously evaluated once the power has decreased from 90 to 80% due to reduction of two animals per treatment. However, our results demonstrated differences in grazing and ruminating behavior that could improve dairy heifers’ management under grazing. Thus, future studies should be done to confirm the present results.
In summary, HOL and HG heifers grazing intensively managed temperate-season pasture had similar intake, digestibility, performance, and metabolic parameters. Although they presented differences in feeding behavior, primarily in grazing characteristics, these effects did not affect performance or intake parameters.

Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s11250-022-03106-w.

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Author contribution  All the authors contributed to the study’s conception and design. Material preparation, data collection, and analysis were performed by Marcelo de Barros Abreu, Marcos Inacio Marcondes, Polyna Pizzi Rotta, Andreia Ferreira Machado, and Valber Carlos Morais. Marcelo de Barros Abreu wrote the first draft of the manuscript and all the authors commented on previous versions of the manuscript. Finally, all the authors read and approved the final manuscript.

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Data availability  The datasets generated and/or analyzed in this study are available from the corresponding author on reasonable request.

Declarations

Ethical committee  Standard procedures for Animal Care and Handling stated UFV guidelines were approved by the Committee on the Ethics under the protocol number CEUA 48/2018.

Conflict of interest  The authors declare no competing interests.

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