

MARCELO DE BARROS ABREU

**PERFORMANCE AND INGESTIVE BEHAVIOR OF HOLSTEIN AND  
GIROLANDO HEIFERS GRAZING TEMPERATE FORAGES**

Dissertation submitted to the Animal Science  
Graduate Program of the Universidade Federal de  
Viçosa as partial fulfillment of the requirements to  
obtain the degree of *Magister Scientiae*.

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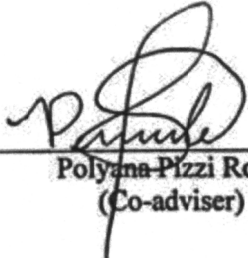
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## **BIOGRAFIA**

Marcelo de Barros Abreu, filho de Marcelo Silva Pires de Abreu e Elisângela Bezerra de Barros, nasceu em Viçosa / MG - Brasil em 27 de fevereiro de 1992.

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## ABSTRACT

ABREU, Marcelo de Barros, M.Sc., Universidade Federal de Viçosa, February, 2019. **Performance and ingestive behavior of Holstein and Girolando heifers grazing temperate forages.** Adviser: Fernanda Helena Martins Chizzotti. Co-advisers: Marcos Inácio Marcondes and Polyana Pizzi Rotta.

This study aimed to investigate the difference between Holstein and Holstein x Gyr breeds on performance and ingestive behavior of dairy heifers grazing an intensively managed temperate pasture. The experiment was conducted during 89 days, divided into 14 days for adaptation plus three periods of 25 days. The heifers were divided into two treatments: Holstein (HOL. n = 7) with body weight (BW) of  $235 \pm 31.7$  kg and Holstein  $\times$  Gyr (HG. n = 7) with BW of  $247 \pm 34.0$  kg. All heifers grazed 1.5 ha divided into 25 paddocks (577 m<sup>2</sup>) of ryegrass (*Lolium multiflorum* Scherb) and Bristle Oats (*Avena strigose* Lam) consortium. Heifers were supplemented individually daily with corn meal at 0,33% of BW plus 5 kg/d of corn silage as partial mixed ration (PMR). From d 13 to 15 of each period, the evaluation of ingestive behavior was performed. The digestibility trial was done between days 16 and 24 of each period. The body measurements were taken and heifers were weighed at d 0 and at days 23, 24, and 25 of each period. At experimental days 0 and 75, an ultrasound device was used to measure carcass parameters. Blood samples were collected on d 25 of each period. At the end of the experimental period, ruminal fluid samples were obtained using an esophageic tube. Data were analysed as a completely randomized design and period was added, when necessary, as a repeated measured to the model. The grazing duration (130.73 vs 179.70), and grazing criteria (32.74 vs 59.58) were greater for HG heifers. HOL heifers had greater grazing frequency (8.03 vs 7.71), and greater bite rate (min/d) (20.01 vs 17.30) than HG heifers. Rumination characteristics were not affected by breed. The ADG was not affected by breed (1.03 kg/d for HOL and 1.17 kg/d for HG). HOL and HG heifers had similar intake and digestibility responses. The body measurements, carcass, blood, rumen, and nitrogen parameters were also not affected by the breed. Although breeds had slight differences in ingestive behavior, primarily in grazing characteristics, these effects did not affect performance or intake parameters. Therefore, HOL and HG replacement heifers managed under temperate pasture in tropical countries have adequate and similar performance.

## RESUMO

ABREU, Marcelo de Barros, M.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Performance and ingestive behavior of Holstein and Girolando heifers grazing temperate forages.** Orientadora: Fernanda Helena Martins Chizzotti. Coorientadores: Marcos Inácio Marcondes e Polyana Pizzi Rotta.

Objetivou-se investigar a performance e comportamento de novilhas das raças Holstein e Girolando em pastagem temperada intensivamente manejadas. O experimento foi conduzido durante 89 dias, dividido em 14 dias para adaptação e três períodos de 25 dias cada. As novilhas foram divididas em dois tratamentos: Holandês (HOL. N = 7) com peso corporal (PC) de  $235 \pm 31,7$  kg e Holstein x Gir (*called* Girolando) (HG. N = 7) com PC de  $247 \pm 34,0$  kg. Todas as novilhas tiveram acesso a 1,5 ha, divididos em 25 piquetes ( $580 \text{ m}^2$ ) de consórcio de azevém (*Lolium multiflorum* Scherb) e aveia de cerda (*Avena strigose* Lam). As novilhas foram suplementadas diariamente com milho moído a 0,33% do PC e 5 kg/dia de silagem de milho, fornecidos como partial mixed ration (PMR). A partir do 13 ao 15 dia de cada período, foram realizadas as avaliações do comportamento ingestivo. O ensaio de digestibilidade foi realizado entre os dias 16 e 24 de cada período. As medidas corporais foram realizadas e as novilhas foram pesadas no dia 0 e nos dias 23, 24 e 25 de cada período. Nos dias experimentais 0 e 75, um aparelho de ultra-som foi usado para medir os parâmetros de carcaça. Amostras de sangue foram coletadas no dia 25 de cada período. No final do período experimental, as amostras de líquido ruminal foram obtidas utilizando tubo esofágico. Os dados foram analisados em delineamento inteiramente casualizado e o período foi adicionado, quando necessário, como medida repetida ao modelo. A duração do pastejo (130,73 vs 179,70), e os critérios de pastejo (32,74 vs 59,58) foram maiores para as novilhas HG. As novilhas HOL apresentaram maior frequência de pastejo (8,03 vs 7,71) e maior taxa de mordida (min/d) (20,01 vs 17,30) que as novilhas HG. As características de ruminação não foram afetadas pela raça. O GMD não foi afetado pela raça (1,03 kg/d para HOL e 1,17 kg/d para HG). As novilhas HOL e HG tiveram respostas similares de ingestão e digestibilidade. As medidas corporais, carcaça, sangue, rúmen e parâmetros de nitrogênio também não foram afetados pela raça. Embora as raças tenham pequenas diferenças no comportamento ingestivo, principalmente nas características de pastejo, esses efeitos não afetaram o desempenho nem os parâmetros de ingestão. Portanto, as novilhas de substituição HOL e HG manejadas sob pastagem temperada em países tropicais apresentam desempenho adequado e similar.

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## **INTRODUCTION**

In most tropical countries, milk production is based predominantly on grazing systems (Costa et al., 2013; Bernardes and do Rêgo, 2014). This characteristic is a result of the greater productivity of tropical forages and of the reduced production cost in these systems, mainly when compared with confined systems (Aguirre-Villegas et al., 2017). In addition, the use of grazing systems has grown worldwide due to the fact that consumers have increasingly come to believe that animals express their natural behavior with improved animal welfare when raised in these systems (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). Moreover, there is a decrease in forage nutritional quality, mainly crude protein content, which becomes a limiting factor for animal performance (Paulino et al., 2008). In this sense, strategies for feeding livestock during wintertime, for instance, grain supplementation, use of silages, hay, and temperate forages, become mandatory (Clark et al., 2018).

Subtropical countries such as New Zealand, Australia, and western Europe developed very efficient temperate grazing systems (Roche et al., 2017), where most of the animal requirements are supplied by the pasture (Clark et al., 2018). Tropical forage reduces their quality and production in the dry season (Detmann et al., 2009). However, this season coincides precisely with reduction of temperature, luminosity, and rainfall (Vitor et al., 2009), which may be an inadequate condition for tropical forage growth. Nonetheless, temperate and luminosity in winter time in tropical counties are generally adequate for temperate forage growth (Carvalho et al., 2010), 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.

Replacement animals programs in dairy farms aim to supply the nutritional requirements to support the growth of replacement heifers with reduced feed cost through efficient nutritional management (Mohd Nor et al., 2015; Hoffman, 2016). Thus, the genetic potential is determinant for the success of these programs (Heinrichs, 1993). In the case of tropical countries, such as Brazil, Mexico, and Colombia (Ferro et al., 2010; Mellado et al., 2010; Andrés et al., 2012), Holstein × Gyr crossbred (HG, also called Girolando breed) have become the most important breed in grazing systems (Santos et al., 2012; Henrique et al., 2018). This breed is well adapted to grazing within high temperatures and ectoparasites incidence (Pereira et al., 2013; Otto et al., 2018). Some studies have already demonstrated that, when grazing in tropical conditions, HG cows had better performance and reproduction when compared with Holstein-Friesian cows (Guimarães et al., 2002; McManus et al., 2008). Furthermore, Morais et al. (2018b; a) showed that Holstein × Gyr heifers had better performance when compared with Holstein-Friesian heifers when grazing tropical pastures during the summer. The findings of Morais et al. (2018b; a) are in agreement with Machado et al. (2018), that demonstrated that HOL heifers were found to have decreased performance when grazing an intensively managed pasture during the summer. Nonetheless, we found no study comparing the performance of these two breeds grazing temperate forage simultaneously during wintertime. Although we acknowledge that temperate forages are widely used for HOL heifers and lactating cows (O'Brien et al., 2014; Washburn and Mullen, 2014; Vibart et al., 2017), there is limited knowledge about Holstein × Gyr performance grazing temperate forages.

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

performance, and ingestive behavior of HG and HOL replacement heifers grazing temperate forages during winter time in Brazil.

## **MATERIAL AND METHODS**

### *Site and weather conditions*

The experiment was carried out at the Dairy Cattle Teaching, Research and Extension Unit (UEPE-GL) of the Animal Science Department of the Federal University of Viçosa (UFV). The geographical coordinates of the experiment are 20° 47' 18'' S, and 42° 51' 45'' W, with an altitude of 728 m. Data from temperature and humidity through the experimental period are presented in Figure 1.

### *Treatments, and Experimental design*

The experiment was conducted from June to September 2017. The experimental was divided three periods 25 days, preceded of 14 days of adaptation. All heifers were treated against endo- and ectoparasites with 1 mL/50 kg body weight (BW) of Dectomax (Pfizer USA, Doramectin 1%) before the start of the trial.

The heifers were divided into two treatments: Holstein (HOL, n = 7) with BW of 235 ± 31.7 kg and F1 Holstein × Gyr (HG, n = 7) with BW of 247 ± 34.0 kg and were 12 to 14 months of age. All heifers (HOL: Holstein and HG:Holstein × Gyr) grazed the same area (the same paddocks) of Ryegrass (*Lolium multifolium* Scherb) and Bristle oats (*Avena strigosa* Lam) consortium. The 1.5-hectare area was established with a seeding density of 35 kg of ryegrass and 35 kg of Bristle oat per hectare. The planting was performed on May 1st, 2017. The seeding was done in rows, with a row spacing of 50 cm. During the establishment, the pasture was irrigated and fertilized with 200 kg of N and K<sub>2</sub>O/hectare/year. The area was

divided into 25 paddocks of 580 m<sup>2</sup> (Supplementary Figure S1). All paddocks had free access to a resting area with shade (3 m<sup>2</sup>/animal), water, and mineral supplement ad libitum (Table 2).

At the beginning of the experimental period, the availability of pasture dry matter was previously estimated based on literature data (Carvalho et al., 2010), and we expected enough dry matter 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, 5 kg of corn silage (as fed-basis) 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 pm, heifers were fed individually with corn silage and the finely ground corn as a partial mixed ration (PMR). The PMR was consumed by all heifers within a maximum period of 1 hour without orts being detected for any heifers during the experimental period. The quantity of finely ground corn was adjusted by period, based on the animals' weight at the beginning of each period. During the first period, four heifers (2 in each treatment) were removed from the experiment due to health problems with babesiosis and risk of death, remaining 5 animals per treatment.

#### *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. (2016). Pre-grazing herbage accumulation was determined in each paddock on days 21 to 24 of each experimental period. Samples were collected at the actual post-grazing canopy height. Two 1.0 x 1.5 m (width x length) exclusion cages were placed in representative areas (based on height and morphological structure) immediately before the beginning of a new grazing cycle in each paddock. Pasture samples were taken by clipping the area within the exclusion cages in the residual height. 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. Every period, a composite sample was prepared by mixing equal amounts of DM from individual samples obtained on d 21 to 24 and stored for chemical analyses.

### *Ingestive behavior*

Every period, from d 13 to 15, the evaluation of ingestive behavior was performed during 24 h, totaling 72 h, in each period. The activities grazing time, rumination time, and resting time (idle time, water intake, socialization, walking, and mineral and PMR intake) were recorded every ten minutes, as described by Martin and Bateson (1993). The grazing time activity was recorded when the animal was ingesting or selecting the forage. The rumination activity was recorded when the animal was ruminating and no other action was in play.

The quantification of the number of meals was obtained by the minimum interval between visits to consider the next feeding visit as a new meal, also called the meal criteria (Tolkamp et al., 1998; Yeates et al., 2001). The methodology used in this study was described in detail by DeVries et al. (2003). Briefly, we considered the meal criteria as the point of intersected between the interval distribution curve between meals and distribution curve of the intra meal intervals (Figure 2). The meal criteria were determined by animal and by a period. Hence, the frequency of meals (events/d) was calculated from the number of intervals that exceeded the criterion by adding one. The meal duration (min/d) was estimated from the time of the first hit to the interval that reached the meal criteria. The sum of the duration of all meals represented the total daily mealtime (min/d).

Moreover, on 13 d, immediately after initiating a new grazing cycle in a fresh paddock, the bite rate was accessed 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). At least two successive counts per experimental period were performed on each period.

### *Digestibility trial*

Between days 16 and 24 of each period, 15 g of chromium oxide was infused by the esophagec probe in each animal to estimate fecal excretion. On days 21 to 24, feces and urine samples were collected at 0600, 1000, 1200, and 1800 h, respectively. Approximately 300 g of feces were sampled directly from the rectum. The same procedures of drying and grinding done with forage samples were applied to fecal samples. Urine samples were taken by vulva stimulation, and 50 mL of urine were sampled and immediately frozen at  $-20^{\circ}\text{C}$ . After the fourth collection day, a pooled sample was obtained by mixing 15 mL of urine of each sampling day. From the pooled sample, 10 mL were diluted into 40 mL of sulfuric acid (0.036N) and stored at  $-20^{\circ}\text{C}$  to prevent purine derivatives degradation (Valadares et al., 1999). A 50 mL sample of the concentrated urine was also stored at  $-20^{\circ}\text{C}$ .

### *Performance and Body measurements*

The heifers were weighed at d 0 and on days 23, 24, and 25 of each experimental period, always at 0600, average daily gain (ADG) was calculated for each experimental period. The body's measurements thoracic circumference, body length, rump length, and withers height were measured using hipometer on d 0, and on d 25 of each experimental period.

### *Blood metabolites*

Blood samples were collected on d 25 of each experimental period by puncturing the jugular vein. After sampling, samples were centrifuged at  $1,304 \times g$  for 15 minutes and then serum samples were frozen at  $-20^{\circ}\text{C}$ . Serum concentrations of glucose (K082), triglycerides (K117), total protein (K031), albumin (K040) and urea (K056) were measured using kits from Bioclin Diagnostics (Belo Horizonte, Brazil). The serum concentrations of IGF1 was

determined by chemiluminescence method using Liason XL chemiluminescence analyzer. An automatic biochemical apparatus (Mindray BS-200E; Shenzhen Mindray Bio-Medical Electronics Co. Ltd.) was used for all analyses. Serum urea nitrogen (SUN) was estimated as 46.67% of the total serum urea.

### *Carcass Characteristics*

On day 0 and 75, an ultrasound device was used to measure the *gluteus medius* and the *biceps femoris* muscle intercessions, located between the ischial and the ileal tuberosities, by scanning between the 12th and 13th ribs and the rump in the P8 region. We used an 18-cm linear array ultrasound instrument (Aloka SSD-500V, Aloka Co., Ltd., Tokyo, Japan) operated at a frequency of 3.5 MHz. A standoff (Aloka long standoff guide-beef, Aloka Co., Ltd. Tokyo, Japan) and vegetable oil were used for adequate acoustic contact between the transducer, the standoff, and the animals' skin. Ultrasound images were recorded and later analyzed for back fat thickness (BFT) and loin depth (LD) using the BioSoft Toolbox® II for 200 Beef (Biotronics Inc., Ames, Iowa, USA) software.

### *Ruminal Fluid*

On d 75, ruminal fluid samples were obtained using a gastric probe with the aid of a vacuum pump and kitasato, as described by Bouda et al. (2000). The first 300 mL of rumen sample was discharged to avoid saliva contamination, and then pH was immediately measured using a digital potentiometer (Ph-221. Lutron Electronics. Taiwan). Then, two 50 mL samples were frozen at  $-20^{\circ}\text{C}$  for further ammonia nitrogen (NAR).

### *Chemical analysis*

Samples of forage, corn silage, finely ground corn, and feces were analyzed for dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), ether extract (EE), and ash by (AOAC International. 2005; methods 934.01; 990.13; 2003.05; 942.05, respectively). The 2 mm samples were used for analysis of indigestible neutral detergent fiber (iNDF). The samples were incubated into the rumen of a ruminally-fistulated cow for a period of 288 h, using non-woven textile bags (100 g/m<sup>2</sup>), and NDF was determined from the post-incubation material (Valente et al., 2011). Additionally, feces samples also were analysed for chromic oxide (method INCT-CA M-005/1), according to Detmann et al., (2012). Lastly, the Non-fibrous carbohydrates (NFC) was calculated as proposed by Weiss (1999).

The concentration of NAR in the ruminal liquid was evaluated by colorimetric technique (INCT-CA method N-006/1; Detmann et al., 2012).

Estimates of fecal excretion (FE) was determined according to (Detmann et al., 2001), which was accessed through to Equation 1:

$$FE = \frac{\text{Infusion of chromium oxide g/day}}{\text{Concentration chromium oxide in the faeces } \frac{g}{g} DM} \quad [1]$$

The pasture intake (PI) was estimated using indigestible NDF (iNDF) as an internal marker (Detmann et al., 2001). The pasture intake (PI) was obtained according to Equation 2:

$$PI = \frac{iNDF \text{ fecal excretion} - \text{corn silage } iNDF \text{ intake} - \text{concentrate } iNDF \text{ intake}}{iNDF \text{ pasture } g/g DM} \quad [2]$$

Allantoin in urine was analyzed by the colorimetric method (Chen & Gomes, 1992). Uric acid and creatinine were analyzed using kits Bioclin® (K0139 and K067, Belo Horizonte, Brazil), determined by the automated biochemical analyzer (BS200E Mindray, Shenzhen, China).

*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 d 0 were tested as covariates and removed from the models as they were all non-significant ( $P > 0.05$ ). For all response variables excepting carcass traits, pH ruminal, and ammonia nitrogen (which were measured only at the end of the experiment) period was included as repeated measurements in the model as follows:

$$Y_{ijk} = \mu + B_i + \delta_{ij} + P_k + (B \times P)_{ik} + \varepsilon_{ijk}$$

Where:  $Y_{ijk}$ = observation  $ijk$ ;  $\mu$ = the overall mean;  $B$  = the effect of treatment (breed)  $i$ ;  $\delta_{ij}$  = random error with mean 0 and variance  $\sigma_{\delta}^2$ , the variance between animals within treatment and it is equal to the covariance between repeated measurements within animals;  $P_k$ = the effect of period  $k$ ;  $B \times P_{ik}$ = the effect of interaction between treatment  $i$  and period  $k$ ; and  $\varepsilon_{ijk}$ = random error with the mean 0 and variance  $\sigma^2$ , the variance between measurements within animals. 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  $P \leq 0.05$ .

## RESULTS

The accumulated herbage (kg DM/ha/cycle) was 912.13, 615.69, and 1013.10 respectively in periods 1, 2 and 3 (Table 1), respectively. The pre-grazing height was 21.57, 35.14, and 32.14 cm for periods 1, 2, and 3, respectively (Table 1). The post-grazing height (cm) were 10.26, 13.42, and 15.33 for period 1, 2, and 3, respectively (Table 1).

Intake and digestibility parameters were not affected by breed ( $P > 0.05$ ; Table 3). The crude protein intake (CPI), ether extract intake (EEI) were the greater in the first period and decreased in the second and third periods for all breeds ( $P < 0.05$ ; Table 3). The non-fibrous carbohydrates intake (NFCI) was greater in Period 3 when compared to the first and second

periods (Table 3). A breed  $\times$  period interaction was observed for non-fibrous carbohydrate digestibility (NFC) ( $P < 0.05$ , Table 3), and Holstein animals had greater digestibility in Periods 1 and 2 (Figure 3), however this difference in NFC did not affect intake or performance ( $P > 0.05$ ).

The ADG was not affected by breed ( $P > 0.05$ ), and HOL and HG had ADG of 1.03 and 1.17 kg/d, respectively (Table 4). The ADG and body length gain were greater in the first period when compared with the second and third periods ( $P < 0.05$ , Table 4). The body measurements changes were also not affected by breed ( $P > 0.05$ ). Wither and rump weight gains were neither affected by breed nor period ( $P > 0.05$ ). The rump width gain (RWG) was greater ( $P < 0.05$ ) in the first and second periods when compared to the third period (Table 4). Despite the analysis of variance found a significant period effect on thoracic perimeter gain (TPG) ( $P < 0.05$ ; Table 4), the least square mean Student's test could not identify difference among periods for RWG ( $P > 0.05$ ). The carcass parameters were also not affected by breed ( $P > 0.05$ ; Table 4).

No breed effect was observed on IGF-1 concentrations ( $P < 0.05$ ; Table 5). We also did not observe breed effect ( $P < 0.05$ ) on serum levels of Glucose, serum urea nitrogen (SUN), triglycerides, total protein, and albumin (Table 5). SUN levels were greater in Period 2 when compared with other periods ( $P < 0.05$ ; Table 5). Furthermore, we observed a breed  $\times$  period interaction ( $P < 0.05$ ) for the serum albumin level, and HG heifers had a greater albumin level in Period 3 (Table 5).

Microbial protein yield (g/d) and efficiency of nitrogen use (ENU, g/g) were not affected by breed ( $P < 0.05$ ; Table 5). In addition, the microbial protein yield was greater in Period 1 when compared with Period 3 ( $P < 0.05$ ; Table 5). ENU was greater in Period 2 when compared with other periods ( $P < 0.05$ , Table 5). No breed effect was observed on rumen pH or ammonia concentration ( $P > 0.05$ , Table 5).

A breed x period interaction ( $P < 0.05$ ) was observed for grazing time ( $P = 0.04$ , Table 6), and HOL heifers had a greater grazing time in Period 3 but did not differ from HG in the first and second periods (Figure 4). Breed did not influence the grazing daily meal time (min/d,  $P > 0.05$ ; Table 6). The frequency of grazing was greater for HOL heifers (8.03 events/day) when compared with HG (7.71 events/day) ( $P < 0.05$ ; Table 6). The grazing meal duration was greater for HG heifers (179.71 min/day) when compared with HOL (130.73 min/day) ( $P < 0.05$ , Table 6). The meal criteria (min) was affected by breed, and it was greater for HG (59.58) when compared with HOL heifers (32.74; Table 6).

The ruminating time was not affected by breed ( $P > 0.05$ ; Table 6). However, rumination time increased from Period 1 to Period 2, and from Period 2 to Period 3 ( $P < 0.05$ ; Table 6). The ruminating time (min/d) was not affected by breed, but a significant reduction ( $P < 0.05$ ) was observed in Period 3 when compared with Periods 1 and 2 (Table 6). The ruminating frequency (events/d) was not affected by breed. In addition, there was a reduction in ruminating frequency ( $P < 0.05$ ) in Period 3 when compared with Period 1 (Table 6). We also did not observe a breed effect on ruminating duration (min/d) ( $P > 0.05$ ). There was a reduction in the meal duration in Period 3 when compared with the first two periods ( $P < 0.05$ ; Table 5). Lastly, we did not observe breed or period effects on ruminating criteria (min) ( $P > 0.05$ ; Table 6).

A breed x treatment interaction was observed for resting time ( $P < 0.05$ ; Table 6), and HOL heifers had greater resting time in Period 1 (Figure 4) when compared with HG heifers. No differences between breeds were observed in the following periods. In addition, we observed a breed effect ( $P < 0.05$ ) on bite rate, and HOL heifers (20.01 min/day) had a greater bite rate when compared with HG heifers (15.3 min/day; Table 6).

## **DISCUSSION**

Previous literature data suggests that Girolando crossbred (Holstein × Gyr) animals are more adapted to tropical conditions than purebred animals such as Holstein-Friesian (Santos et

al., 2012), and usually are related to greater production and reproduction outcomes in grazing systems in tropical areas (Madalena, 1996; Santana, Jr et al., 2015; Weller et al., 2016). However, all those studies were conducted with tropical forages during warmer months (Guimarães et al., 2002; Santana, Jr et al., 2015). Nevertheless, due to reported greater grazing ability of HG animals (Madalena, 1996; Santos et al., 2012), 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, in this study, breed did not influence performance, intake, metabolism, or digestibility of the animals. These results indicate that weather conditions during winter in tropical countries are adequate for HOL heifers grazing temperate pastures (Figure 1).

Regarding performance data, previous studies (Paiva et al., 1992; Flôres et al., 2004) reported that increasing Holstein-Friesen genetic composition on crossbred herds is related to a reduction on the ADG of replacement heifers, when grazing tropical forages during the summer time, which was linked mostly with reduced pasture intake (West, 2003). In a recent study, Morais et al. (2018b) verified that HOL heifers had lower ADG compared with HG when grazing intensively managed tropical pastures. Nevertheless, Rutter et al. (2002) showed that Holstein-Friesian heifers grazing ryegrass or white clover pastures had adequate performance in temperate conditions weather, which was similar to the performance of our animals.

In any replacement heifers program, nutritional strategies aim at shortening age at first calving through heifers' performance manipulation, which is also linked to increased profitability (Hoffman et al., 1996; Ettema and Santos, 2004; Meyer et al., 2014). Furthermore, heifers with ADG between 750 to 1.17 g/d would reach first calving at 24 months with adequate BW (Place et al., 1998; Heinrichs et al., 2017). Nevertheless, McManus et al. (2008) demonstrated that in HG heifers, BW was not the only trait associated with puberty. Thus, in HG replacement heifers' programs, a performance strategy should also be followed by a

reproduction strategy to shorten age at puberty. In our study, although all HOL animals reached puberty at the end of the trial (visual heat observation, *data not shown*), only one HG heifer demonstrated heat. Therefore, future studies relating performance and reproduction efficiency in HG animals are warranted.

A reduction in DMI by HOL heifers grazing tropical pastures during summer time 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 forage allowance (kg DM pasture/kg BW) is mainly influenced by the grazing pressure, that is the relationship between animal weight and forage mass per area. Thus, the greater the grazing pressure, the lower the forage allowance (Mccartor and Rouquette, 1997; 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 lower accumulate herbage per BW (Table 1). On the other hand, in the third period, there was an adequate pasture re-growth and herbage accumulation, that contributed to lower grazing pressure, increasing the forage allowance (Table 1). 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% (Table 1) indicates that the animals consumed pasture beyond the programmed post-grazing height goal. When evaluating the effect of intensive grazing, Dale et al., (2017) showed that the greater the herbage allowance the lower the grazing efficiency (Table 1). Hence, the decrease in forage allowance (kg DM of pasture/BW) in Period 2 resulted in the greater grazing efficiency (114%), which might be due to the lower forage allowance (Table 1) in this period.

Although total and pasture DMI was not affected by breed or period (Table 3), CP intake was greater in period 1 when compared with other periods. This greater intake was likely

caused by the better forage quality in Period 1 (Table 2), which provided greater CP supply and lower NDF content. Likewise, the greater EE intake was observed in the first period was due to greater pasture intake (Table 3). Lastly, the increase in NFCI in Periods 2 and 3 was associated with an increase in forage NFC during these periods (Table 2). The greater DMI (g / kg BW) in Period 1 may be associated with the greater pasture allowance in that period (Table 2), which is the main driver of pasture intake (Vazquez and Smith, 2000). Although the performance data showed good performance during all periods, notably the heifers had some extent of feed restriction in periods 2 and 3 (Table 1). Thus, we speculate that both HG and HOL could have had even greater performance in case of higher forage allowance. In this sense, future studies should focus on greater forage allowance during winter times in tropical countries to verify this hypothesis.

Although we observed similar performance between breeds, the grazing behavior was affected. HOL heifers had higher grazing frequency, which was likely associated with their lower meal criteria and meal duration (Table 6). Tolkamp et al. (1998) showed that lower meal criteria induced greater meal frequency in dairy cows. Therefore, the greater frequency of grazing could be compensation of the lower meal duration of HOL heifers, once both breeds had the same grazing time (Table 6). In this sense, the low winter temperatures (Figure 1) affected some behavior parameters of HOL heifers, but grazing time was not different from HG heifers. Nevertheless, Rutter et al. (2002) observed a grazing time of HOL heifers of 436 h/d in England, which is still 19% greater than the average grazing time of our animals. We speculate that the addition of PMR in the diet reduced the grazing time of the animals. In addition, the breeds had the same pattern of grazing distribution throughout the day (Figure 5a), which is a higher grazing frequency during the day, as observed by Olivo et al. (2006). Lastly, the higher bite rate (15% lower, min/d) of HOL compared to HG (20.0 vs 17.3) might be linked to the greater meal duration of HG heifers, which spend a greater time their meal to achieve the same

DMI (Table 6). 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 to 69 min/d. In summary, the grazing behavior of HG was characterized by the lower number of longer periods of grazing with lower bite rates, and the grazing behavior of HOL was characterized by a greater number of short periods of grazing with a higher bite rate.

Although it has been previously suggested that grazing behavior has a direct effect on the rumination process (Yang et al., 2001; Nasrollahi et al., 2016; Beauchemin, 2018), this effect was not translated into changes in rumination patterns between HOL and HG heifers in the present study. Additionally, the ruminating frequency of animals (Figure 5b) is consistent with those presented by Zanine et al. (2002), 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 pasture when compared to temperate pastures.

#### *Considerations about pasture management during winter time in tropical areas*

Our objective was to evaluate the performance, behavior, and metabolism of HOL and HG heifers grazing temperate pasture during the winter season in a tropical climate (Brazil). We expected a pasture DM production/d of at least 90.8 kgDM/paddock (Carvalho et al., 2010) however, this DM production was not achieved. Therefore, the decreasing of accumulated herbage in the second period might be associated to the overgrazing observed in the first period, which might have limited regrowth and pasture DM production in Period 2 (Brougham, 1957; Lee et al., 2008), and may be confirmed by the lower pre- and post-grazing height (10.26 cm) in period 1 compared to the pre-defined pre- and post-grazing heights of 30 and 15 cm, respectively (Table 1).

The 25 paddocks designed in this study were based on a previous study with black oats and ryegrass, where Tonato et al. (2014) observed that 25 days was sufficient to completely pasture regrowth. The increase in the accumulation herbage in the third period was likely associated with an adequate PosGH (13.42 cm) on Period 2 (Table 1). Therefore, further studies should focus on determining the adequate grazing intervals and management practices of a mixture of black oats and ryegrass during winter time in tropical areas (Brazil).

## **CONCLUSIONS**

In summary, HOL and HG heifers grazing intensively managed temperate-season pasture had similar intake, digestibility, performance, and metabolic parameters. Although they presented slight differences in ingestive behavior, primarily in grazing characteristics, these effects did not affect performance or intake parameters. Therefore, HOL and HG replacement heifers managed under winter pasture in tropical countries have adequate and similar performance.

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## **Ethics statement**

This research was submitted to the Ethics Committee for Production Animals of the Federal University of Viçosa and was approved by of CEUAP/UFV.

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## TABLES CAPTIONS

*Table 1 Characteristics of herbage during experiment periods*

Item	Period			SEM
	1	2	3	
Accumulated herbage (kg DM/ha/cycle)	913.23	615.69	1013,10	206.69
Accumulated herbage (kg DM/paddock/cycle)	52.69	35.52	58.45	13.50
Herbage allowance (kg DM/animal/day)	5.26	3.55	5.84	1.32
Forage allowance (kg DM of pasture/kg BW)	0.35	0.21	0.31	0.07
Grazing efficiency <sup>1</sup> (%)	105	114	81	17.05
PreGH <sup>2</sup> (cm)	21.57	35.44	32.14	7.24
PosGH <sup>3</sup> (cm)	10.26	13.42	15.33	2.56

<sup>1</sup>Calculate as = total pasture DMI (sum of all animals)/ accumulate herbage (kg/paddock/cycle) (Sollenberger et al., 2005)

**Table 2** Chemical composition (DM basis) of pasture, corn silage, and corn meal during experimental periods

Item	Pasture			Corn silage			Finely ground corn		
	Period			Period			Period		
	1	2	3	1	2	3	1	2	3
DM (%)	18.07	17.88	17.63	28.09	27.35	31.02	92.22	93.14	92.20
NDF <sup>1</sup>	39.47	44.40	43.21	46.75	48.64	47.61	16.62	12.12	14.12
iNDF <sup>2</sup>	5.39	9.18	10.10	21.29	23.77	19.65	2.08	1.54	2.04
CP <sup>3</sup>	30.37	24.27	21.48	7.54	7.26	6.69	6.74	6.38	8.71
EE <sup>4</sup>	4.45	3.33	3.47	2.43	1.85	2.43	3.84	4.43	4.13
Ash	12.25	11.47	10.42	5.34	5.31	7.2	1.70	1.86	1.78
NFC <sup>5</sup>	13.46	16.53	21.42	37.94	36.94	36.07	71.10	73.67	71.26
Mineral composition <sup>6</sup>	-	-	-	-	-	-	-	-	-

<sup>1</sup> Neutral detergent fiber.

<sup>2</sup> Indigestible neutral detergent fiber.

<sup>3</sup> Crude protein.

<sup>4</sup> Ether extract.

<sup>6</sup> Mineral composition: salt – 49.66 g/kg; dicalcium phosphate – 47 g/kg; limestone - 1,71 g/kg; zinc sulphate – 7,25 mg/kg; ferrous sulphate – 4,05 mg/kg; copper sulphate – 2,39 mg/kg; manganese sulphate – 2,15 mg/kg; cobalt sulphate – 0,2 mg/kg; sodium sulphate – 0,16 mg/kg; potassium iodate 0,08 mg/kg

**Table 3** Intake and digestibility of nutrients of Holstein and Holstein x Gyr heifers grazing temperate forages.

Item	Breed		Period			SEM	P-value		
	HOL	HG	1	2	3		Breed	Per	Breed×Per
<i>Intake, kg or Mcal/d</i>									
DM <sup>1</sup> , kg/day	6.98	7.43	7.82	6.57	7.23	0.94	0.737	0.260	0.766
Pasture <sup>2</sup> , kg/day	4.57	5.01	5.59	4.07	4.74	0.89	0.739	0.149	0.780
CP <sup>3</sup> , kg/day	1.34	1.47	1.85 <sup>a</sup>	1.16 <sup>b</sup>	1.20 <sup>b</sup>	0.23	0.714	0.004	0.720
NDF <sup>4</sup> , kg/day	2.81	2.99	3.06	2.70	2.94	0.36	0.739	0.611	0.800
EE <sup>5</sup> , kg/day	0.24	0.26	0.31 <sup>a</sup>	0.20 <sup>b</sup>	0.24 <sup>b</sup>	0.03	0.718	0.005	0.698
NFC <sup>6</sup> , kg/day	1.95	2.02	1.81 <sup>b</sup>	1.93 <sup>a</sup>	2.22 <sup>a</sup>	0.18	0.787	0.033	0.825
ME <sup>7</sup> , Mcal/day	20.95	20.79	23.83	18.89	19.90	2.91	0.968	0.275	0.761
<i>Intake, g/kg of BW</i>									
DM <sup>1</sup>	2.360	2.42	2.83 <sup>a</sup>	2.14 <sup>b</sup>	2.19 <sup>b</sup>	0.291	0.890	0.035	0.816
Pasture <sup>9</sup>	1.53	1.64	2.01 <sup>a</sup>	1.31 <sup>b</sup>	1.43 <sup>b</sup>	0.28	0.799	0.038	0.830
NDF <sup>4</sup>	0.95	0.97	1.11	0.88	0.89	0.12	0.890	0.095	0.844
<i>Digestibility, g/kg</i>									
DM <sup>1</sup>	646.2	635.6	683.4	597.2	642.3	0.02	0.758	0.053	0.897
CP <sup>3</sup>	698.4	676.4	748.4	652.4	661.4	0.02	0.553	0.129	0.749
NDF <sup>4</sup>	611.8	594.6	639.7	595.3	574.6	0.01	0.472	0.101	0.912
EE <sup>5</sup>	597.0	535.3	625.9	517.6	555.0	0.03	0.136	0.102	0.628
NFC <sup>6</sup>	862.6	807.9	853.3	800.6	851.8	0.02	0.112	0.185	0.004

<sup>1</sup> Total Dry Matter.

<sup>2</sup> Crude Protein.

<sup>3</sup> Neutral Detergent Fiber.

<sup>4</sup> Ether Extract.

<sup>5</sup> Non-Fibrous Carbohydrates.

<sup>6</sup>Metabolizable Energy.

<sup>a,b,c</sup> Values within a row with different superscripts differ significantly at  $P < 0.05$ .

**Table 4** Performance, body and carcass measurements of Holstein and Holstein x Gyr heifers grazing temperate forages.

Item	Breed		Period			SEM	P-value		
	HOL	HG	1	2	3		Breed	Period	Breed×Per
ADG <sup>1</sup> , kg/d	1.03	1.17	1.46 <sup>a</sup>	0.98 <sup>b</sup>	0.87 <sup>b</sup>	0.050	0.077	<0.001	0.709
WHG <sup>2</sup> , cm/d	0.11	0.11	0.09	0.12	0.11	0.006	0.777	0.600	0.427
RHG <sup>3</sup> , cm/d	0.08	0.07	0.08	0.09	0.05	0.011	0.662	0.204	0.271
BLG <sup>4</sup> , cm/d	0.39	0.27	0.04 <sup>a</sup>	0.02 <sup>b</sup>	0.03 <sup>b</sup>	0.054	0.139	<0.001	0.334
RWG <sup>5</sup> , cm/d	0.22	0.22	0.29 <sup>a</sup>	0.25 <sup>a</sup>	0.11 <sup>b</sup>	0.025	0.802	0.004	0.881
TPG <sup>6</sup> , cm/d	0.24	0.19	0.25	0.19	0.20	0.062	0.601	0.033	0.428
Ribeye area, cm×cm	11.58	10.94	-	-	-	2.121	0.836	-	-
Loin Depth, cm	7.58	7.26	-	-	-	2.788	0.937	-	-
Back fat thickness, mm	0.36	0.33	-	-	-	0.212	0.923	-	-
Ribeye fat thickness, mm	0.52	0.53	-	-	-	0.233	0.962	-	-

<sup>1</sup> Average Daily Gain.

<sup>2</sup> Withers Height Gain.

<sup>3</sup> Rump Height Gain.

<sup>4</sup> Body Length Gain.

<sup>5</sup> Rump Width Gain.

<sup>6</sup> Thoracic Perimeter Gain.

<sup>a,b</sup> Values within a row with different superscripts differ significantly at P<0.05.

**Table 5** Metabolic, ruminal, and nitrogen use parameters of Holstein and Holstein x Gyr heifers grazing temperate forages.

Item	Breed		Period			SEM	P-value		
	HOL	HG	1	2	3		Breed	Period	Breed×Per
IGF-1 (ng/dL)	290.47	303.53	277.10	293.10	320.80	23.84	0.708	0.293	0.916
Glucose (mg/dL)	82.47	82.13	86.67	82.56	77.66	3.87	0.950	0.386	0.707
SUN <sup>1</sup>	35.22	38.55	35.43 <sup>b</sup>	43.07 <sup>a</sup>	32.13 <sup>b</sup>	1.93	0.231	0.009	0.094
Triglicerydes (mg/dL)	27.72	27.33	31.46 <sup>a</sup>	24.69 <sup>b</sup>	26.43 <sup>ab</sup>	1.44	0.850	0.064	0.712
Total protein (g/dL)	7.10	6.70	6.73	6.93	7.03	6.39	0.141	0.507	0.064
Albumina (g/dL)	3.16	3.26	3.14 <sup>b</sup>	3.19 <sup>ab</sup>	3.28 <sup>a</sup>	7.56	0.239	0.003	0.009
Microbial protein (g/d)	962.73	1078.33	1209.77 <sup>a</sup>	1126.83 <sup>ab</sup>	724.99 <sup>b</sup>	168.06	0.624	0.020	0.289
Nitrogen efficiency use (%)	0.74	0.68	0.66 <sup>b</sup>	0.87 <sup>a</sup>	0.58 <sup>b</sup>	0.05	0.393	0.019	0.440
Ruminal pH (%)	7.21	7.29	-	-	-	0.08	0.491	-	-
Ruminal ammonia (mg/dL)	13.55	12.48	-	-	-	1.711	0.667	-	-

<sup>1</sup> Serum urea nitrogen.

<sup>a,b</sup> Values within a row with different superscripts differ significantly at P<0.05

**Table 6** Behavior activities of Holstein and Holstein x Gyr heifers grazing temperate forages.

Item <sup>1</sup>	Breed		Period			SEM	P-value		
	HOL	HG	1	2	3		Breed	Period	Breed×Per
Grazing characteristics									
<i>Grazing time, min/d</i>	376.44	356.44	412.33	318.00	369.00	14.02	0.342	<0.001	0.040
<i>Daily meal time, min/d</i>	1005.83	935.78	893.00	1040.42	979.00	38.517	0.202	0.101	0.385
<i>Grazing frequency, events/d</i>	8.03	7.71	7.10	6.28	7.23	0.610	0.037	0.425	0.825
<i>Meal duration, min/d</i>	130.73	179.70	137.87	177.33	150.44	9.64	0.040	0.217	0.941
<i>Grazing criteria min/d<sup>1</sup></i>	32.74	59.58	39.07	50.33	43.83	0.076	0.022	0.708	0.751
Ruminating characteristics									
<i>Ruminating time, min/d</i>	372.22	405.56	347.00 <sup>c</sup>	380.67 <sup>b</sup>	439.00 <sup>a</sup>	13.754	0.119	<0.001	0.140
<i>Daily ruminating time, min/d</i>	560.96	440.24	678.03 <sup>a</sup>	632.43 <sup>a</sup>	191.33 <sup>b</sup>	50.849	0.119	<0.001	0.584
<i>Ruminating frequency, events/d</i>	3.77	3.76	4.87 <sup>a</sup>	3.83 <sup>ab</sup>	2.60 <sup>b</sup>	0.375	0.974	0.006	0.066
<i>Ruminating duration, min/d</i>	125.75	114.87	134.73 <sup>a</sup>	151.22 <sup>a</sup>	74.98 <sup>b</sup>	9.64	0.442	<0.001	0.070
<i>Ruminating criteria, min/d<sup>1</sup></i>	76.18	83.53	79.77	85.47	74.44	0.087	0.753	0.924	0.302
Resting time, min/d	690.89	683.78	688.33	741.67	632.00	20.91	0.816	<0.001	0.018
Bite rate, min/d	20.01	17.30	19.92	17.20	18.50	0.688	0.010	0.091	0.148

<sup>1</sup> Meal criteria (min/d) calculated as 10<sup>^</sup>meal criteria.

<sup>a,b,c</sup> Values within a row with different superscripts differ significantly at P<0.05

## FIGURE CAPTIONS

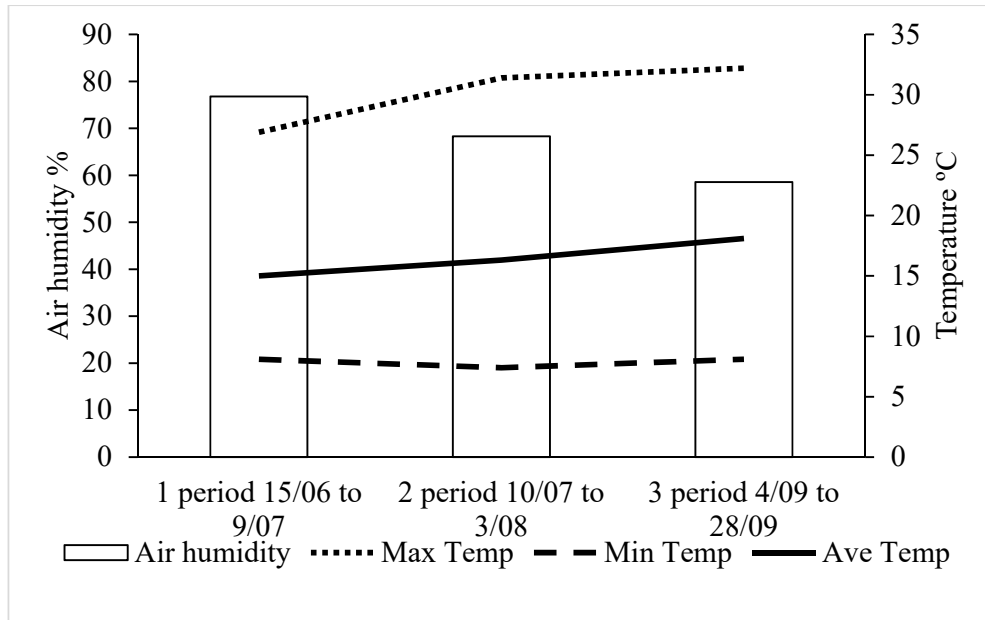
**Figure 1:** Air humidity (%), minimum, average, and maximum (°C) temperatures during three experimental periods where Holstein and Holstein × Gyr heifers grazed temperate forages

**Figure 2:** Examples of the expected frequency distribution of the intervals between grazing (A,B) and ruminating (C,D) events of Holstein (B,D) and Holstein x Gyr (A,C) heifers grazing temperate forages fitted with a mixed distribution model. The intersection between curves was used as meal criteria to calculate total daily meal time, meal frequency and meal duration.

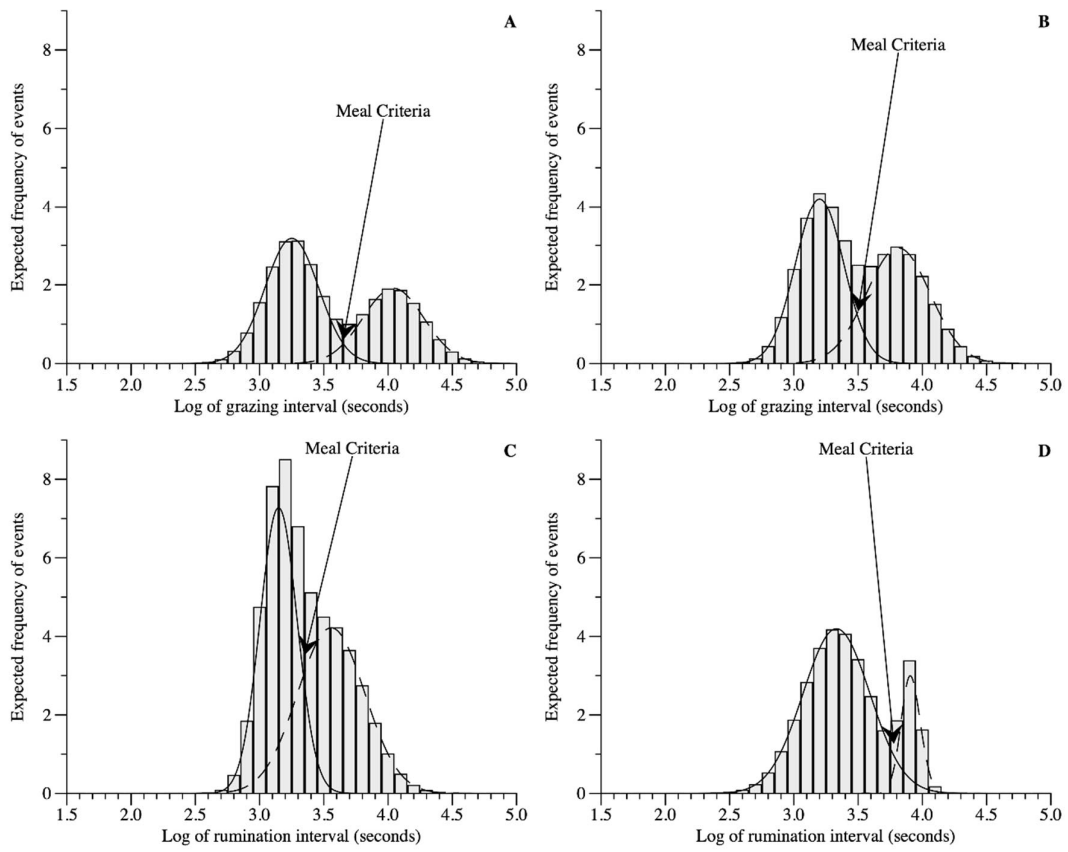
**Figure 3:** Digestibility of non-fibrous carbohydrates of Holstein ( ■ ) and Holstein × Gyr ( - ○ - ) heifers grazing temperate forages. Periods marked with \* indicate a difference between breeds at  $P < 0.05$ .

**Figure 4:** Grazing (A), ruminating (B), and resting (C) times of Holstein ( ■ ) and Holstein × Gyr ( - ○ - ) heifers grazing temperate forages. Periods marked with \* indicate the difference between breeds at  $P < 0.05$ .

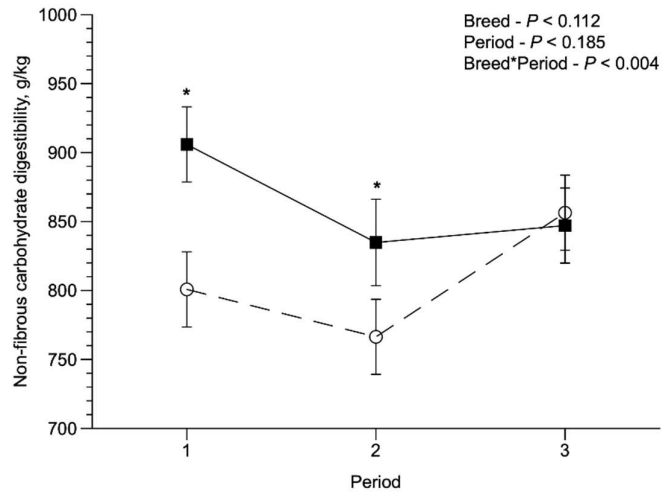
**Figure 5:** Percentage of Holstein (red lines) or Holstein × Gyr (black line) grazing (A) or ruminating (B) obtained through the average of three periods of 72 hours of observation.



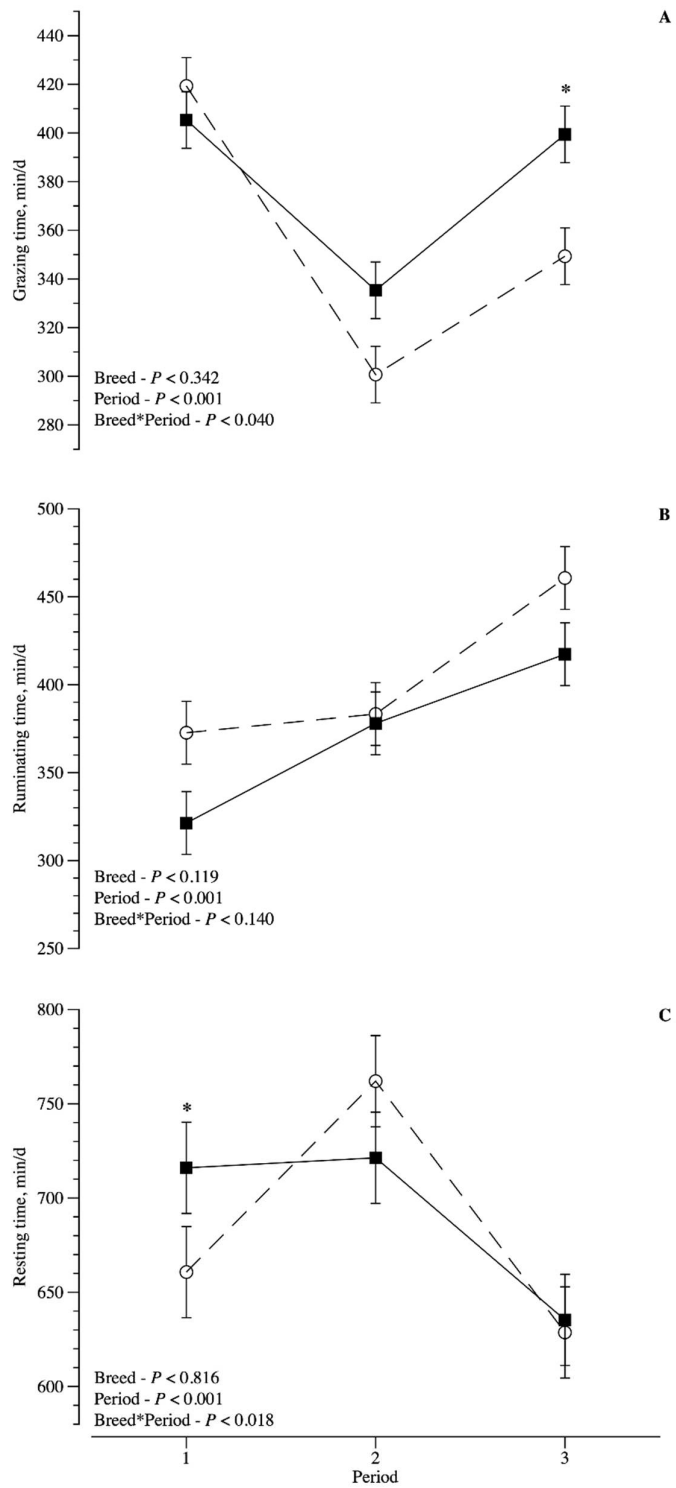
**Figure 1: Abreu et al.**



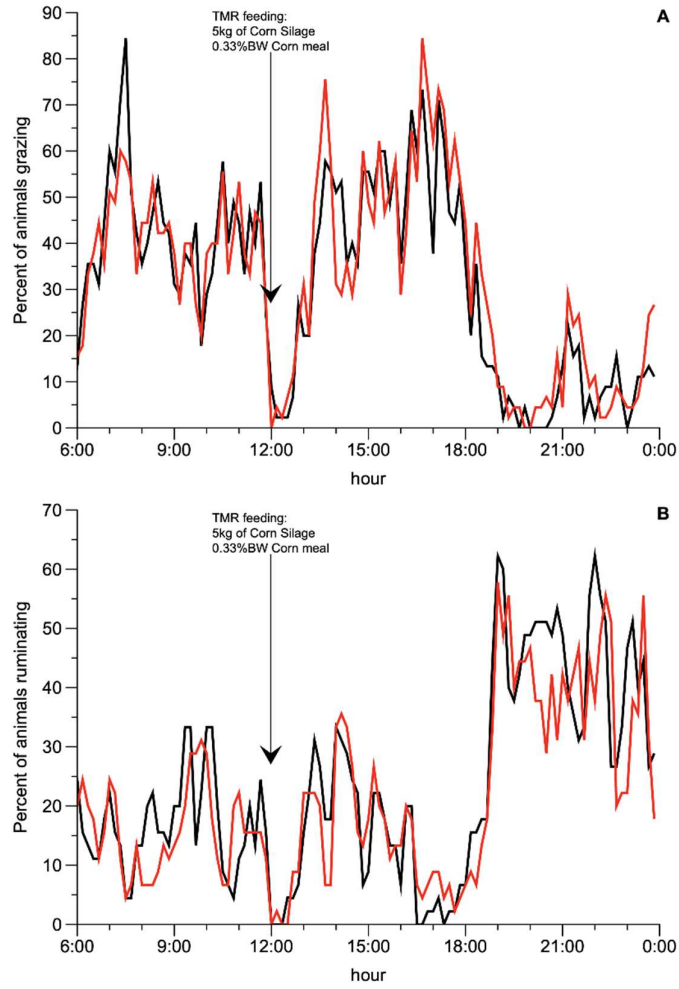
**Figure 2: Abreu et al.**



*Figure 3: Abreu et al.*



**Figure 4: Abreu et al.**



*Figure 5: Abreu et al.*