DAIANA FRANCISCA QUIRINO

BEHAVIOR, PERFORMANCE, AND TICK INCIDENCE IN GIROLANDO AND HOLSTEIN GRAZING HEIFERS

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

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Marcos Inácio Marcondes
(Co-adviser)

Luciana Navajas Rennó
(Co-adviser)

Alex Lopes da Silva

Camila Soares Cunha

Polyana Pizzi Rotta (Adviser)

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BIOGRAPHY

DAIANA FRANCISCA QUIRINO VILLANOVA, daughter of Wander Clécio Peres Quirino and Irani Auais Campos Quirino was born in Antônio Carlos, MG-Brazil on September 16, 1984.

Villanova, started bachelor's degree in Agronomy at Instituto Federal de Educação Ciência e Tecnologia Campus Barbacena in 2012 and obtained Bachelor of Science degree in February of 2017. In March of 2017, Villanova started the Master Science Program in Animal Science at Universidade Federal de Viçosa.

On February 25th of 2019, Villanova defended hers Master's dissertation to obtain the Magister Scientiae degree in Animal Science.

ABSTRACT

VILLANOVA, Daiana Francisca Quirino, M.Sc. Universidade Federal de Viçosa, February, 2019. **Behavior, performance, and tick incidence in Girolando and Holstein grazing heifers**. Adviser: Polyana Pizzi Rotta. Co-advisers: Marcos Inácio Marcondes, Luciana Navajas Rennó and Simone Eliza Facioni Guimarães.

Performance studies on young dairy cattle in tropical pastures are still deficient in literature, mainly studies comparing different breeds. The aim of this study was to evaluate the behavior, performance and tick incidence in Girolando and Holstein heifers in intermittent grazing system in Guinea grass (*Panicum maximum* Jacq. cv Mombaça). Sixteen heifers were divided in two groups according to body weight (BW), one with 258.6 ± 24.79 kg and other with 157.1 ± 24.99 kg. Each group consisted of 4 Girolando (½ Holstein × ½ Gyr) and 4 Holstein heifers. The experimental period lasted 84 d subdivided into 4 periods. To estimate the average daily gain (ADG), animals were weighing at beginning and at end of experiment. The counting and collection of ticks were out carried each period. Behavior analyzes were performed during 48 h with observations every 10 min. The behavioral variables considered were: ruminating, grazing, resting, and concentrate intake time. For digestibility analyzes, titanium dioxide was used to estimate concentrate intake, chromic oxide to estimate fecal excretion and FDNi as internal marker to estimate forage intake. Samples were collected to estimate dry matter (DM), crude protein (CP), ether extract (EE), ashes, neutral detergent fiber (NDF) and indigestible NDF (iNDF). Blood samples were taken at the end of experiment to evaluate the blood concentrations of glucose (G), urea (U), total protein (TP), albumin (Alb), insulin-like growth factor 1 (IGF- 1), triiodothyronine (T3), and thyroxine (T4). Statistical analyzes were performed using the GLIMMIX SAS procedure and differences were considered when P values <0.05, and values 0.05 < P < 0.10 were considered as trend. Differences were observed for forage intake (FDMI) and NDF intake, both with greater values to Girolando heifers. We observed a trend to greater concentrate intake to Holstein heifers. Holstein animals tended to have a greater DMI/BW. We observed a trend of greater digestibility of CP and NDF to Girolando heifers. The ADG was greater to Girolando heifers, as well as feed efficiency. The rumination time was greater to Girolando heifers, while resting time was greater to Holstein heifers. The concentrate intake time was also greater for Holstein heifers. The tick count and weight was greater for Holstein. Blood concentrations of U, Alb, IGF-1, T3 and T4 were greater to Girolando heifers. The greatest Girolando animals' performance might be related to better nutrients use from forage due greater forage

intake and greater rumination time. It is possible that Holstein animals suffered heat stress, impairing their performance.

RESUMO

VILLANOVA, Daiana Francisca Quirino, M.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Comportamento, desempenho e incidência de carrapatos em novilhas Girolando e Holandês a pasto.** Orientadora: Polyana Pizzi Rotta. Coorientadores: Marcos Inácio Marcondes, Luciana Navajas Rennó e Simone Eliza Facioni Guimarães.

Estudos sobre o desempenho de novilhas leiteiras em pastagens tropicais são escassos, principalmente comparando diferentes raças. Dessa forma, esse trabalho teve por objetivo avaliar o comportamento, desempenho e incidência de carrapatos em novilhas Girolando e Holandês em sistema de pastejo intermitente em capim Mombaça (*Panicum maximum* Jacq. cv. Mombaça). Dezesseis novilhas foram divididas em dois grupos de acordo com o peso corporal (PC), um grupo com 258,6 ± 24,79 kg e outro com 157,1 ± 24,99 kg. Cada grupo era composto por 4 animais Girolando (½ Holstein × ½ Gir) e 4 Holandês. O período experimental foi de 84 dias subdividido em 4 períodos de 21 dias cada. Para calcular o ganho médio diário (GMD) os animais foram pesados no início e final do experimento. A contagem e coleta de carrapatos foram realizadas a cada período. As análises de comportamento foram realizadas durante 48 h com observações a cada 10 min, a cada período. As variáveis comportamentais consideradas foram ruminação, pastejo, ócio e consumo de concentrado. Para os cálculos de digestibilidade foram utilizados dióxido de titânio para estimar o consumo de concentrado, óxido crômico para estimar a produção fecal e FDNi como indicador interno para estimar o consumo de forragem. Amostras dos alimentos e amostras *spot* de fezes foram coletadas para estimar os teores de matéria seca (MS), proteína bruta (PB), extrato etéreo (EE), matéria mineral (MM), fibra insolúvel em detergente neutro (FDN) e fibra indigestível em detergente neutro (FDNi). Coletas de sangue foram realizadas ao final do experimento para avaliar as concentrações sanguíneas de glicose (G), ureia (U), proteína total (PT) e albumina (Alb), e dos hormônios fator de crescimento semelhante à insulina tipo 1 (IGF-1), triiodotironina (T3) e tiroxina (T4). Análises estatísticas foram feitas utilizando o procedimento GLIMMIX do SAS e as diferenças foram consideradas quando valores de $P < 0,05$, e valores $0,05 < P < 0,10$ foram considerados como tendência. Foram observadas diferenças para o consumo de forragem (CFMS) e para o consumo de FDN ambos com maiores valores para novilhas Girolando. Foi observada tendência de maior consumo de concentrado para novilhas Holandesas. Os animais Holandeses apresentaram tendência de maior CMS/PC. Ainda, tendência de maior digestibilidade da PB e FDN foram observadas para novilhas Girolando. O GMD foi maior para novilhas Girolando, assim como a eficiência alimentar. O tempo de

ruminação foi mais elevado para as novilhas Girolando enquanto o tempo em ócio foi superior para as novilhas Holandesas. O tempo de consumo de concentrado também foi maior para novilhas Holandesas. A contagem e pesagem de carrapatos foram maiores para novilhas Holandesas. As concentrações sanguíneas de U, Alb, IGF-1, T3 e T4 foram mais elevadas para novilhas Girolando. O maior desempenho dos animais Girolando pode estar relacionado à melhor utilização dos nutrientes da forragem devido ao seu maior consumo e maior tempo de ruminação. É possível que animais da raça Holandesa tenham sofrido estresse térmico.

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INTRODUCTION

Dairy heifers are very important to maintain the farm's productivity mainly due their cattle replacement role (Paciullo et al., 2011). Performance of young dairy cattle may be influenced by quantity and quality of feed provided as well as environmental conditions, and genetic characteristics (Garcia et al., 2011). In tropical climate regions the pasture has been used as the main animals' feed source due to the diversity of species and forage productive potential. However, it is necessary intensify these systems by improving the land use and consequently mitigate environmental impacts (Dini et al., 2012; Macdonald et al., 2017).

In tropical conditions solar irradiation, high air humidity and temperatures are associated to an inadequate feeding management which may impair animal welfare and production, being the main results the low ADG and consequently elevated age at first calving (Paciullo et al., 2011). Additionally, infestations by ectoparasites as bovine tick, *Rhipicephalus* (*Boophilus*) *microplus* may impact the performance of adult's animals due to secondary illness caused by them representing threats to herd production (Léger et al., 2013).

Crossbred animals are commonly used in pasture-based dairy systems in tropical and subtropical areas (Mellado et al., 2011; Washburn and Mullen, 2014). Holstein and Gir crosses combining the Holstein milk production and Gir adaptability and hardiness (Fraga et al., 2016) representing desirable productive characteristics in warm climates (Santos et al., 2011). However, the genetic of Holstein and crossbreed animals have been modified over the years (Washburn and Mullen, 2014), and the mechanisms responsible for the different behavioral and performance responses of purebred or crossbreed young animals in tropical pasture-based systems still unclear.

Considering the statements above, further studies aiming to rear young animals in grazing systems should be developed to understand the performance responses of

Holstein and Girolando (Holstein × Gir) breeds. Additionally, by comprehensions of the mechanisms involved on these responses, it will be possible to determine the adequate management of forage species and animals, reducing the costs of the heifer's growth period and consequently increasing the farmer´s productivity.

The hypothesis of this study is that Girolando heifers will perform better in relation to Holstein heifers due greater grazing time and intake, and lower incidence of ticks. Thus, the aim of this study was to evaluate the performance and behavior of Holstein and Girolando heifers kept in an intermittent grazing system in Guinea grass (*Panicum maximum* Jacq. cv. Mombaça) and evaluate animals' tick infestation by counting.

MATERIALS AND METHODS

All procedures with use of animals were submitted to the Ethics Committee for Use of Animals of the Department of Animal Science of the Universidade Federal de Viçosa-MG and was approved under protocol number 24.

Location and Experimental Period

The experiment was conducted at Universidade Federal de Viçosa, southwest of Minas Gerais from December to April, during summer season in Brazil. The animals underwent an adaptation period to diets and management during 45 d prior the experiment. The experimental period lasted 84 d with four periods for collection with 21 d each (Figure 1).

Animals and Diets

Sixteen heifers were divided into two groups according to BW, one with 258.6 ± 24.79 kg and another group with 157.1 ± 24.99 kg. Each group was composed

by 4 Holstein and 4 Girolando (½ Holstein × ½ Gir) animals. Treatments considered in this study were the 2 breeds: Holstein and Girolando blocked according to BW.

Rotational grazing was conducted on 32 Guinea grass (*Panicum maximum* Jacq. cv. Mombaça) paddocks, using 1 d grazing period, and fertilized with 200 kg of N/hectare/year and 150 kg of K_2O/ hectare/year. The average pasture area was 800 m² and artificial shade of 60 m² in each paddock, which was equipped with feeders and drinking fountains. Distinct paddocks were used for each heifer's group to avoid competition during concentrate supply.

Each heifer group received daily supplementation, always at 1200 h. The supplement was based on soybean meal (22%), corn meal (75%) and urea + ammonium sulfate (3%). We offered a proportion of 0.5% BW on DM basis and updated according to animal weighing every 21 d. Mineral salt was offered to heifers *ad libitum*. Samples of feed that composed the concentrate were collected directly from animal's feed factory, prior mixing ingredients. The animals were driving to next paddock daily after the concentrate supply and its consumption.

Environmental Measurements

Data on weather conditions (average, maximum, and minimum temperatures, humidity, and rainfall) were obtained from Viçosa Weather Station (Table 1). The temperature humidity index (**THI**) was calculated using the average values of temperature and humidity, as described by Thom (1959):

$$\text{THI} = (\%RH/100) \times (T_a - 14.4) + (0.8 \times T_a) + 46.6$$

in which: THI = temperature humidity index; %RH = percent relative humidity; Ta = average air temperature in °C.

Performance and Tick Count

To estimate the ADG, we used the average of 3 initials BW and 3 final BW measurements as following described. Animals were weighed for 3 consecutive days at beginning and at the end of experiment (Figure 1). Heifers were removed from pasture at 1800 fasted for 12 h with access to water and weighed. After weighing, animals were placed in paddocks until the next time to fast.

On the first day of each period, intermediate weightings were conducted during the experiment to adjust the concentrate supply without fasted. At the same days, tick count was performed (Figure 1). Tick count was performed in the left side of the animal's body. Only the adult female ticks, with diameter of 4.5 mm or more were counted (Wharton and Utech, 1970) and taken from the animal and weighed using an analytical scale immediately after the end of the count. After countingticks, we multiplied the value by two (2 sides of the animal), which resulted an estimate of the total ticks per animal (Wharton and Utech, 1970). All animals received preventive treatment with anti-parasite in the pour-on form (Eprinex® Pour-On, Boehringer Ingelhein, São Paulo, São Paulo, Brazil), as recommended by manufacturer, after tick collection.

Animal Behavior

Behavior evaluation was done from d-8 to d-9 of each experimental period. Animals were simultaneously observed during 48 h (two days; Figure 1). The sampling routine with a sampling interval of 10 min (Martin and Bateson, 1993) was used for the following behavior categories: grazing, ruminating, resting, and concentrate intake time, totalizing 288 observations per animal in each period. The grazing behavior was considered when the animal was ingesting or selecting forage. The rumination activity occurs when the cud was being re-chewing without feed ingestion. Resting behavior

was considered when the animal was not feeding or ruminating and it was related to the non-occurrence of rumination and feeding activities and their occurrence was observed when the animal was standing (standing or lying down) or sleeping. The concentrate intake activity was considered when the animal was eating in the bunker.

As reported by Beauchemin (2018), feeding time differs from eating time because feeding time includes meals and inactivity periods while the animal is feeding, and eating time represents only time spent ingesting feed. Meals represents eating events with short interval within a meal, and the meal criteria is defined as the longest non-feeding interval within a meal (Bailey et al., 2012). The beginning of first feeding event until an interval between events was considered the meal duration (min/meal) (Kargar et al., 2018). In our study, we used behavior data collected every 10 min to calculate meal criteria. This method was used due to the collection data of grazing be visually realized, and was performed to do not interfere in animal natural behavior, which could occur if it was done every minute as found on literature to confined animals.

We used the methodology described by DeVries et al. (2003) to estimate meal criteria, meal time, meal frequency, and meal duration. Briefly, intervals between events (grazing or ruminating) were calculated and log10 transformed using Excel spreadsheets. Meal criteria was determined per animal per period as the point at which the distribution curve of intrameal intervals is intersected by distribution curve of intermeal intervals (Figure 2). To estimate meal criteria we used the statistical software R (ver. 2.13; R Foundation for Statistical Computing) and MIXDIST R package (http://www.math.mcmaster.ca/peter/mix/mix.html) as described by Bailey et al. (2012). Data from meal criteria were used to calculate meal frequency (meals/d) counting the interval numbers that exceeded the criterion and adding one. To calculate meal duration (min/meal) start time of first bout until end of last feeding bout were considered. Total meal time was calculated by sum of two meal durations described above.

Digestibility and Laboratorial Analysis

To estimate individual concentrate intake, titanium dioxide was used mixed to the concentrate immediately prior to delivery in the amount of 10 g of titanium dioxide per animal/d, at 1000 h (Figure 1). To estimate fecal excretion, the chromic oxide was used as marker. Chromic oxide was infused (packaged in paper), directly into the animal's esophagus with the aid of a probe, in the amount of 15 g per animal/d. To estimate forage intake we used iNDF as internal marker. Both chromium oxide infusion and titanium dioxide supply were done during 8 d for each period (from d-10 to d-18). Spot feces samples were taken during the last 3 infusion days (Figure 1) of each period at 0600, 1200, and 1800 h, totalizing 9 samples per animal, per period.

To estimate the composition and availability of grazing stratum, 2 isolation cages (1.0 × 1.5 m) were placed in paddocks before animals' entrance, in a representative place of pasture height and density (Figure 1). On following day after animals leaving each paddock, the average paddocks height was obtained in different 10 points using a graduated rule and the forage inside the cage was cut at the same height of the pasture consumed (Brandao et al., 2018). Aliquots of this material were withdrawn for bromatological analyzes.

Samples of forage and feces were pre dried in a forced-air drying oven at 55°C for 72 h. Concentrate, forage, and feces samples were grounded in a Willey mill (model TE-680, brand TECNAL, Piracicaba, São Paulo, Brazil) in 2 and 1 mm screen. The samples grounded in a 2 mm screen were used to analyze iNDF by the INCT-CA method F-008/ and samples grounded in a 1 mm screen were used to analyze DM by the INCT-CA G-003/1 method, CP by the INCT-CA N-001/1 method, EE by the INCT-CA G-004/1, ashes by the INCT-CA M-001/1, NDF by the INCT-CA F-001/1 method, as described by Detmann et al. (2012).

The total digestible nutrient (TDN) contents were estimated as described by Weiss, (1999):

$$TDN = dCP + 2.25 \times dEE + dNDF + dNFC$$

dCP = digestible crude protein; dEE = digestible ether extract; dNFC = digestible non fibrous carbohydrates ; dNDF = digestible neutral detergent fiber.

To estimate digestible energy (DE) in the diet, the digestible fraction of each component was multiplied by its respective caloric value (ARC, 1980):

$$DE = 5.6 \times dCP + 9.4 \times dEE + 4.2 \times dNFC + 4.2 \times dNDF$$

in which DE = digestible energy (Mcal/kg);

To estimate metabolizable energy, we used equation described by Galyean et al. (2016):

$$ME = 0.9611 \times DE - 0.2999$$

in which ME = metabolizable energy; DE = digestible energy. All terms were expressed as megacalories per kg of DM.

Blood Samples

Blood samples were collected on the last period (d-80; Figure 1), by jugular vein puncture, using vacuum tubes with clot activator and gel for serum separation (BD Vacutainer® SST® II Advance®, São Paulo, Brazil), which was identified and stored in ice until centrifugation (2,700×g for 20 min, 4°C). After centrifugation, the blood serum was separated and placed in micro-tubes (1.5 mL) and frozen at -20° C for further analysis. Blood samples were used to quantify urea (K056), glucose (K082), total protein (K031), albumin (K040), triglycerides (K117) that were evaluated by the automatic biochemical analyzer (Mindray, BS200E, Shenzhen, China) using Bioclin determination kits. The IGF-1 was analyzed by chemiluminnescence in the UniCell Dxl Access Imunoassay System (Beckman Coulter Inc., Brea, USA). In addition, total T3

(**T3**) and total T4 (**T4**) contents were analyzed by kits Beckman (ref. number 33830 and 33800 Beckman Coulter®, Brea, USA) in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA).

Statistical Analysis

Statistical analysis was performed using the GLIMMIX procedure of SAS (SAS University Edition). Data were analyzed following a randomized block design model:

$$Y_{ijklm} = \mu + T_i + P_k + (T \times P)_{ik} + B_l + \varepsilon_{ijklm}$$

where: Y_{ijkm} = individual; μ = the overall mean; T_i = the fixed effect of treatment (breed) i; P_k= the fixed effect of period k; $T \times P_{ik}$ = the fixed effect of interaction between treatment *i* and period *k;* B_l = the random effect of block *l;* and ε_{ijklm} = random error with the mean 0 and variance σ^2, the variance between measurements within animals. Fifteen covariance structures were tested and for each response variable. Thus, we used the covariance structure that provided the best fit based on lower AIC. Data on tick counts and weight followed non-normal distribution, then all available distributions in GLIMMIX procedure were tested using the same model described above, and the SHIFTED T(3) distribution was used.

To analyze blood parameters, ADG and FE data that had single observation, we used following a randomized block design model:

$$Y_{ijklm} = \mu + T_i + (T \times P)_{ik} + B_l + \varepsilon_{ijklm}$$

where: Y_{ijkm} = individual; μ = the overall mean; T_i = the fixed effect of treatment (breed) i; $T \times P_{ik}$ = the fixed effect of interaction between treatment *i* and period *k;* B_l = the random effect of block *l;* and ε_{ijklm} = random error with the mean 0 and variance σ^2, the

variance between measurements within animals. Differences were declared when P < 0.05 and a trend was reported when 0.05 < P < 0.10.

RESULTS

Forage Allowance and Chemical Composition

Pre-grazing and post-grazing height varied from 66.44 to 74.19, and 42.40 to 45.30, respectively (Table 2). Accumulated herbage per paddock (kg/DM), herbage allowance (kg DM/animal/d) and grazing efficiency (%) also varied among periods (Table 2). Forage chemical composition underwent changes throughout periods (Table 2).

Intake, Digestibility, and Animal Performance

There was no difference ($P = 0.22$; Table 3) between breeds to DMI, however, there were differences among experimental periods ($P < 0.05$; Table 3). The greatest DMI was observed in the fourth period. Forage DMI (**FDMI**) was different ($P < 0.05$) between breeds and among periods ($P < 0.05$), Girolando heifers had FDMI 11.7% greater than Holstein. We observed a trend ($P = 0.07$) to greater concentrate intake to Holstein (1.40 kg/d) than Girolando heifers (1.18 kg/d). The NDF intake (**NDFI**) was greater ($P < 0.05$; Table 3) to Girolando heifers and the greatest ($P < 0.05$) NDFI was observed in the fourth period. Crude protein intake (**CPI**) was not different ($P = 0.91$) between breeds but effect ($P < 0.05$; Table 3) was observed among periods. There was no breed effect ($P = 0.16$) to TDN intake (**TNDI;** $P = 0.16$) and ME intake (**MEI**; $P = 0.24$). However there was effect among periods to NCFI ($P < 0.05$) and MEI ($P <$

0.05). We observed a greater NFCI ($P < 0.05$) and MEI ($P < 0.05$) in the fourth period as previously described to NDFI and CPI.

We observed a trend ($P = 0.05$) to greater DMI/BW (Table 3) to Holstein (2.31 %) than Girolando heifers (2.17 %). However, there was difference ($P = 0.01$) among periods. Breeds did not influence ($P = 0.28$) FDMI per BW (**FDMI/BW**) nor NDFI per BW ($P = 0.55$; **NDFI/BW**) but this variable was affected ($P = 0.02$) by periods which the greatest NDFI/BW observed in the first period.

There was no difference ($P = 0.28$) between breeds to DM digestibility (Table 3), however there was effect among periods ($P = 0.04$), where the fourth period presented the greatest value (Table 2). We also observed a trend to NDF ($P = 0.06$) and CP ($P = 0.05$) digestibilities being greater to Girolando heifers (Table 3).

The final BW of Girolando was 311.50 kg and for Holstein was 260.39 kg. The ADG was different between breeds ($P = 0.01$; Figure 3) with greater values for Girolando heifers (0.87 kg) comparing to Holstein heifers (0.54 kg) corresponding to 37.9 % more gain to Girolando animals. There was difference ($P < 0.05$) between breeds to feed efficiency (**FE**) with greater values (14.98%) observed for Girolando comparing to Holstein heifers (9.14 %; Figure 3).

Animal Behavior

Grazing time was not different ($P = 0.49$) between breeds (Table 4), but we observed an effect among periods ($P < 0.05$) with the greatest value to the second period. Additionally, the grazing behavior throughout the day was distinct between breeds. We observed that all heifers had preference to graze during morning (0500 to 0900; Appendix 1; Figure 5). However, in the afternoon (1300 to 1700 h; Appendix 1; Figure 6) the percentage of Holstein heifers grazing was lower than Girolando. We did not observe differences between breeds (Table 4) to meal criteria ($P = 0.30$), meal time

(P = 0.29) nor to bouts events (P = 0.61), however there were effects among periods, which values in the fourth period were lower to meal criteria (P = 0.02). Meal duration tended to differ (P = 0.06) between breeds, and was greater to Girolando (254.63 min/d) than Holstein heifers (230.10 min/d; Table 4). We observed difference (P < 0.05) between breeds to ruminating time, which was 18.43 % lower for Holstein heifers than Girolando heifers (Table 4). Rumination criteria was different (P = 0.03) between breeds and greater value was observed to Holstein heifers. There was no difference between breeds to rumination total time (P = 0.79) and rumination bouts (P = 0.37). We observed differences (P = 0.02) between breeds to rumination length which was greater to Holstein heifers. Resting time differed (P < 0.05) between breeds on what Holstein showed 11.5% greater resting time than Girolando heifers. There was a trend (P = 0.05) to greater concentrate intake time to Holstein (31.25 min/d) than Girolando (26.71 min/d). There were differences among periods (P < 0.05) to concentrate intake in which greater values for the first (30.62 min/d) and second (32.5 min/d) periods were observed.

Tick Incidence

The tick incidence was greater (P < 0.05) to Holstein (9.8 ticks/animal) than Girolando heifers (2.56 ticks/animal; Figure 4). There was difference (P < 0.05) among periods, and the greatest value was observed during the third period. Consequently, tick weigh also differed (P = 0.05) between breeds with greater values to Holstein heifers.

Blood Parameters

There was difference (P = 0.01) between breeds for urea serum concentration, where greater value was observed to Girolando (Table 5). The serum concentrations of glucose (P = 0.35), total protein (P = 0.25), and triglycerides (P =0.16) did not differ

between breeds. We observed differences between breeds for IGF1 ($P < 0.05$), total T3 ($P = 0.01$), and total T4 ($P = 0.01$) which the greater values were observed for Girolando heifers.

DISCUSSION

Our hypothesis in this study was that Holstein heifers would have the lowest DMI in tropical conditions, but Girolando and Holstein heifers had similar DMI. According to Santos et al. (2012), Holstein heifers are not adapted to pasture systems, and hence have intake and performance compromised. However, in present study we did not find breed effects on DMI, but performance was affected.

There is a relationship between BW and intake (Allen, 2014) as observed in this study. As BW increased there is an increase in internal organs feed capacity (Santana Junior et al., 2013) thus, DMI also increased, it was observed mainly in the fourth period besides no DMI differences between breeds were found. As reported by Hoffman et al. (2008) there is an increase in NDFI with the BW increase, which Girolando being heavier than Holstein heifers. This is a plausible explanation to differences found in NDFI due differences found in animals' BW.

Girolando heifers showed a greater intake capacity for forage and NDF, while we observed a trend to greater concentrate intake to Holstein heifers. As Holstein heifers had lower forage intake, the animals tended to consume more concentrated feed to meet the daily nutrient requirements, and them there was no difference in DMI between breeds. Additionally, Holstein heifers probably selected the diet (Rutter, 2006), searching for plant parts with lower NDF content due the lower FDMI observed to these animals. In addition, greater rumination time was observed for Girolando (418.91 min/d) than Holstein heifers (341.72 min/d) that are directly related to fiber content

ingested (Hejcmanova et al., 2009) and appears to be proportional to cell wall content of bulky feed (Van Soest, 1994). As Girolando heifers had greater ADG, the ruminal capacity, intake and rumination increased (Santana Junior et al., 2013) at higher rates than Holstein heifers, this fact may explain the difference observed between breeds for ruminating time, once we did not find differences to DMI.

There is a decrease in DMI/BW of heifers according to animal´s growth (Hoffman et al., 2008). This information might help explain a trend to greater DMI/BW for Holstein heifers (2.3 %), once these animals were lighter than Girolando.

As dietary NDF increase there is an increase in NDFD (Souza et al., 2018), we observed a trend to great NDFD to Girolando than Holstein heifers and it might be caused by a greater FDMI and consequently greater NDFI for Girolando heifers.

Average daily gain for Girolando heifers (0.87 kg) was 37% greater than Holstein (0.54 kg). These results are similar to those found to Garcia et al. (2011), which observed an ADG for Girolando animals in Guinea grass (*Panicum maximum* Jacq. cv. Mombaça) pasture of 0.85 kg. The results observed for Holstein heifers are similar to those reported by Machado (2018) which observed ADG of 0.57 kg using supplementation of 25% CP in the same grass at the same season. Supplement content up to 24% CP improve ADG of crossbred heifers due to better N utilization by rumen microorganism and consequently obtain great animal performance (Moraes et al., 2006).

Blood parameters as total protein, albumin and urea may be used as indicator to health status (Cecchinato et al., 2018), and animals' productive priorities (Huntington and Archibeque, 2000) in addition, these parameters are used to evaluate the diet protein balance (Aguilar et al., 2012; Cecchinato et al., 2018). Our findings for total protein and albumin are within normal concentrations for cattle that ranges from 6.6 to 7.5 g/dL and from 2.7 to 4.1 g/dL (González and Silva, 2006) respectively.

We observed greater urea concentration to Girolando (29.82 mg/dL) than Holstein (22.7 mg/dL) and these values were within the reference values that ranged from 17 to 45 mg/dL (González and Silva, 2006). As ruminating time was greater for Girolando heifers, the salivary flux may be greater and improve urea recycling, once we did not find difference between breeds to CPI. According to Tan and Murphy (2004), salivary secretion and blood urea concentration determine the urea amount transferred into rumen. Urea is synthetized in the liver to prevent N toxicity and is used as N source to rumen microorganism mainly by means of saliva recycling (Aguilar et al., 2012; Schwab and Broderick, 2017) and improving microbial protein production essential more AA may be synthetized and used by host (Tan and Murphy, 2004). It is possible that greater urea blood concentration observed for Girolando heifers could have contributed to greater performance as we observed in this study.

We observed greater FE to Girolando (14.98%) than Holstein heifers (9.14%); this observation may be related to a greater FDMI to Girolando breed, greater IGF-1 blood concentrations and more adaptability to grazing system and environment. The greater IGF-1 blood concentration observed in Girolando animals contribute to understand the greater ADG and FE comparing to Holstein heifers. Once growth and cell differentiation are controlled by IGF-1, it plays a role in post-natal growth, performance, mammary gland development,reproduction, and lactation in dairy cows (Mullen et al., 2011; Gobikrushanth et al., 2018). Serum IGF-1 also contribute to ADG, body size, and FE (Mullen et al., 2011).

There is a substantially decreased in ruminating time, DMI, growth and FE Soriani et al. (2013) when animals are outside of its thermos-neutral zone (Oliveira and Ferreira, 2016). The depression of ruminating time for Holstein heifers might be related to a slow digesta rate passage impairing motility and ruminal activity (Silanikove, 1992). In heat stress besides DMI reduction the energy maintenance may increase due

mechanisms by heat dissipation (Bernabucci et al., 2010). Lower performance to Holstein heifers may be related to high environmental temperatures over the thermo-neutral zone to this breed. A measurement used to describe environmental conditions that lead the heat stress is THI (Polsky and von Keyserlingk, 2017). Our THI ranged from 68.8 to 72.0 and might be caused moderate sings of heat stress in Holstein heifers (De Rensis et al., 2015). The incidence of solar radiation may be another cause to heat increase mainly in graze systems, but due to the difficulty of quantification it has been inconsiderate (Bernabucci et al., 2010). As reported by Hansen (2004) Polsky and von Keyserlingk (2017), Zebu have greater thermo-tolerance than European cattle due genes thermo-tolerant acquired during genetic adaptation.

Environmental conditions also may contribute to increase resting time mainly in the afternoon, on what temperatures and humidity outside range of animal's thermal comfort. Animals may reduce grazing and rumination activities raising the search for shadow (Pereira et al., 2017), as we observed in our study when grazing activity occurred mainly at early morning and late afternoon (Zanine et al., 2009). Additionality, animals did not graze for a long time or stopped grazing (Kilgour, 2012) in these situations. In our study, Holstein heifers spent more time (712.50 min/d) in resting behavior than Girolando heifers (628.75 min/d) probably due thermal discomfort.

Despite nutritional factors, it was suggested by Spicer et al. (1990) that serum IGF-1 may be affected by environmental conditions in which cows in summer season had lower milk concentration of IGF-1. Besides IGF-1, T3, and T4 are important hormones associated with metabolic homeostasis and to animal's adaptation environment. We observed low concentrations of these hormones in Holstein heifers that may be under heat stress as reported by Bernabucci et al. (2010). Animals under heat stress reduce feed intake to control body temperature resulted from feeding,

digestion, absorption, and metabolism (Zereu, 2016), impairing the animals performance.

There are many factors that affect meal time duration, among them diet composition and access to feed are more relevant (Beauchemin, 2018). We observed a greater meal time over 850 min/d to heifers kept in pasture, with an average of 3.8 bouts/d. Meal duration was greater to Girolando heifers and is associated with searching and feed intake. Charlton and Rutter (2017) and Shepley et al. (2017) reported that animals in pasture systems spent more time foraging or grazing than animals receiving a TMR diet. In fact, meal time vary according to diet compounds and feed management.

Rumination criteria and rumination length (min/bouts) was greater for Holstein than Girolando Heifers. Thus, Holstein heifers spent more time per event to ruminate less content than Girolando once we observed lower FDMI to Holstein heifer. In this study, we observed a greater rumination time at night for both breeds (Figure 6, appendix) and this data is in agreement with Zanine et al. (2009) evaluating Girolando cows kept in grazing system. White et al. (2017) conducted a meta-analysis and found a ruminating time of 456 min/d on average ranging from 236 a 610 min/d to dairy cows. In this study, ruminating time was 341.72 min/d for Holstein and 418.91 min/d to Girolando heifers in agreement with data observed by authors cited. There are large variations to ruminating time, factors associated with feed management and composition, besides factors inherent to individual animals. According to Gregorini et al. (2013) ruminating time can be controlled by factor as animal's age and breeds and also number and chewing time per ruminal bolus.

It is known that *Bos indicus* cattle have more resistance to tick infestation than *Bos taurus* due to the presence of genes naturally selected during their evolutionary process (Ibelli et al., 2012; Otto et al., 2018) to predict tick resistance in cattle, tick counts can be used (Biegelmeyer et al., 2015). Holstein heifers were more susceptible to ticks incidence

than Girolando although animals received treatment for ticks during our study. An examination of many studies indicate that over one gram in weight of beef cattle can be lost daily for each engorging tick (Jonsson, 2006). However, the levels of infestation observed in our study were low and may not have directly affected the animals' performance once at least 80 ticks/d per animal are necessary to induce secondary illness as Babesiosis (Johnston et al., 1981). Additionally, there is a relationship with nutrition and tick resistance, as the animals had access to a pasture with high quality, it is possible that the ticks incidence had no effects on heifers (Mahoney et al., 1981).

CONCLUSION

The greater Girolando animals' performance might be related to better nutrients use from forage due greater forage intake and greater rumination time. In addition, Girolando animals are better adapted to tropical climate. It is possible that Holstein animals suffered heat stress however more research is needed to elucidate the causes of lower performance of these animals in grazing.

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TABLES AND FIGURES

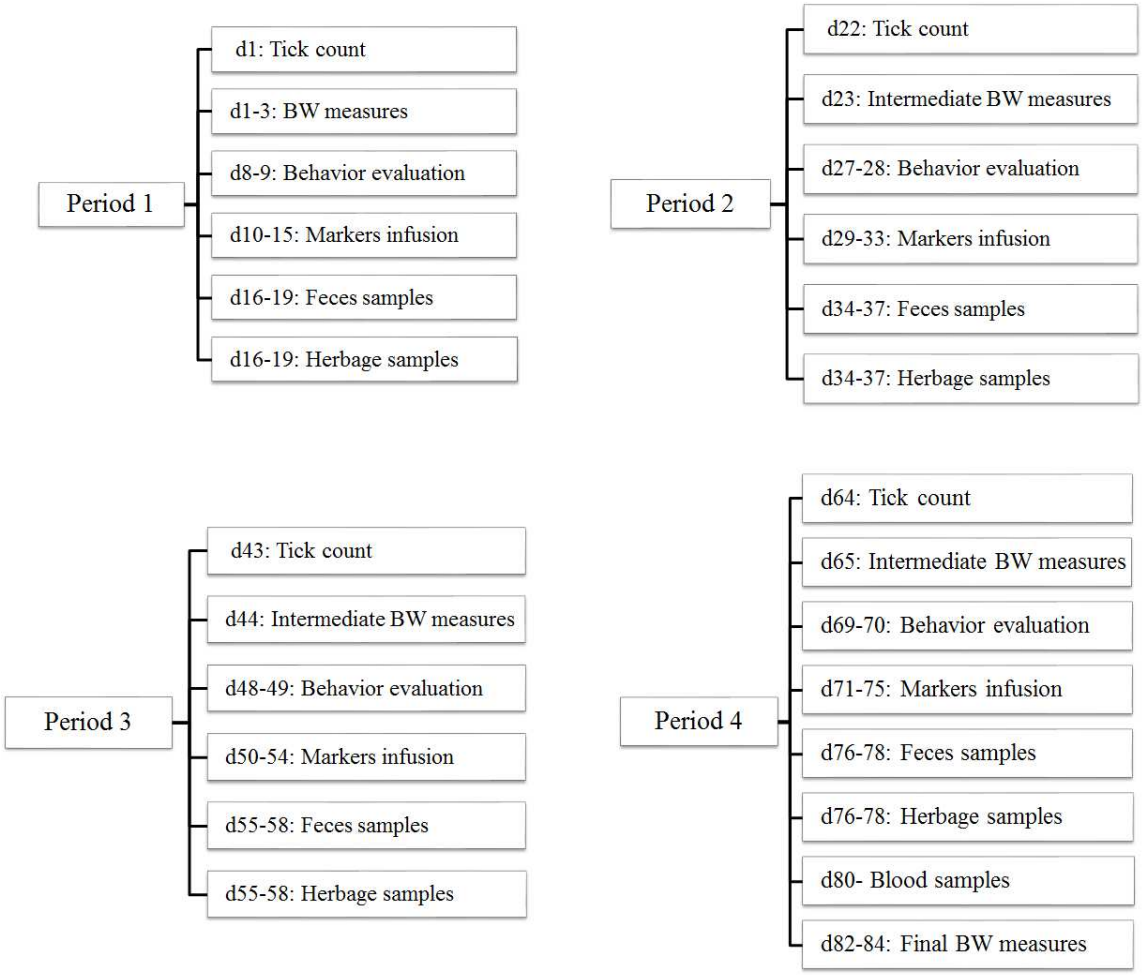

Figure 1. Schematic model of experimental periods. Each period lasted 21 d. [1-84] represents data collection days within each period.

Table 1. Environmental conditions throughout periods

	Period			
Item	1[1]	2[2]	3[3]	4[4]
Avg T (°C)	23.1	22.6	21.3	22.9
Min T (°C)	19.2	18.9	17.8	19.8
Max T (°C)	27.5	29.7	28.2	31.4
Rainfall (mm)	4.2	3.45	2.9	4.9
Relative humidity (%)	79.5	78.5	75.4	75.4
Temperature humidity index (THI)	72.0	71.2	68.8	71.4

[1] January 29, to February 6, 2017; [2]February 2nd, to March 1st, 2017; [3] March 16 to 22, 2017; [4]April 7 to 13, 2017. Data from Viçosa-MG weather station.

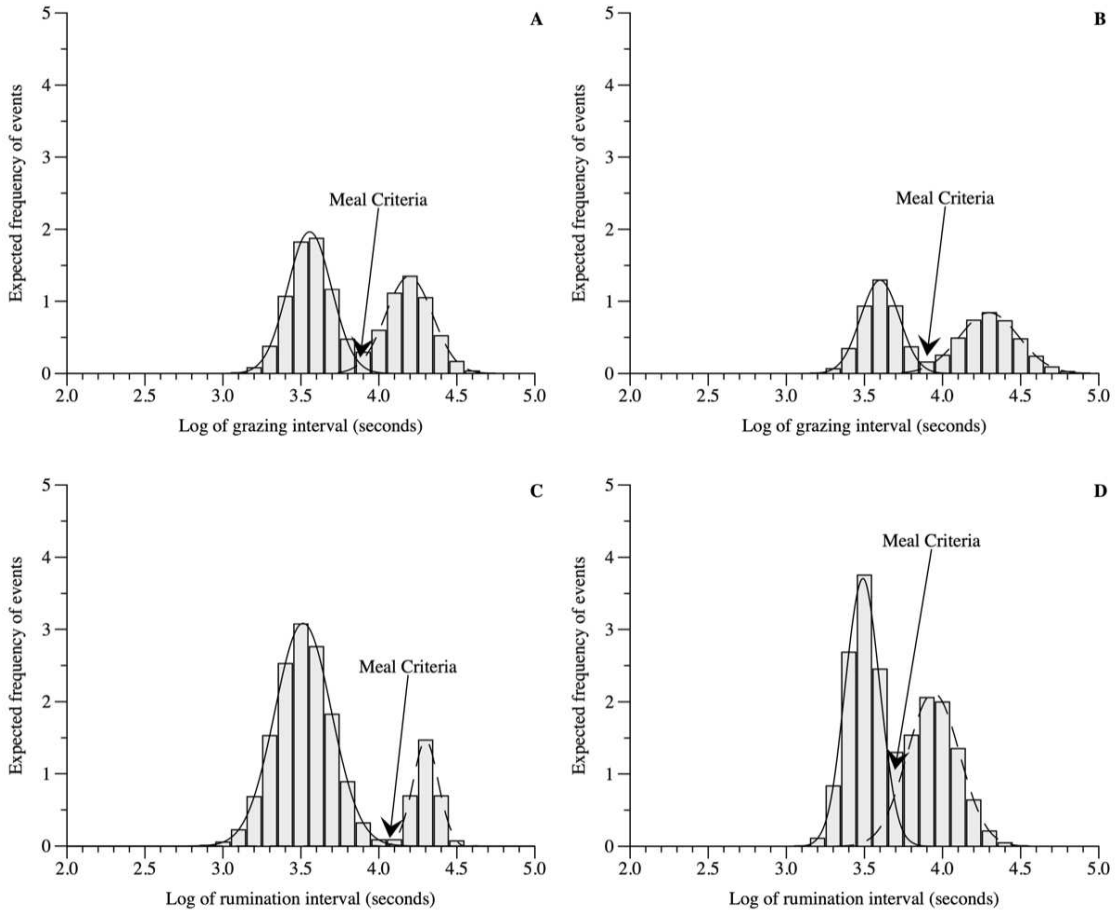

Figure 2. Meal criteria. The intersection of distribution intrameal curve intervals, and distribution curve of intermeal intervals. **A.** Meal criteria to Holstein heifers in grazing behavior; **B**. Meal criteria to Girolando Heifers in grazing behavior; **C**. Rumination critera to Holstein heifers in ruminating behavior; **D.** Rumination criteria to Girolando heifers in ruminating behavior.

Table 2. Accumulated forage, pre and post-grazing height and chemical composition of pasture per period and supplement

Item	*Pasture production*				
	Period				
	1	2	3	4	Supplement
Accumulated herbage (kg DM/ha/cycle)	1235.26	1194.13	1370.76	1423.42	-
Accumulated herbage (kg DM/paddock/cycle)	85.93	82.85	91.36	94.1	-
Herbage DM allowance (kg DM/animal/cycle)	10.74	10.35	11.42	11.76	-
Grazing efficiency (%)	61.3	60.1	58.74	63.34	-
PreGH[1] (cm)	72.64	70.96	74.19	66.44	-
PostGH[2] (cm)	42.40	43.07	45.30	44.56	-
	Chemical composition (%DM)				
Dry matter	28.37	22.18	27.37	25.82	89.35
Neutral Detergent Fiber	69.31	70.06	70.27	66.03	10.99
Indigestible Neutral Detergent Fiber	14.70	15.74	15.87	15.29	1.48
Crude Protein	12.67	12.37	11.80	12.97	26.15
Ether Extract	1.18	0.99	1.54	1.42	3.55
Ash	2.14	3.20	2.06	2.44	2.46

[1]Pre-grazing Height; [2]Post-grazing Height; Cycle 15d.

Table 3. Intake and digestibility of grazing Girolando and Holstein heifers

Item	Treatment			Period				P-value		
	Girolando	Holstein	SEM	P1	P2	P3	P4	breed	Per	breed×Per
Intake, kg/day										
DM	6.06	5.74	1.237	5.64^{b}	5.36^{b}	5.71^{b}	6.89^{a}	0.2220	0.0006	0.3802
FDM[1]	4.87	4.30	0.941	4.42^{b}	4.17^{b}	4.42^{b}	5.35^{a}	0.0023	<.0001	0.3826
CDM[2]	1.18	1.40	0.299	1.20	1.16	1.28	1.53	0.0717	0.1249	0.6169
NDF	3.49	3.12	0.693	3.19^{b}	3.04^{b}	3.25^{b}	3.74^{a}	0.0065	0.0022	0.3472
CP	0.91	0.90	0.184	0.88^{b}	0.83^{b}	0.83^{b}	1.08^{a}	0.9155	0.0012	0.4404
TDN	3.77	3.53	0.819	3.43^{a}	3.23^{b}	3.51^{a}	4.44^{a}	0.1622	<.0001	0.5254
ME (Mcal/kg DMI)	6.05	4.98	3.219	4.73	4.99	5.72	6.61	0.2490	0.4684	0.8596
Intake,% of BW										
DM/BW	2.17	2.31	0.0004	2.35^{a}	2.14^{b}	2.10^{b}	2.36^{a}	0.0521	0.0137	0.1109
FDM/BW	1.74	1.80	0.0003	1.87^{a}	1.70^{b}	1.64^{b}	1.86^{a}	0.2805	0.0049	0.0830
NDF/BW	1.25	1.27	0.0002	1.34^{a}	1.22^{b}	1.19^{b}	1.27^{ab}	0.5521	0.0219	0.2137
Digestibility, g/g										
DM	0.624	0.616	0.010	0.604^{b}	0.613^{b}	0.619^{b}	0.643^{a}	0.2859	0.0046	0.6419
NDF	0.654	0.639	0.010	0.638^{b}	0.635^{b}	0.662^{a}	0.653^{ab}	0.0631	0.0527	0.6076
CP	0.691	0.675	0.012	0.662^{b}	0.66^{b}	0.677^{b}	0.723^{a}	0.0512	<.0001	0.8617

[1]Forage Dry Matter Intake; [2] Concentrate Dry Matter Intake. $^{a–b}$ Means within a row with different letters superscripts differ ($P < 0.05$).

Table 4. Behavior activities of grazing Girolando and Holstein heifers

	Treatments			Period				P- value		
Item	Girolando	Holstein	SEM	P1	P2	P3	P4	breeed	Per	breed×Per
Grazing characteristics										
Grazing time (min/d)	365.16	354.22	11.324	348.75^{b}	404.69^{a}	335.62^{b}	349.69^{b}	0.4992	<.0001	0.2713
Meal criteria (min)	117.99	135.47	0.039	141.85^{a}	132.38^{ab}	138.61^{ab}	95.90^{b}	0.3045	0.0255	0.5378
Meal time (min/d)	929.75	877.03	35.404	927.31^{ab}	822.81^{b}	895.63^{ab}	967.81^{a}	0.2989	0.0663	0.1376
Bouts (events/d)	3.81	3.95	0.191	3.59^{b}	3.87^{ab}	3.84a^{b}	4.21^{a}	0.6115	0.0424	0.4463
Meal duration (min/meal)	254.63	230.10	8.971	258.09^{a}	254.72^{b}	394.36^{ab}	314.10^{ab}	0.0619	0.0547	0.6666
Ruminating characteristics										
Ruminating time (min/d)	418.91	341.72	13.312	397.81^{ab}	400.00^{a}	364.37^{bc}	359.06^{c}	0.0008	0.0602	0.5934
Rumination criteria (min)	93.72	117.99	0.323	105.15	107.60	100.42	102.76	0.0320	0.9532	0.3788
Rumination total time (min/d)	719.08	729.22	26.891	837.22^{a}	696.56^{ab}	811.56^{a}	551.25^{b}	0.7936	0.0024	0.6626
Rumination bouts (events/d)	4.73	4.34	0.305	5.46^{a}	4.43^{ab}	4.81^{a}	3.43^{b}	0.3711	0.0042	0.6308
Rumination length (min/bouts)	157.13	179.56	6.940	164.08	166.17	178.81	164.33	0.0261	0.6742	0.6516
Other behaviors										
Resting time (min/d)	628.75	712.50	23.014	662.50^{b}	602.50^{c}	714.37^{a}	703.12^{ab}	0.0008	0.1589	0.5138
Concentrate intake time (min/d)	26.71	31.25	2.775	30.62^{a}	32.50^{a}	24.68b	28.12^{ab}	0.0590	0.0068	0.0501

$^{a–b}$ Means within a row with different letters superscripts differ (P < 0.05).

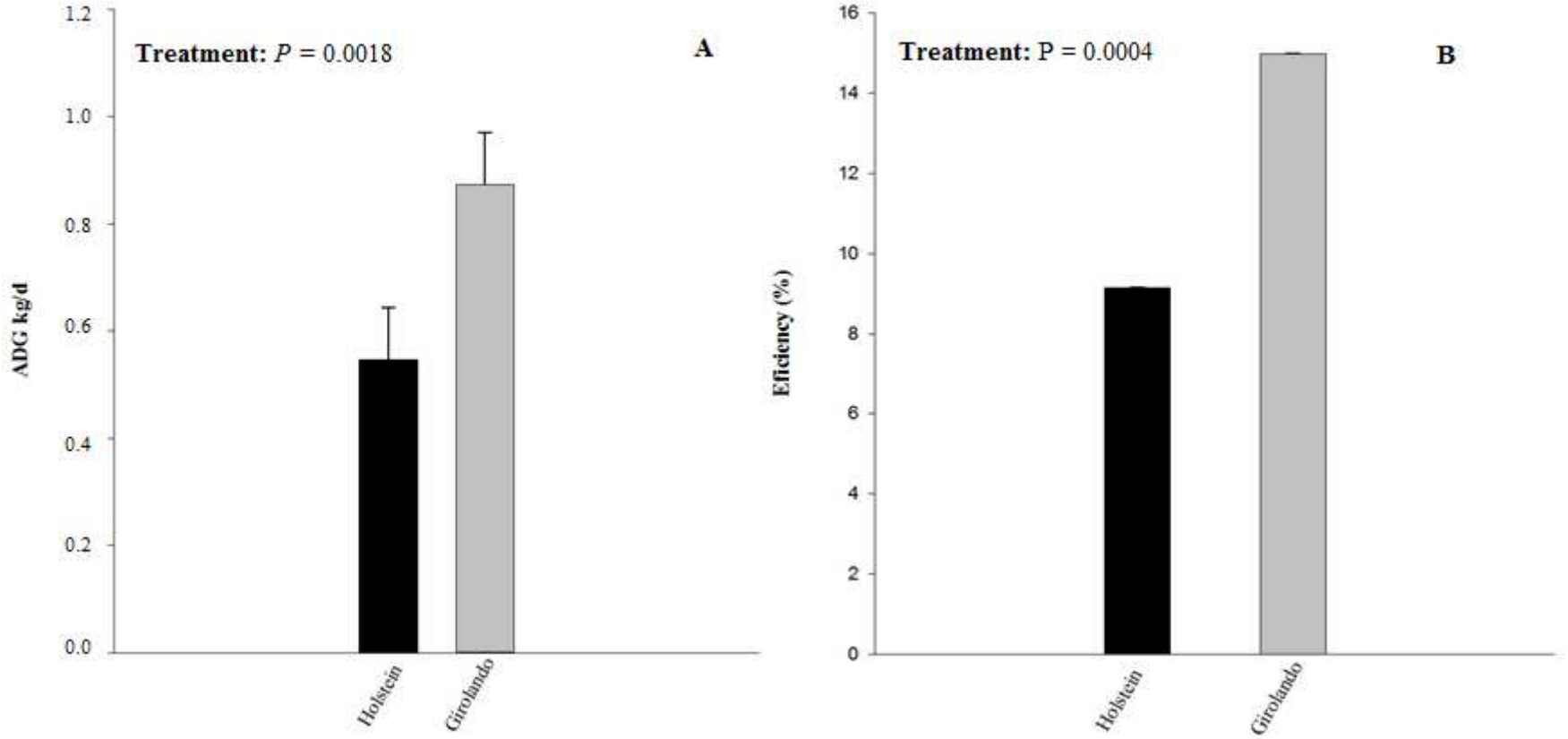

Figure 3. A. Average daily gain of Girolando and Holstein heifers in pasture of Guinea grass (*Panicum maximum* Jacq. cv. Mombaça). **B.** Feed eficiency of Girolando and Holstein heifers in pasture of Guinea grass (*Panicum maximum* Jacq. cv. Mombaça).

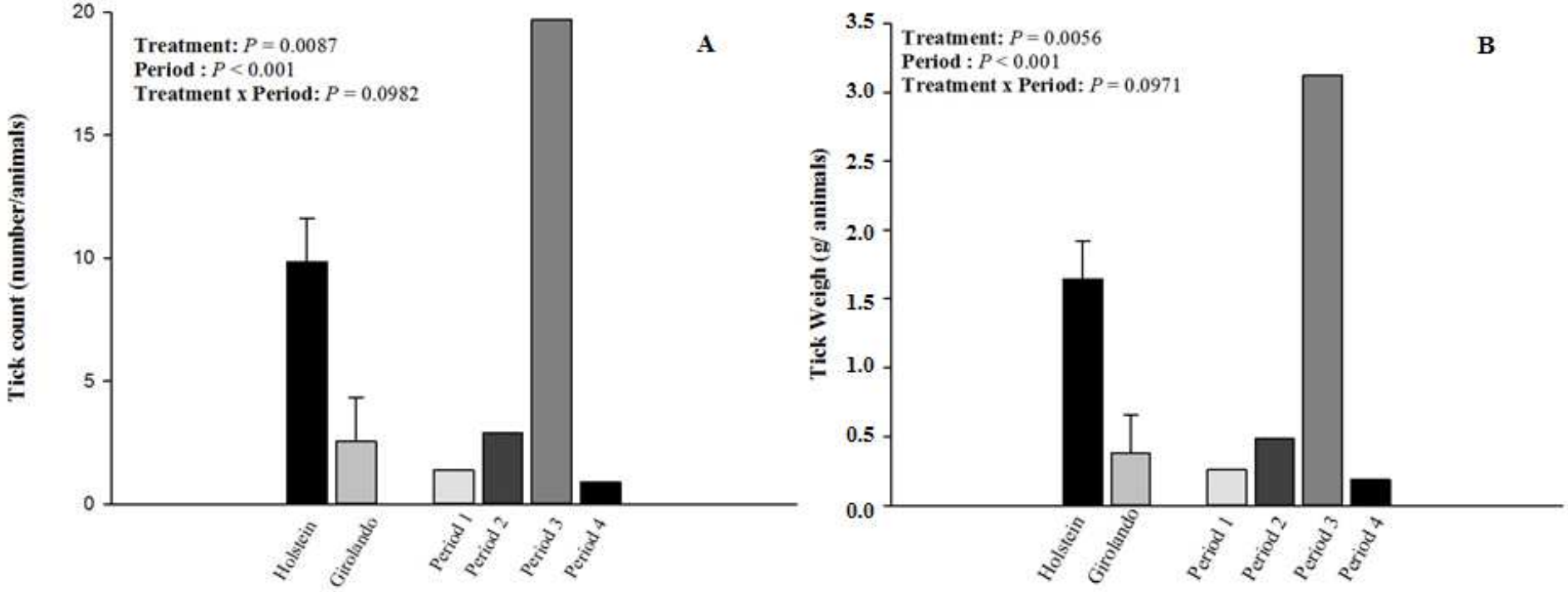

Figure 4. A. Average tick count (number/ animals) per breed and average per periods of Girolando and Holstein heifers in pasture of Guinea grass (*Panicum maximum* Jacq. cv. Mombaça). **B.** Average tick weight (g/animals) per breed and average per periods of Girolando and Holstein heifers in pasture of Guinea grass (*Panicum maximum* Jacq. cv. Mombaça).

Table 5. Blood parameters of Girolando and Holstein heifers under grazing condition

Item	Treatment			P-value
	Girolando	Holstein	SEM	breed
Urea (mg/dL)	29.82[a]	22.70[b]	1.780	0.0142
Glucose (mg/dL)	71.67	69.78	4.485	0.3523
Total protein (g/dL)	6.73	7.22	0.183	0.2535
Albumin (g/dL)	3.29[a]	2.90[b]	0.165	<.0001
Triglycerides (mg/dL)	30.07	24.25	2.783	0.1627
IGF 1 (ng/mL)	270.67[a]	165.94[b]	39.933	<.0001
Total T3 (ng/dL)	2.73[a]	1.43[b]	0.517	0.0162
Total T4 (µ/dL)	9.98[a]	5.51[b]	1.677	0.0164

[a–b] Means within a row with different superscript letters differ ($P < 0.05$).

APPENDIX 1

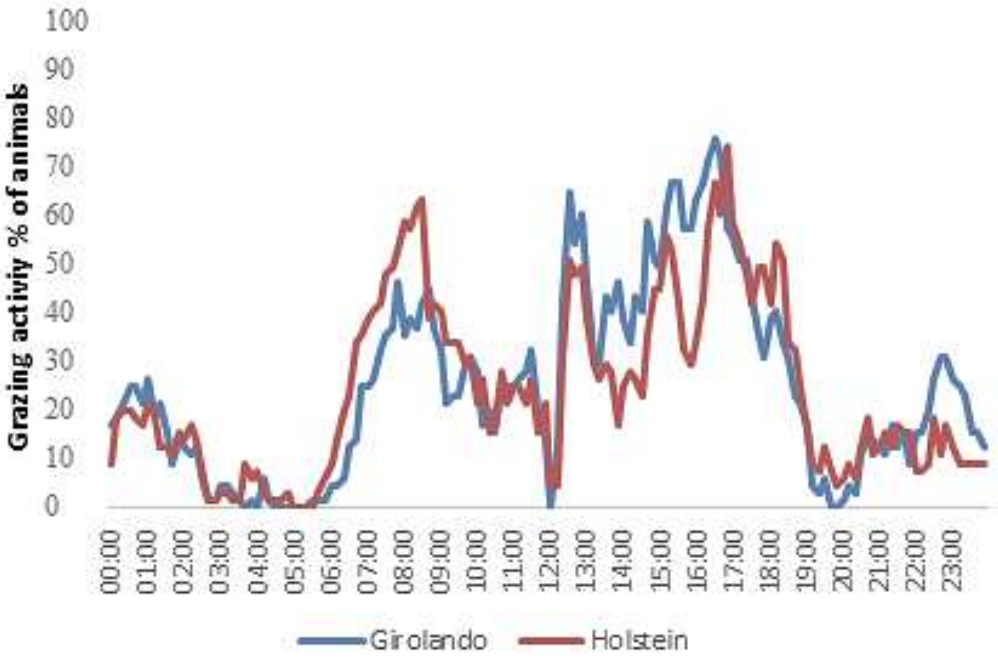

Figure 5. Animals' percentage in grazing activity during 24 h in Guinea grass (*Panicum maximum* Jacq. cv. Mombaça) pasture. Average data from the four experimental periods.

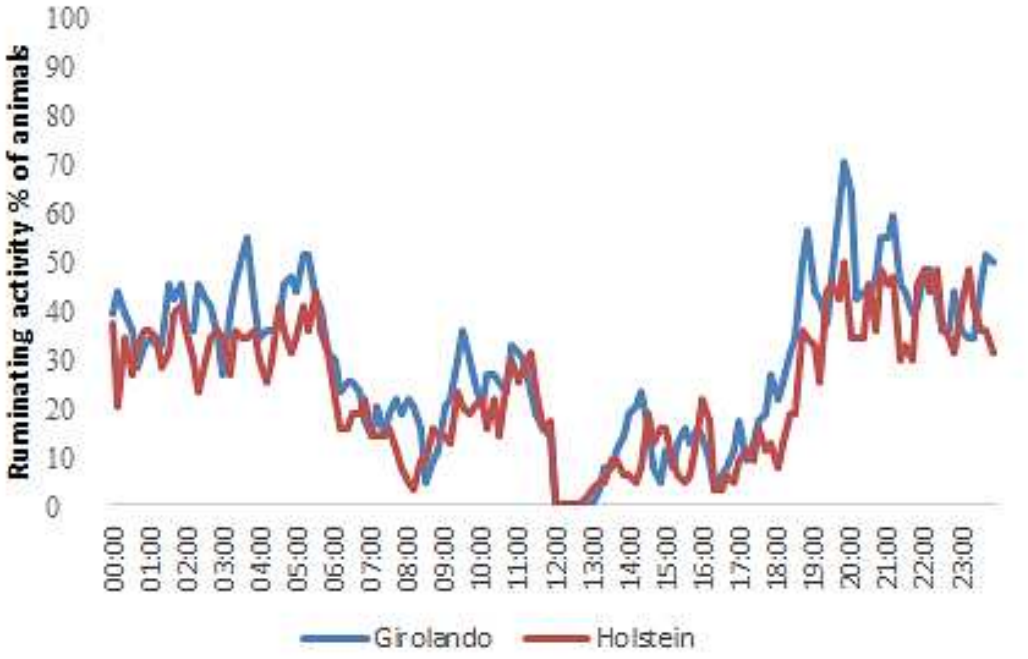

Figure 6. Animals' percentage in ruminating activity during 24 h in Guinea grass (*Panicum maximum* Jacq. cv. Mombaça) pasture. Average data from the four experimental periods.

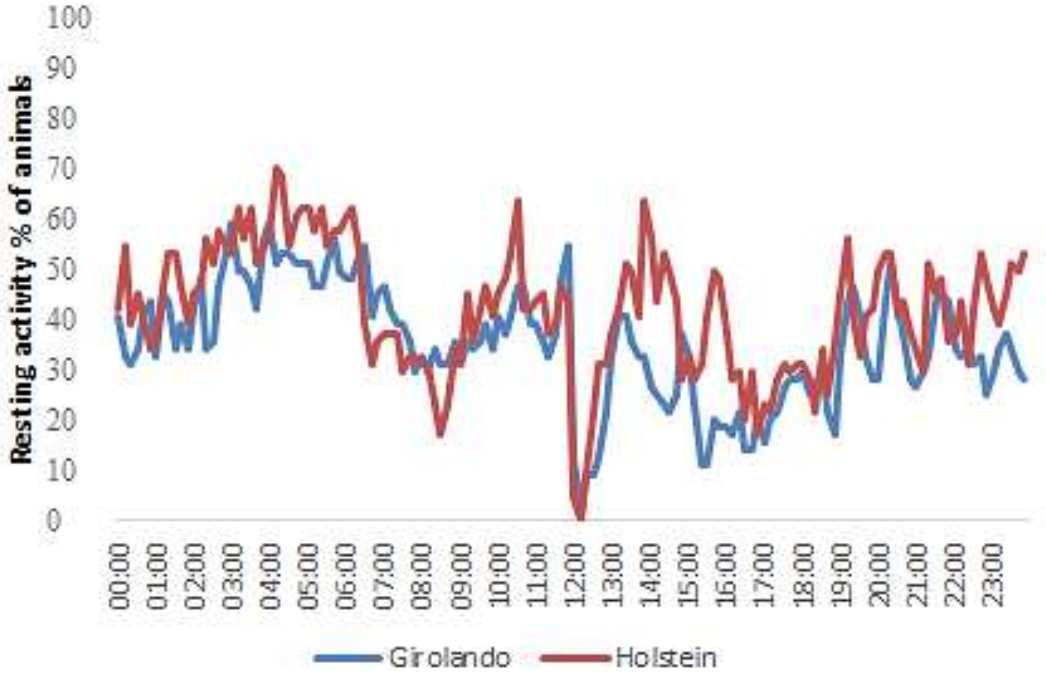

Figure 7. Animals' percentage in resting activity during 24 h in Guinea grass (*Panicum maximum* Jacq. cv. Mombaça) pasture. Average data from the four experimental periods.

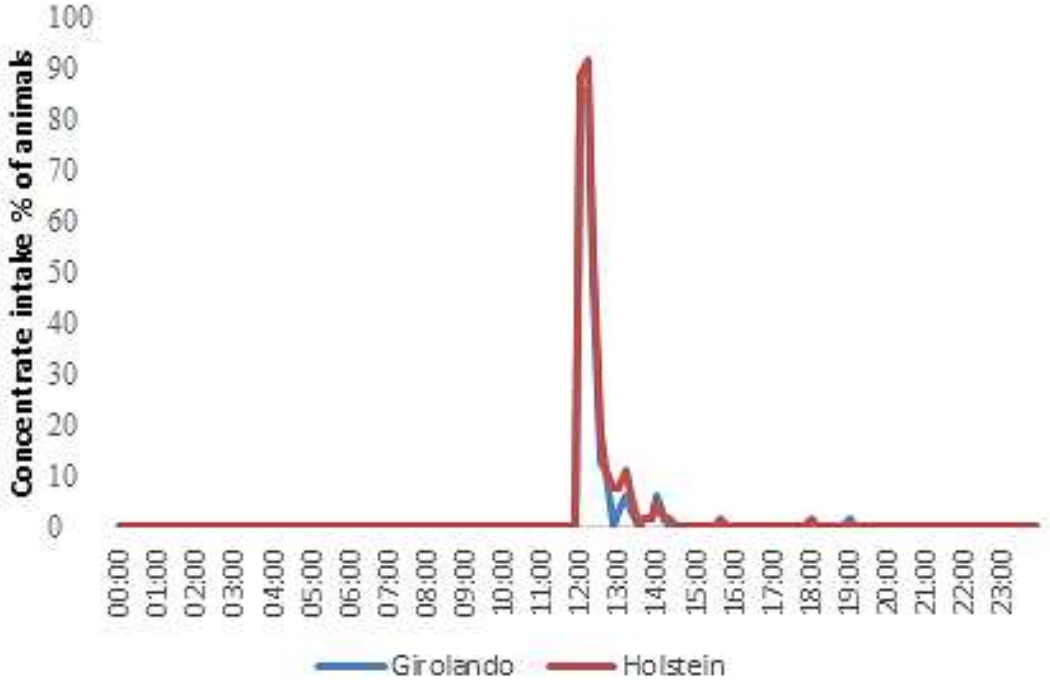

Figure 8. Animals' percentage in concentrate intake activity during 24 h in Guinea grass (*Panicum maximum* Jacq. cv. Mombaça) pasture. Average data from the four experimental periods.