WAGNER DA SILVA MACHADO

SUPPLEMENTATION STRATEGIES AFFECT FEED INTAKE AND PERFORMANCE OF GRAZING HOLSTEIN 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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APPROVED: February 26, 2018.

Marcio de Souza Duarte

Joanis Tilemahos Zervoudakis

Edenio Detmann

Marcos Inácio Marcondes
(Adviser)

DEDICATION

No more but, however or nevertheless, because everything in our life have some why. So why should I tell my dear how my efforts and tears are rooted here in this staccato work, if, at my side you are attendant and shining? Well of course, is you who follow me, at strange and unique way, would know whom I would dedicate! So, mom, one more well achievement to you I proud announce and one more defeat here I lay out. You would know if our life were different, and no one more than you know how painful to me, do everything to you, bringing conquests, without your smile shining my life from inside. So, when I hear a bird or a meow, making my career my love-life I'm choosing live my own life like you dreamed your life to yourself. Now I'm living your dream of life. An what a wonderful dream!

So, it's for you!
It's for you that never abandoned me
And to whom fought for me.
It's for you that never sleep
And even with painful feel smile to me
It's for you that when was terrified
with upon human power cherish me at night.

It's for you my sublime warrior, that I dedicate this work.

I also dedicate this work to everyone how were at my side: My father, Adelaide, Lili, Ilsinho, Marilene (sweet family); Marcos Marcondes, my mentor, our Dairy Cattle team; to the support of CAPES, FUNARBE, UEPE-GL, DZO-UFV and everyone who have at some way helped.

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Thanks for Edenio Detmann, Joanis Zervoudakis and Marcio Duarte for enrich my work!

Thanks for him... my father
Without anything more to say,
On more thank you, or even to dedicate.
Your presence and personal support
Made myself a man, and sometimes foolish

Thank you for staying there for me,
For love me and support me!
And for never left my side,
Even when I wanted to run away!

We're two in one, separated by the space, but not by our love!
Thank you too!

BIOGRAPHY

Wagner da Silva Machado, son of Uilson de Souza Machado and Lilia da Silva Machado, was born in Rio de Janeiro, RJ - Brazil on August 21, 1985.

He started the undergrad in Animal Science at Universidade Federal de Viçosa in 2009 and obtained Bachelor of Science degree in Animal Science in July of 2015. In March of 2016, he started the Master Science program in Animal Science at the same University. He submitted his dissertation on February of 2018, to obtain the Master Science degree in Animal Science.

SUMMARY

ABSTRACT

MACHADO, Wagner da Silva, M.Sc., Universidade Federal de Viçosa, February, 2018. **Supplementation strategies affect feed intake and performance of grazing holstein heifers.** Adviser: Marcos Inácio Marcondes. Co-adviser: Polyana Pizzi Rota.

Literature lacks studies investigating performance of grazing Holstein heifers on tropical conditions, under different supplementation regime. Thus, we aimed to evaluate intake, performance and digestibility of grazing Holstein heifers under 3 supplementation strategies during the rainy and rainy-dry transition seasons. Eighteen Holstein heifers with average age and initial live weight of 12.57 ±2.54 mo and 218.76 ±47.6 kg, respectively were submitted to a randomized block design, with 6 replicates on a rotational grazing system of *Panicum maximum* cv. Mombaça pasture. Treatments were: control (CON; mineral salt ad libitum); energy supplement (ENE; corn meal as supplement); and protein supplement PRO (corn and soybean meal, 25% crude protein (CP)). Supplements were fed at 0.5% BW. The experiment lasted 120 days subdivided into 3 periods. Titanium dioxide and indigestible neutral detergent fiber (iNDF) were used to estimate intakes and digestibility of the nutrients. Body weight, withers height, thoracic circumference, body length, and ultrasound of ribeye area, and ribeye fat thickness measurements were taken every period. Body condition scores (BCS) was taken twice. MIXED procedure of SAS including period as repeated measurements was used and significance was declared at $P \leq 0.05$. DM intake, CP intake (CPI) and DE intake were greater in PRO ($P<0.05$) compared to CON and ENE. Supplementation strategy affected forage DM intake (%BW, $P = 0.049$), and a supplementation strategy × period interaction was observed for NDF intake (%BW), mainly due to a greater NDFI/BW in the period 3 to CON. The PRO had greater ($P<0.05$) grass DM digestibility compared to CON. ENE had lower CP digestibility compared to PRO and CON ($P<0.01$). Average daily gain (ADG) and thoracic circumference gain were the greatest ($P<0.05$) in PRO. BCS was greater ($P = 0.02$) on PRO (3.43) than CON (3.24) and ENE (3.03). PRO animals had the best performance, consequently they better expressed the associative effects of forage and supplement. Its recommended that Holstein heifers in rotational grazing system with Mombaça grass should be protein supplemented during the rainy and rainy-dry transition seasons to maximize performance.

RESUMO

MACHADO, Wagner da Silva, M.Sc., Universidade Federal de Viçosa, fevereiro de 2018. **Efeitos das estratégias de suplementação no consumo e performance de novilhas Holandesas.** Orientador: Marcos Inácio Marcondes. Coorientadora: Polyana Pizzi Rota.

O desempenho de novilhas Holandesas a pasto em condições tropicais sob diferentes regimes de suplementação ainda não é largamente estudado. Deste modo, avaliou-se o consumo, desempenho e digestibilidade de novilhas Holandesas a pasto recebendo três diferentes tipos de suplementação no período das águas e de transição águas-seca. Dezoito novilhas Holandesas com idade e peso inicial de 12.57 ± 2.54 meses e 218.76 ±47.6 kg respectivamente, foram submetidas a um delineamento em blocos casualisados, com 6 repetições em sistema de pastejo rotacionado de *Panicum maximum* cv. Mombaça. Os suplementos avaliados foram: controle (CON; sal mineral ad libitum); suplementação energética (ENE; fubá de milho como suplemento); e suplementação proteica (PRO; Mistura de fubá de milho e farelo de soja, 25% de proteína bruta (PB). O experimento durou 120 dias, subdividido em 3 períodos. Dióxido de titânio e fibra em detergente neutro indigestível (FDNi) foram utilizados para estimar o consumo e digestibilidade dos nutrientes. As mensurações relativas ao peso corporal, altura de cernelha, circunferência torácica, comprimento corporal e as medidas de ultrassom da área do olho de lombo e da espessura da gordura do lombo foram tomadas a cada período. Os escores de condição corporal (ECC) foram avaliados duas vezes durante o período experimental. Os dados foram avaliados em procedimento MISTO no SAS incluindo período como medidas repetidas no tempo e a significância foi declarada em P ≤ 0,05. Os consumos de: matéria seca (MS), proteína bruta (PB) e de energia digestível (CED) foram maiores para o suplemento PRO (P <0,05) comparados com CON e ENE. A suplementação afetou o consumo de MS de pasto (% BW, P = 0,049) e a interação suplementação x período no consumo de FDN (% PC), possivelmente ocorreu devido a um consumo de forragem maior (%PC) no período 3 do CON. O PRO proporcionou maior digestibilidade da MS do pasto (P =0,04) em comparação com CON. O aporte de energia via suplemento ENE possibilitou menor digestibilidade da PB comparado com os suplementos PRO e CON (P<0.01). O ganho diário médio (GMD) e o ganho de circunferência torácica foram maiores (P <0,05) quando se forneceu suplementação protéica aos animais (PRO). O ECC foi maior (P=0,02) para os animais suplementados com proteína (PRO -3,43) comparados com os animais que receberam apenas suplementação minera (CON -3,24) e energética (ENE -3,03). Para suplementação de Novilhas Holandesas em sistema de pastejo

rotacionado de Capim Mombaça no período das águas e de transição águas-seca, recomenda-se o fornecimento de suplementos proteicos (25% PB) (0,5% PC) para obtenção de melhor desempenho produtivo dos animais.

INTRODUCTION

The public concern about animal welfare is currently leading to changes in animal production systems. Thus, studies within good care, which allows animals to express their natural behavior, are desirable and have received considerable attention nowadays (Harper and Henson, 2001; Špinka, 2006). Ruminants in natural living environments such as pastures can face obstacles such as extreme weather conditions, detrimental social behaviors, parasites exposure, diseases or infections, which could impair the animals' performance (Špinka, 2006; von Keyserlingk et al., 2009). In that sense, Holstein heifers on pastures should not only be able to express their natural behavior, but also to meet their nutritional requirements, susceptibility to the environment, climate and diseases.

At tropical pasture conditions, with low crude protein (CP) and energy contents, cattle should be supplemented to optimize performance (Paulino et al., 2004). Thus, supplementation strategies for grazing beef systems are already established, especially concerning protein supplementation in low-quality pastures (Boddey et al., 2004; Paulino et al., 2004). Figueiras et al., (2010) demonstrated positive responses of protein supplementation on grazing Holstein x Zebu steers (*Brachiaria decumbens*), and Detmann et al., (2016) found that the increased protein levels of supplement on grazing Holstein x Zebu steers (*Brachiaria decumbens*) granted a quadratic outcome on average daily gain (ADG), final body weight (FBW) and necessary days to reach 450 kg of live weight. However, the majority of studies with beef cattle supplementation was performed with *Brachiaria* grasses (Bicalho et al., 2014; Oliveira et al., 2014; Santos et al., 2017), which have low protein content even during the rainy season. Studies with Mombaça grass, with higher levels of energy and protein, even for beef cattle are scarce (Peripolli et al., 2013).

To our knowledge, there is no information about how Holstein heifers perform in tropical pasture environments, supplemented or not. It is well stablished that Holstein cows in pasture-based dairy systems have limitations that go through voluntary dry matter intake (DMI) to the forage nutrients levels, and adequate supplementation is needed to meet their production requirements (Bargo et al., 2002a; b; Kennedy et al., 2003). On the other hand, the performance level should provide conditions to breed young Holstein heifers, hence reducing age at first-parity and increasing economic profitability (Ettema and Santos, 2004). Thus, our hypothesis was that Holstein heifers will have a better performance in a high-quality tropical pasture-based system when supplemented with energetic concentrate, when compared to protein supplemented or non-supplemented animals. Therefore, this study evaluated the effects of concentrate supplementation (mineral salt supplemented, energy-supplemented, protein-supplemented) to dairy Holstein heifers on performance, muscle development, thermogenic, intake, and digestibility in a Mombaça grass (*Panicum maximum* cv. Mombaça) pasture.

MATERIAL AND METHODS

The study was conducted in the Dairy Cattle Teaching, Research and Extension unit of the Viçosa Federal University, Viçosa-MG, Brazil. Eighteen Holstein heifers were divided in two blocks and submitted to a completely randomized block design by initial body weight [BW, 188.46 ± 56.51 kg and 239.59 ± 27.30 kg for the first and the second block, respectively (mean ± SD)]. Three supplementations were randomly assigned to the animals [6 replications]. The experimental area consisted of 30 paddocks [816 m^2 of pasture area and 60 m^2 of shadow each] with a pre-established *Panicum maximum* cv. Mombaça pasture. The pasture implantation occured two years before the experiment. From the implantation to the experiment, the experimental area was managed with dairy heifers in a rotational system, irrigated and fertilized with 200 kg of N/hectare/year and 150 kg of K_2O/ hectare/year. The experimental period ranged from January 14 until May 15, 2015, corresponding to the rainy and rainy-dry transition seasons, and was divided into three measurement periods of 40 days each during summer and autumn. The animals from all treatments within one block grazed at the same paddock in a one-day rotational system, with *ad libitum* access to water and mineral salt, and the supplement was offered each day at 12:00 h, in a proportion of 0.5% BW [dry matter (DM) basis]. Prior to the experiment, a 45-days adaptation period was used, with the same management strategy adopted during the experiment. The paddocks were subdivided into two groups of 15 paddocks for each animal block. The pre-grazing height (PreGH) target was 70 cm, while the post-grazing height (PostGH) target was 35 cm. When the animals were not able to reach the paddock's PostGH, put and take heifers, with similar body weight as experimental animals, were used after the experimental animals left the paddock to reach PostGH of 35 cm. However, an unexpected decrease in rainfall in the third period determined the pre and post-grazing height, resulting in an unforeseen fall in the forage DM availability periods (Table 1, 2; Fig. 1). Pre-grazing herbage yield was determined in each grazing paddock during the digestibility sampling period, using exclusion cages [1.0 x 1.5 m, breadth x length]. The cages were placed at the representative areas (height and morphological structure) immediately before the animals obtained access to the paddock. The grass from each cage was harvested at the same PostGH and morphological structure as the remaining grass swards immediately after the animals left the paddock and weighted.

During the adaptation period a mixture of corn and soybean meal (18 % CP) was fed to all animals in a proportion of 0.5% BW (DM basis). After 45 days of adaptation period, the following treatments were assigned to animals: control [CON; animals were fed only *ad libitum* mineral mixture]; energy supplement [ENE: animals were supplemented with corn meal offered in a proportion of 0.5% BW (DM basis) and *ad libitum* mineral mixture]; protein supplement [PRO: animals were supplemented with a mixture of corn and soybean meal, 25% CP, offered in a proportion of 0.5% BW (DM basis) and *ad labium* mineral mixture].

The animals were weighed (after 12h of total fast), body measurements were taken [withers height, thoracic circumference, and body length], and the supplementation feeding corrected every 14 days according to their current body weight. All animals were separated daily at 1200h for supplement feeding to guarantee total supplement intake. All animals were conducted to individual feeders in a stable area. The CON animals were conducted together and kept separated until the animals finished their meals. The animals entered the new paddock every day just after supplement supply.

To estimate fecal excretion, 10 g/animal/day of titanium dioxide were infused orally for eight days, starting at d 34, 74, and 114 in the first, second, and third period, respectively, at 11:45 h. During the last three days of each infusion period, feces samples were taken at 6:00 h, 12:00 h, and 18:00 h, respectively. Forage, supplement and feces samples were submitted to the same chemical analyses previously described. The samples were oven-dried (55°C) for 72 h and then ground to 2 and 1 mm, using a knife mill [Willey, model TE-680, from TECNAL, Brazil] (Detmann et al., 2012). Pooled samples by period and animal of each forage, supplement and animals' feces were proportionally mixed up during the sampling days. The 1 mm samples were analyzed for DM (AOAC et al., 2005; method 934.01), neutral detergent fiber (NDF) (Detmann et al., 2012; INCT-CA method F-002/1); ash (AOAC et al., 2005; method 942.05); CP (AOAC et al., 2005; method 990.13). The 2 mm samples were analyzed for indigestible neutral detergent fiber (iNDF), incubated into the rumen of a cow over a period of 288 h, using non-woven textile bags (100 g/m^2), and NDF was determined in the post-incubation material (Valente et al., 2011). Feces were also analyzed with regards to titanium dioxide content, according to description of Detmann et al., (2012), at INCT-CA M-007/1 method.

At d 33, 73, and 113, heifers were ultrasonically scanned between the 12th and 13th ribs and the rump in the P8 region to measure the *gluteus medius* and the *biceps femoris* muscles intercession, located between the ischial and the ileal tuberosities; we used an 18-cm linear array ultrasound instrument (Aloka SSD-500V, Aloka Co., Ltda., Tokio, 196 Japan) operated at a frequency of 3.5 MHz. A standoff and vegetable oil were used to guarantee adequate acoustic contact between the transducer and the standoff as well as between these and the animals' skin. Ultrasound images were recorded and later analyzed for back fat thickness (BFT), loin depth (LD), and ribeye area (REA), using the BioSoft Toolbox® II for 200 Beef (Biotronics Inc., Ames, Iowa, USA) software. The BFT is presented in millimeters, while LD and REA are presented as squared centimeters.

Infrared thermogenic photographs of each animal´s eyes were taken at d 60 and 100 at 15:00 h to evaluate the effects of supplementation on heat production (Gomes et al., 2016). The body condition scores (BCS) were taken on the same day by three trained people, and the averages of the three measurements were used to compose the body condition score.

All variables were analyzed using the procedure MIXED of SAS (Statistical Analysis System version 9.2). Data were analyzed as a completely randomized block design, and period was included as repeated measurements in the model when necessary:

$Y_{ijke} = \mu + T_i + \beta_j + (T\beta)_{ij} + \varepsilon_{(ij)k}$,

where Y_{ijke} = dependent variable, μ = general constant, T_i = fixed effect of the treatment i, β_j = random effect of the block j, $(T\beta)_{ij}$ = random effect of interaction between treatment i and block j (removed from the model after identifying that it was non-significant for all variables), $\varepsilon_{(ij)k}$ = random error among measurements with mean 0 and variance σ^2. The interaction was tested and wasn't significant, and it was removed from the model. When necessary, least square means were compared using the Fisher's least significant difference and differences were considered significant at $p \leq 0.05$.

RESULTS

The accumulated herbage (kg DM/ha/cycle), herbage available per paddock (kg DM/paddock/cycle), herbage allowance (kg DM/animal/cycle), PreGH and PostGH decreased, and grazing efficiency (%) increased throughout experimental periods (Table 1).

The forage DM was higher in the second period, and lower in the third period and NDF and iNDF were higher in the second period, and lower in the first period (Table 2). The CP was higher in the third period, and lower in the second period and ash decreases from first to third periods. (Table 2).

We did not observe supplementation strategy × period interactions for intake measurements (P > 0.05), excepting CPI (P = 0.012; Fig. 2) and NDFI when expressed as g/kg of body weight (%BW) (NDFI/BW; P = 0.035; Fig. 3). The PRO had greater CPI (1.224, 1.187 and 1.538 ± 0.144 kg/day for first, second and third periods respectively [mean ± SE]) in all periods compared to CON (0.997, 0.968 and 1.214 ± 0.144 kg/day for first, second and third periods respectively) and ENE (0.962, 0.850 and 0.888 ± 0.144 kg/day for first, second and third periods respectively). In the third period, the ENE strategy had the lowest CPI comparing to CON and PRO (Fig. 2). The NDFI/BW of PRO and ENE decreased in the third period compared to CON (Fig. 3).

Supplementation strategies affected all intake variables. The PRO supplementation strategy had greater DM intake (DMI) (7.172 ± 0.686 kg/day; P = 0.045) compared to ENE (5.904 ± 0.683 kg/day); greater forage DM intake (FDMI) (5.747 ± 0.535 kg/day; P = 0.006) compared to ENE (4.823 ± 0.535 kg/day); greater NDF intake (NDFI) (3.574 ± 0.253 kg/day; P = 0.012) compared to ENE (2.895 ± 0.253 kg/day); and greater digestible energy intake (DEI) (4.827 ± 0.520 kg/day; P = 0.045) compared to ENE (3.937 ± 0.518 kg/day) and CON (4.032 ± 0.530 kg/day) (Table 3).

The supplementation strategies also affected forage DMI (FDMI/BW). The CON supplementation strategy had greater FDMI/BW (26.31 ± 2.01 g/kg of BW; P = 0.049) compared to PRO (22.02 ± 1.89 g/kg of BW) and ENE (24.01 ± 1,86 kg/day).

A period effect was observed, and DMI (DMI/BW), FDMI/BW, CPI (CPI/BW), and DEI (DEI/BW) were greater in the first period when compared to the second and the third periods (Table 3).

The supplementation strategies did affect digestibility of DM (DMD) and CP (CPD) ($P<0.05$; Table 3). The PRO promoted a greater DMD (0.723 ± 0.008 g/g of DM) when compared to CON (0.689 ± 0.008 g/g of DM). The ENE negatively affected CPD (0.761 ± 0.013 g/g of DM) compared to PRO (0.817 ± 0.013 g/g of DM) and CON (0.801 ± 0.013 g/g of DM). Nevertheless, there were period effects for all digestibility variables. The DMD was lower in the second period (0.685 ± 0.008 g/g of DM) compared to the first (0.714 ± 0.008 g/g of DM) and third periods (0.714 ± 0.008 g/g of DM). The NDF digestibility (NDFD) and CPD were greater in the third period (0.718 ± 0.007 and 0.816 ± 0.013 g/g of DM to NDFD and CPD respectively) compared to the first (0.680 ± 0.007 and 0.787 ± 0.013 g/g of DM to NDFD and CPD, respectively) and second periods (0.670 ± 0.007 and 0.775 ± 0.013 g/g of DM to NDFD and CPD, respectively).

The average daily gain (ADG) and thoracic circumference gain (TCG) were greater for PRO (0.570 ± 0.051 kg/day and 0.110 ± 0.020 cm/day to ADG and TCG, respectively) ($P<0.05$) when compared to CON (0.308 ± 0.052 kg/day and 0.061 ± 0.021 cm/day to ADG and TCG, respectively) and ENE (0.346 ± 0.051 kg/day and 0.052 ± 0.021 cm/day to ADG and TCG, respectively). Additionally, there was a period effect on both ADG and TCG, and a greater performance was observed in the first (0.544 ± 0.065 kg/day and 0.112 ± 0.021 cm/day to ADG and TCG, respectively) and second (0.512 ± 0.065 kg/day and 0.087 ± 0.021 cm/day to ADG and TCG, respectively) periods when compared to third period (0.169 ± 0.065 kg/day and 0.024 ± 0.021 cm/day to ADG and TCG respectively).

There was a supplementation strategy × period interaction for LD and BFT ($P<0.05$; Fig. 4). The LD was greater to PRO only in the second and third periods (55.073 and 66.006 ± 5.575 cm for second and third periods, respectively) compared to CON (44.977 and 55.077 ± 5.575 cm for second and third periods, respectively). The BFT was greater to ENE compared to CON in the second period, and PRO animals had greater BFT than CON in the third period. The supplementation strategy did not influence ribeye eye area (REA), ribeye fat thickness (RFT), back fat thickness (BFT) (Table 4).

Eventually, the supplementation strategy did not affect animals' eye temperature, although in the third period all temperature measurements were lower ($P < 0.01$; Table 5). The BCS was greater to PRO ($P=0.02$) (3.429 ± 0.113) compared to CON and ENE (3.238 and 3.037 ± 0.113 to CON and ENE respectively).

DISCUSSION

The weather condition of the experiment period (Fig. 1) led the pasture to lower herbage availability during the second and third period (Table 1). Silveira et al., (2010) showed in a similar geographic and weather conditions as our study, that decreasing precipitation and temperature will promote less water availability in the soil, decreasing forage production, especially Mombaça grass. The authors pointed out that this condition affected herbage total production, tillers and number of new leaves. In these swards characteristics, there will be an increase oinf NDF, iNDF and CP, as was observed in our study (Table 2).

Protein supplementation resulted in greater DMI compared to ENE supplementation. We suggest that protein supplementation provided a better ruminal environment, with the N from the supplement supporting the rumen microbiome development, thereby contributing to a greater DMI and ADG (Table 3, 4). Ortiz et al. (2002) had similar results when comparing Zebu steers supplemented with protein (PS) containing slow release urea (22%CP) and a commercial supplement (CS) with 18% CP. The PS promoted greater DMI and ADG and, according to the authors, PS provided greater microbial protein synthesis.

There is a strong relationship between nitrogen intake and DMI (Figueiras et al., 2016). Mombaça grass has a rapid growth ratio, with an intensive energy concentration. That characteristic associated with the unbalance protein:energy ratio of the diet caused by ENE supplementation in our study, impacted DMI, resulting in differences in animal performance. Detmann et al., (2014) pointed that the unbalanced diet regard to protein:energy could lead to an increased metabolic discomfort when the energy of the diets is in excess. In that sense, the forage replacement by the supplement usually is greater when energetic supplements are supplied, as it was observed in our study (Table 3 and 6). The substitution rates caused by the ENE unbalanced diet is clear when we observe greater NDFI and FDMI of the PRO animals compared to ENE. In addition, De Oliveira Franco et al., (2017) demonstrated that grazing animals fed nitrogen sources had an increased nitrogen balance, efficiency of nitrogen used and higher IGF1 serum concentration, promoting an anabolic effect. Perhaps, the effect of protein:energy ratio of PRO diet led those animals to higher serum concentration of IGF-1, with this same anabolic effect, resulting on greater DMI and consequently greater ADG.

The PRO supplementation strategy provided greater CPI in all periods compared to CON and ENE (Fig. 2). Most likely, the protein from supplement has stimulated forage intake, affecting final DMI and DEI when PRO are compared to CON and ENE. To our knowledge, there is no literature data on the effects of protein vs energy supplementation on dairy Holstein heifers at high-quality tropical pasture on intake and intake behavior. Moraes et al., (2006) also evaluating protein levels in the supplement for grazing crossbred (Holstein × Zebu) steers (1 kg of DM/ animal with 8, 16 and 24% of CP supplement; *Panicum maximum* cv. Mombaça) observed a linear effect of protein supplementation on ADG. Although the authors did not evaluate pasture intake, the similar responses present on this study suggests that protein supplementation will have the same impact on crossbred animals.

There were differences between ENE compared to PRO and CON on FDMI and NDFI. It is notorious how ENE supplement strategy affected animals' intake. The substitution rate of ENE animals (Table 6) is likely the main reason for the effects observed on FDMI and NDFI. With a decrease in forage quality across periods (Table 2), and lower herbage allowance (Table 1), ENE animals increased the substitution rate, instead of compensating that on intake of forage. Differently, PRO animals showed the opposite behavior. Hannah et al., (1991) and Detmann et al., (2004) mentioned that with a decrease in forage allowance, the effect on forage intake behavior (substitution rates) could be minimized by feeding protein supplements, and this theory was confirmed in our study. Additionally, as result of the higher density of ENE diet provided by the supplement, DEI and DMI did not differ among ENE and CON, despite of higher FDMI of CON. The PRO animals consumed more digestible energy and CP than CON, probably due to the same reason (the nutrients concentration provided by the supplement) mentioned above.

The high energy density from corn in ENE supplement may have influenced to a decrease in forage intake, which was reflected in similar DEI values between CON and ENE (Table 6). Dixon and Stockdale (2000) reported that substitution rates could happen when specific nutrients interact in the rumen. When the forage has a good protein source, higher animal performance may be limited by the diet's energy density (Dixon and Stockdale, 2000; Moraes et al., 2006) and the supplementation could be formulated with low-protein feedstuffs such as corn meal.

Nevertheless, the interaction between supplement and animal FDMI should be carefully observed to control situations where the supplemented animals decrease their DMI even when the grass have high quality, as observed for ENE animals in the third period (Table 6). The substitution rates usually range from 0 when the supplement did not affect forage intake, to 1.0, where the forage intake decrease by the same amount as the supplement intake (DM basis) (Bargo et al., 2003). In our case, the substitution rate was calculated based on CON FDMI. Here, ENE had a substitution rate of 0.60, 1.21 and 1.88 of substitution rates for first, second and third periods respectively, and substitution rates of 0.27, 0.53 and 0.17 to the first, second and third periods respectively to PRO (Table 6) due to pasture characteristics and the supplement effects on substitution rate described above.

That means the ENE had more intensive depression on FDMI probably because of nutrients interact inside the rumen, due to a possible increased retention time (Grigsby et al., 1993) or the effects on grazing behavior as the substitution rates described above

(Hannah et al., 1991; Detmann et al., 2004). The ENE supplementation strategy was based in corn meal exclusively and may not provide limiting nutrients for fiber digestion such as nitrogen or sulfur (De Moraes et al., 2006). Furthermore, the competition for essential subtract inside rumen between fibrolytic and amylolytic bacteria's will further the amylolytic bacteria because of an faster degradation rate of starch (Dixon and Stockdale, 2000). That will increase the retention time of fibrous residue and consequently decreased forage intake (Grigsby et al., 1993; De Moraes et al., 2006; Carli Costa et al., 2009).

The CP from the supplement of ENE (Table 2) and the observed substitution rates (Table 6) may elucidate how DEI between CON and ENE were not different, as well as why CPI was greater for CON then ENE. The ENE supplement (corn meal) has a lower amino acids' quality when compared to PRO, with slower ruminal fiber degradation rate and consequently higher ruminal retention time (Hoover, 1986; Grigsby et al., 1993; Dixon and Stockdale, 2000). This fact might have contributed to lower CPI for ENE, once that the forage had higher %CP and ENE had lower FDMI. Furthermore, supplements always have greater digestible energy than forage (Grigsby et al., 1993). Comparing both CON and ENE, CON compensate their absence of supplementation with greater FDMI, and ENE had their DEI increased by the higher DE from supplement, compensating ENE lower FDMI and balancing their DEI.

The observed substitution rates among our supplementation strategies also helped to understand the supplementation strategy x period interaction in CPI (Fig. 2). There was a greater CPI in PRO in all periods (Fig. 2; Table 3), and no differences between CON and ENE were observed in the first and the second periods, but ENE CPI was lower than CON in the third period. As seen in Table 6, ENE substitution rates were greater in the second and the third periods, which may also be a consequence of lower forage allowance in that same period (Table 1). The PreGH of 54.51 cm in third period was the lowest compared to other periods. Palhano et al. (2007) evaluated the intake behavior of Holstein heifers gazing Mombaça and the sward characteristics, and showed that when the PreGH was 60cm, the CP was 19% and the animals decreased FDMI compared to PreGH of 80, 100, 120, 140 cm. Even though, the authors showed an increase time speared gazing with less herbage intake by animals, due to the lower herbage allowance. It is possible that the same happened especially with ENE animals in our study, once CON animals were already more adapted to grazing condition due to absence of supplementation. Nevertheless, new studies must have done to evaluate Holstein heifers' grazing behavior in different strategies supplementation.

When intake measurements were expressed as percentage of BW, only FDMI/BW was affected by the supplementation strategy. The FDMI/BW was greater for CON compared to ENE and PRO, probably due to a compensatory behavior trying to compensate the lack of supplement in this treatment. It was our hypothesis that ENE supplementation would stimulate forage digestion, leading to an increase in FDMI; however, this behavior was not observed, and we rejected our hypothesis. The same effect was found by Silveira et al. (2008) when animals exclusively fed hay had a greater hay intake (%BW) compared to animals with energy or protein supplementation. Furthermore, PRO was not different from ENE in terms of FDMI/BW, probably because of a greater body gain (Table 4), which decreased the proportion of FDMI per kg of BW since substitution rates in PRO were not as intense as in ENE (Table 6). Similarly, Silveira et al. (2008) showed greater hay DMI/BW for the control group (as described above); according to their data, the supplemented groups decreased hay DMI/BW by about 33% when compared to the supplemented groups and in our study, the decrease in forage intake (%BW) was 15.32% and 11.53% for PRO and ENE, respectively.

There was a period effect on intake measurements expressed by BW, which was greater in the first period compared to the second and the third periods (Table 3). We observed a lower DMI (%BW) in the third period due to a lower forage availability. The greater sward height in the first period (Tables 1, 2) was an effect of the climate, with higher temperatures and rainfall stimulating pasture growth (Fig. 1). Thus, grazing efficiency was lower in the first period (68.81%), indicating a greater grass selection by the animals, which might have led to the decreased DMI/BW.

Nevertheless, there was a supplementation strategy x period interaction in NDFI/BW (Fig. 3). The main difference occurred in the third period, where CON had a greater NDFI/BW compared to PRO and ENE. The possible explanation could be the forage substitution rate of PRO and ENE, associated to the compensatory behavior of non-supplemented animal (CON), leading to a greater FDMI (%BW). On the other hand, the NDFD of the third period was greater, which may have stimulated CON animals to have a greater FDMI/BW and NDFI/BW than those in PRO and ENE supplementation. The better NDFD in this period is likely associated to a low grass growth rate. With the absence of rainfall and with lower temperatures, grass re-growth was at the limit of the number of animals possible per paddock, with a grazing efficiency of 104.25% (Table 1), indicating that the animals obtained access to the paddock with smaller and more digestible leaves. The grazing efficiency above 100% also indicated that animals had to graze below the pre-defined post grazing height (33.45 cm), which may be linked to an excessive removal of steams and a better quality during re-growth and during the digestibility sampling period (Palhano et al., 2007). At this condition, the grass had a better digestibility of DM, NDF, and CP (Table 3).

The supplementation strategies did affect DMD and CPD. The PRO supplementation had a greater DMD compared to CON, and we suspect that protein supplementation could have provided a better degradability comparing the PRO and CON diets. Indeed, supplements (concentrates) have higher digestibility than forages (Grigsby et al., 1993) and it is expected to the protein supplemented increases DMD and CPD. As was observed by De Oliveira Franco et al., (2017), the supplementation was the main responsible for increases on DMD and CPD. Once we did not observe differences among CON and PRO to NDFD, the supplements intake is likely the responsible for an increase in DMD and CPD of PRO compared to CON. On the other hand, the CPD differences of ENE compared to PRO and CON may stand at the ruminal environment provided by ENE supplement. The greater proliferation of non-fiber carbohydrate-fermenting microorganisms may increase the nitrogen need (Hoover, 1986). When N availability in the rumen is not in synchronism, it could result in nitrogen limitation for microbial protein synthesis, which may affect the digestibility of the nutrients (Dixon and Stockdale, 2000; De Moraes et al., 2006; Carli Costa et al., 2009). In addition, there is no data in the literature that had evaluated the DMD and CPD of Mombaça grazing animals when supplemented. Nevertheless, Silveira et al. (2008), observed an increase in organic matter digestibility when ruminal degradable protein went from 61.17% to 69.73%. Furthermore, the CPD was greater in PRO supplementation strategy compared to ENE, but PRO did not differ of CON. We suspect that the greater CPD of CON compared to ENE is mainly due to changes in microorganisms' population in the rumen ENE rumen due to bacterial subtract competition (Grigsby et al., 1993; Carli Costa et al., 2009). The energy supplementation could drive to a amylolytic bacterial selection inside the rumen (Hoover, 1986; Grigsby et al., 1993; Carli Costa et al., 2009), compromising the microbial growth and digestibility of protein, as it was described above.

The PRO supplementation strategy positively affected ADG and TCG (Table 4). The larger supply of amino acids in the PRO diet, with greater energy intake, result in a greater ADG in PRO animals. Moraes et al., (2006) observed a linear increase in ADG in crossbred steers fed up to 24% CP in the supplement. According to Moraes et al. (2006), animals fed protein supplement had greater ADG because of synchronism between ruminal fermentable organic matter and N utilization by rumen microorganisms for microbial synthesis and consequently greater amino acid availability for intestine absorption. The results from Moraes et al. (2006) showed that with 24% CP, it is possible to obtain a better performance and body development when a protein supplement is fed even in high quality-tropical pastures such as Mombaça pastures.

Furthermore, there was a period effect on ADG, TCG, and HG, and a lower performance was observed in the third period (Table 4); in addition, there was an effect on BSC in the second evaluation (between second and third periods) (Table 5). These responses confirm the decrease in pasture availability due to climatic conditions in the second half of the experiment; they also show that the animals were not able to maintain their performance to face this environmental challenge.

We observed a supplementation strategy x period interaction in LD, which happened mainly in second and third periods. The LD differences occurred only between PRO and CON, and it was greater to PRO at both periods. These differences may stand at the PRO CPI greater in all periods linked with lower CPI (%BW) at second and third period. The protein supplementation may support a greater protein synthesis in animal's muscles (Rosenvold et al., 2001), which reflected in a bigger LD in PRO animals. We also observed a supplementation strategy x period interaction in BFT, which also occurred mainly in the second and third periods. At second period, BFT differences occurred among CON and ENE, and it was greater to ENE. At third period, PRO animals had greater BFT than CON. It seems that in the first two periods ENE animals focused their performance on fat deposition, which may also be linked the lack of N in the rumen and, thus, high supply of energy but not enough N to support protein deposition. In the third period, it is possible that the fall in pasture availability lead to the use of this fat deposited to support the requirements of those animals. On the other hand, PRO and CON animals had almost constant deposition of BFT, but it was greater for PRO, for the reasons already discussed above. Other reasonable explanation for BFT results of PRO is that the association of protein supplementation of PRO [N source for rumen microbial population

(Carli Costa et al., 2009)] and greater NDFD of third period may have stimulated the propionic acid production in the rumen, that will stimulate more fat synthesis in PRO rather than CON (Rooney and Pflugfelder, 1986; Igarasi et al., 2008).

The supplementation strategy did not affect body temperature, but a period effect was observed. The lower temperature on d 100 perhaps was directly linked to the lower environmental temperature (Fig. 1). The maximum and minimum day temperatures were 31 and 28.4 ºC; 17.5 and 13.5 ºC in d 60 and d 100, respectively. The air temperature at the photography moment was 30 and 27.6 ºC in d 60 and d 100, respectively. We hypothesized that PRO animals, with a greater protein deposition, could have been linked to a greater heat production. Gomes et al. (2016) demonstrated that ocular temperate may be linked to animal's heat production, thus we rejected our hypothesis. Despite environmental temperature helped explain the lower ocular temperature at d 100, more studies should be done to evaluate the interaction between ocular temperature by thermal infrared camera and gazing animals' heat production.

In summary, the PRO supplemented animals showed better body development, with greater intake compared to ENE and greater CPI, DEI, ADG and TCG than both CON and ENE. Better performance of PRO was also observed, and indeed, when compared to CON and ENE, PRO supplementation led animals to better associative benefits between forage and supplement intake. Therefore, dairy heifers should be supplemented when grazing high quality Mombaça grass at rotational pasture system in the rainy and rainy-dry transition seasons.

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Table 1 Herbage characteristics and Height of 15 days (average days of cycle) of grazing activities

Item	Period		
	1[3]	2[4]	3[5]
Accumulated herbage (kg DM/ha/cycle)	1727.34	1485.42	840.12
Accumulated herbage (kg DM/paddock/cycle)	144.41	110.92	82.84
Herbage DM allowance (kg DM/animal/cycle)	8.02	6.16	5.37
Grazing efficiency	68.81%	87.95%	104.25%
PreGH[1] (cm)	75.34	81.51	54.51
PostGH[2] (cm)	51.96	46.56	33.45

[1]Pre-grazing Height; [2]Post-grazing Height.
[3]January 14, 2016 to February 23, 2016; [4]February 24, 2016 to April 4, 2016; [5]April 5, 2016 to May 15,2016.

Table 2 Chemical composition (DM basis) of pasture and supplements throughout periods

Item	Period			Supplements	
	1[4]	2[5]	3[6]	PRO	ENE
DM (%)	25.29	28.24	23.65	87.94	89.08
NDF[1] (%DM)	55.04	59.02	58.84	19.86	12.13
iNDF[2] (%DM)	8.68	9.90	8.92	0.92	0.95
CP[3] (%DM)	16.70	15.52	18.11	25.74	8.09
Ash (%DM)	11.18	10.65	10.53	4.24	1.47

[1]Neutral Detergent Fiber; [2]Indigestable Neutral Detergent Fiber; [3]Crude Protein;
[4]January 14, 2016 to February 23, 2016; [5]February 24, 2016 to April 4, 2016; [6]April 5, 2016 to May 15,2016.

Table 3 Intake and digestibility of grazing Holstein heifers fed no supplement or fed protein or energy supplement during rainy season.

Item	Supplement strategies			Period			SEM	P-value		
	CON[14]	PRO[15]	ENE[16]	P1[17]	P2[18]	P3[19]		Treat	Per	Int
					Intake, kg/day					
DMI[1]	6.314 ab	7.172 a	5.904 b	6.298	6.403	6.689	0,671	0.045	0.340	0.094
FDMI[2]	6.289 a	5.747 a	4.823 b	5.567	5.496	5.795	0.529	0.006	0.528	0.074
NDFI[3]	3.635 a	3.574 a	2.895 b	3.182	3.378	3.544	0.247	0.012	0.088	0.054
CPI[4]	1.060 b	1.316 a	0.900 c	1.061 B	1.002 B	1.214 A	0.144	<0.001	<0.001	0.012
DEI[5]	17.777 b	21.282 a	17.358 b	18.566	18.059	19.796	2.235	0.045	0.089	0.300
CPI/DEI	0.262	0.272	0.228	0.299	0.244	0.270	-	-	-	-
					Intake, g/kg of BW					
DMI/BW[6]	26.080	26.810	28.030	29.040 A	25.750 B	26.130 B	1.413	0.653	0.016	0.168
FDMI/BW[7]	0.026 a	0.022 b	0.023 b	0.025 A	0.022 B	0.023 B	0.002	0.049	0.031	0.088
NDFI/BW[8]	14.920	12.497	13.541	14.985 A	13.664 A	12.309 B	1.123	0.220	0.001	0.035
CPI/BW[9]	4.202	4.634	4.072	4.882 A	3.957 B	4.069 B	0.203	0.221	<0.001	0.211
DEI/BW[10]	0.044	0.074	0.078	0.085 A	0.071 B	0.0678 B	0.003	0.578	0.001	0.070
					Digestibility, g/g					
DMD[11]	0.689 b	0.723 a	0.702 ab	0.714 A	0.685 B	0.714 A	0.008	0.040	<0.001	0.293
NDFD[12]	0.691	0.696	0.682	0.680 B	0.670 B	0.718 A	0.007	0.529	<0.001	0.439
CPD[13]	0.801 a	0.817 a	0.761 b	0.787 B	0.775 B	0.816 A	0.013	<0.001	<0.001	0.407

[1]Total dry matter intake; [2]Forage Dry Matter Intake; [3]Neutral Detergent Fiber Intake; [4]Crude Protein intake; [5]Digestible Energy Intake (Mcal/day); [6]Dry Matter Intake per Body Weight; [7]Forage Dry Matter Intake per Body Weight; [8]Neutral Detergent Fiber Intake per Body Weight; [9]Crude Protein Intake per Body Weight; [10] Digestible Energy Intake per Body Weight (Mcal/day/kg of BW); [11]Dry Matter Digestibility; Neutral Detergent Fiber Digestibility; [13]Crude Protein Digestibility [14]Control Group; [15]Protein Supplement Group; [16]Energetic Supplement Group;
[17]January 14, 2016 to February 23, 2016; [18]February 24, 2016 to April 4, 2016; [19]April 5, 2016 to May 15,2016.
Different lowercase letters in the same row indicate difference between supplement strategy and different uppercase letters in the same row indicate difference between period at $P < 0.05$.

Table 4 Performance and ruminal pH of grazing Holstein heifers fed no supplement or fed protein or energy supplement during rainy season.

Item	Supplementation strategy			Periods			SEM	P-value		
	CON[10]	PRO[11]	ENE[12]	P1[13]	P2[14]	P3[15]		Treat	Per	Int
ADG[1] (kg/d)	0.308 b	0.570 a	0.346 b	0.544 A	0.512 A	0.169 B	0.065	0.007	0.001	0.083
TCG[2] (cm/d)	0.061 b	0.110 a	0.052 b	0.112 A	0.087 A	0.024 B	0.021	0.030	<0.001	0.253
WHG[3] (cm/d)	0.056	0.058	0.055	0.079 A	0.059 A	0.031 B	0.008	0.956	0.015	0.080
BLG[4] (cm/d)	0.096	0.098	0.090	0.138	0.064	0.083	0.025	0.972	0.093	0.290
REA[6] (cm^2)	28.894	32.861	27.661	30.916	29.568	28.932	5.314	0.547	0.056	0.068
RFT[7] (mm)	0.913	0.997	1.028	0.907	1.039	0.991	0.079	0.665	0.254	0.349
BFT[8] (mm)	1.126	1.368	1.319	1.162	1.309	1.342	0.090	0.163	0.122	0.030
LD[9] (cm)	48.708	56.676	50.988	47.427 B	49.690 B	59.255 A	5.575	0.140	<0.001	0.027

[1]Average daily gain; [2]Thoracic circumference gain; [3]Withers Height gain; [4]Body length gain; [6]Ribeye area; [7] Ribeye fat Thickness; [8]Back fat Thickness; [9]Loin deep; [10]Control Group; [11]Protein Supplement Group; [12]Energetic Supplement Group
[13]January 14, 2016 to February 23, 2016; [14]February 24, 2016 to April 4, 2016; [15]April 5, 2016 to May 15,2016.
Different lowercase letters in the same row indicate difference between supplement strategy and different uppercase letters in the same row indicate difference between period at $P < 0.05$.

Table 5 Eye temperature and Body Score Condition (BSC) of grazing of Holstein heifers fed no supplement or fed protein or energy supplement during rainy season.

Item	Treatments			Period		SEM	P-value		
	CON[5]	PRO[6]	ENE[7]	d 60[8]	d 100[9]		Treat	Per	Int
ETmax[1] (ºC)	37.043	37.325	37.550	38.682 A	35.93 B	0.486	0.276	<0.001	0.692
ETmin[2] (ºC)	34.786	35.416	35.241	36.68 A	33.60 B	0.371	0.483	<0.001	0.887
ETavg[3] (ºC)	36.139	36.416	36.483	37.709 A	34.983 B	0.423	0.533	<0.001	0.723
BCS[4]	3.238 b	3.429 a	3.037 c	3.212 B	3.257 A	0.113	0.026	0.531	0.766

[1] Maximum eye temperatures; [2] Minimum eye temperatures; [3]Average eye Temperatures; [4]Body condition score; [5]Control Group; [6]Proteic Supplement Group; [7]Energetic Supplement Group; [8]Medial day between 1st and 2nd period; [9] Medial day between 2nd and 3rd period.

Different lowercase letters in the same row indicate difference between supplement strategy and different uppercase letters in the same row indicate difference between period at $P < 0.05$.

Table 6 Substitution rate of supplementation strategies across periods.

Period	Substitution rate[6]	
	PRO[4]	ENE[5]
1[1]	0.27	0.60
2[2]	0.53	1.21
3[3]	0.17	1.88

[1]January 14, 2016 to February 23, 2016; [2]February 24, 2016 to April 4, 2016; [3]April 5, 2016 to May 15,2016.; [4]Proteic Supplement Group; [5]Energetic Supplement Group;
[6] % of forage DMI = to supplement DMI.

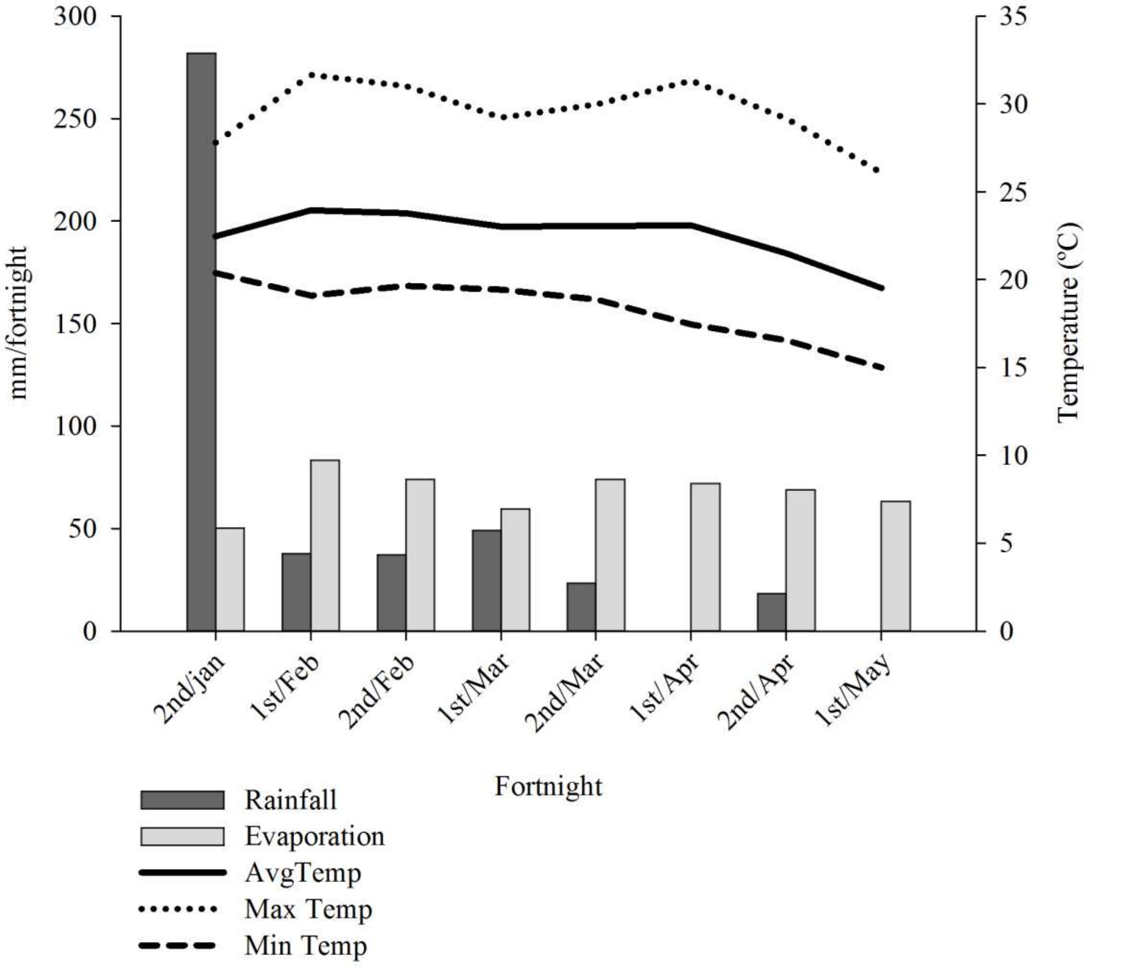

Figure 1 Accumulate fortnightly rainfall and evaporation (mm); minimum, average and maximum (ºC) temperatures during the experiment.

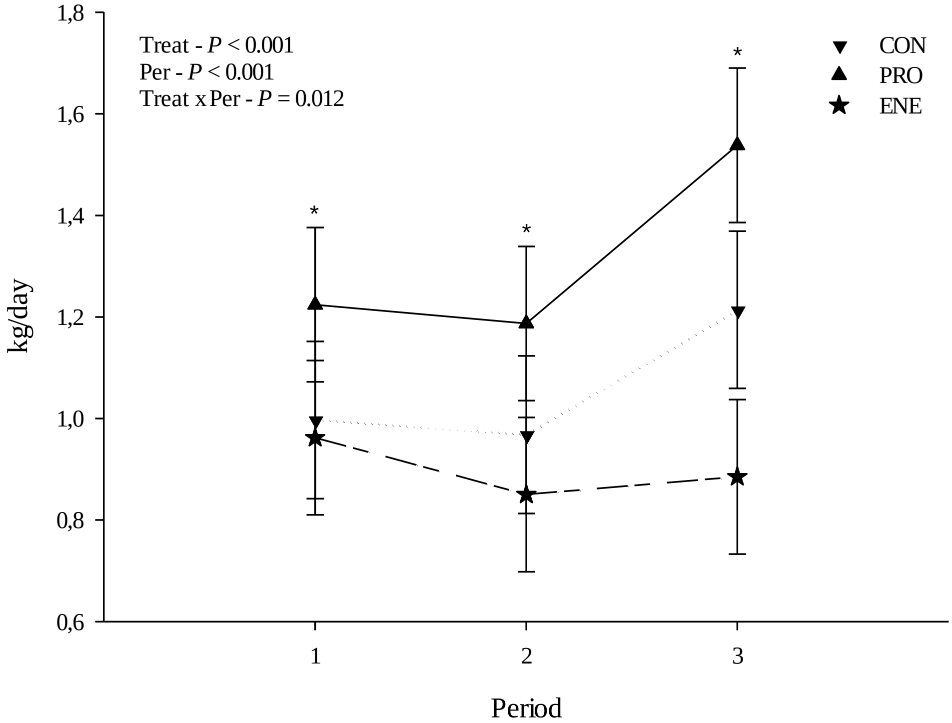

Figure 2 Crude Protein Intake of grazing Holstein heifers fed mineral supplement (CON) or fed protein (PRO) or energy (ENE) supplement through three periods in the rainy season.
*Indicative of significance in the period ($P < 0.05$).

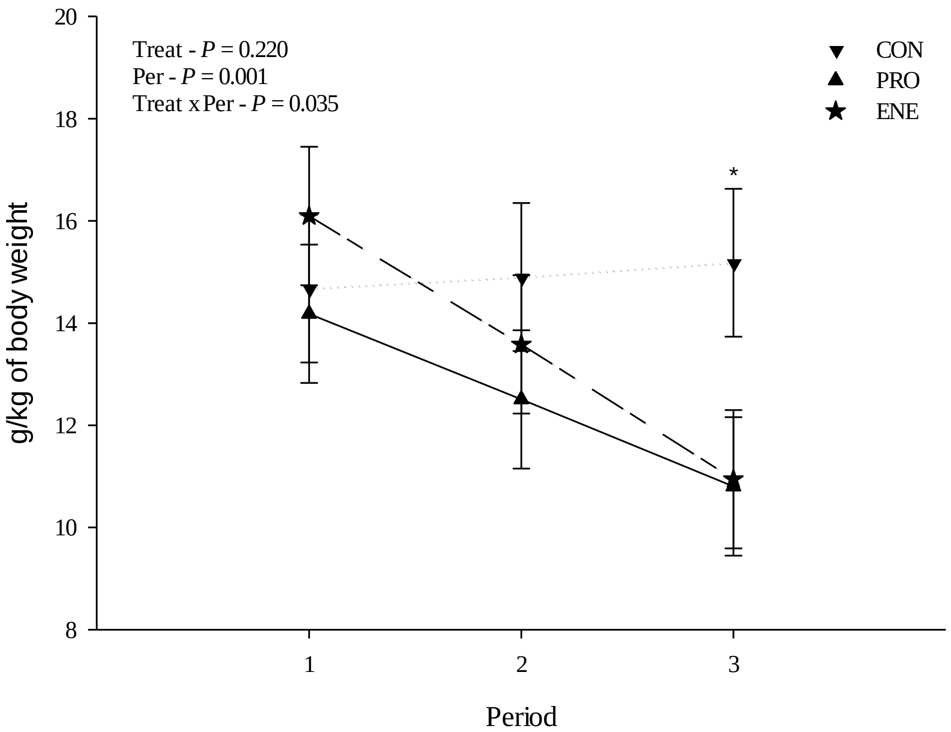

Figure 3 Neutral Detergent Fiber Intake (g/kg BW) of grazing Holstein heifers fed no supplement (CON) or fed protein (PRO) or energy (ENE) supplement through 3 periods in the rainy season.
* Indicative of significance in the period (P < 0.05).

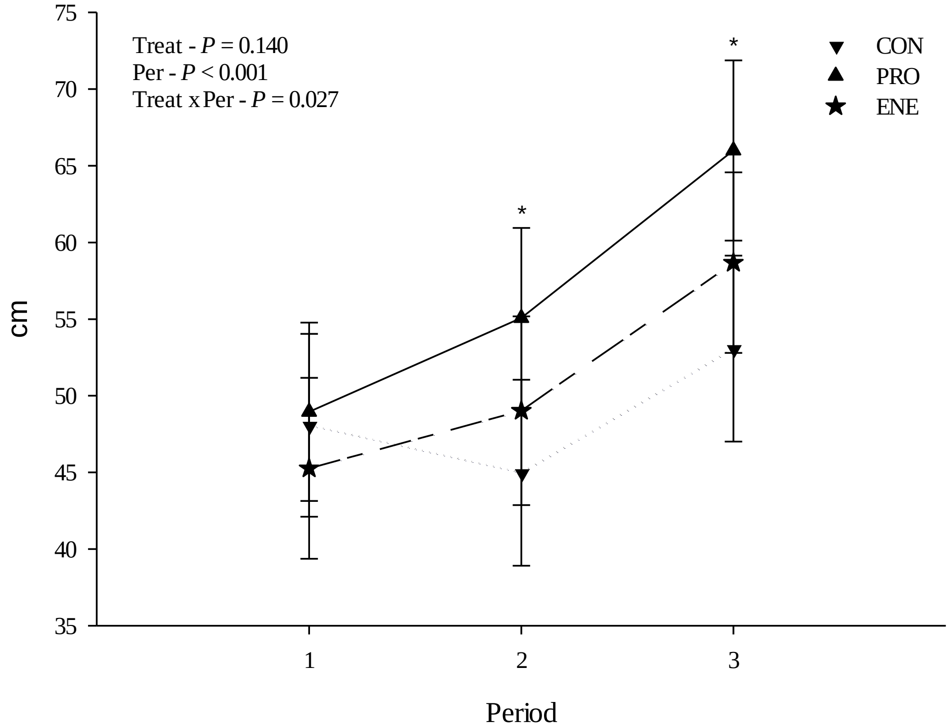

Figure 4 Loin Deph of grazing Holstein heifers fed no supplement (CON) or fed protein (PRO) or energy (ENE) supplement through three periods in the rainy season. * Indicative of significance in the period (P < 0.05).

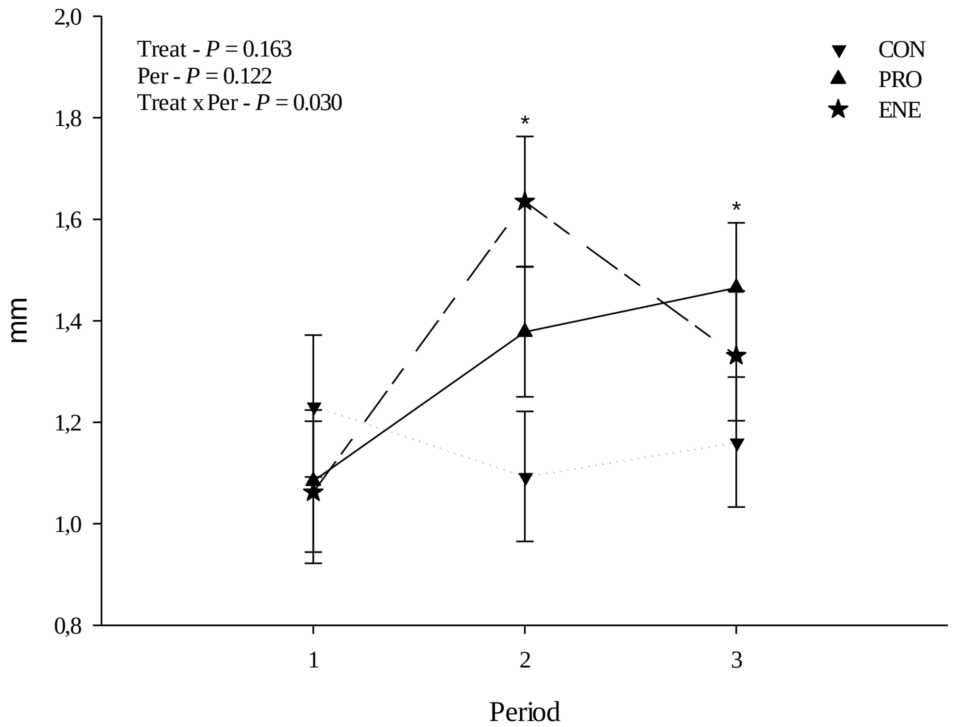

Figure 5 Back Fat Thickness of grazing Holstein heifers fed no supplement (CON) or fed protein (PRO) or energy (ENE) supplement through three periods in the rainy and transition rainy-dry season.
* Indicative of significance in the period (P < 0.05).