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ORIGINAL ARTICLE

Energy and protein requirements for growth of Holstein × Gyr heifers

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Summary

There is little information regarding the nutritional requirements for dairy heifers, leading the majority of nutrient requirement systems to consider dairy heifers to be similar to beef heifers. Therefore, we evaluated the muscle protein metabolism and physical and chemical body composition of growing Holstein × Gyr heifers and estimated the energy and protein requirements. We performed a comparative slaughter experiment with 20 Holstein × Gyr heifers at an initial body weight of 218 ± 36.5 kg and an average age of 12 ± 1.0 months. Four heifers were designated as the reference group, and the 16 remaining heifers were fed *ad libitum*. The 16 heifers were distributed using a completely randomized design in a 2 × 2 factorial arrangement with two roughages (corn silage or sugarcane) and two concentrate levels (30 or 50%) for 112 days. Greater ($p < 0.05$) values for fractional rates of muscle protein synthesis, degradation and accretion were observed for heifers that were fed 50% concentrate. The following equations were obtained to estimate the net energy for gain (NE_g) and net protein for gain (NP_g): NE_g (Mcal/day) = $0.0685 \times EBW^{0.75} \times EBWG^{1.095}$ and NP_g (g/day) = $203.8 \times EBWG - 14.80 \times RE$, respectively, in which EBW is the empty body weight, EBWG is the empty body weight gain and RE is the retained energy. We concluded that increased rates of protein *turnover* are achieved when a greater quality diet is provided. In the future, these results can be used to calculate the nutritional requirements for growth of Holstein × Gyr heifers after equation validation rather than using the recommendations provided by other systems, which use values developed from beef heifers, to determine the nutritional requirements of dairy cattle.

Keywords comparative slaughter, protein *turnover*, sugarcane, tissue deposition

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Introduction

Raising replacement heifers is one of the most important steps in sustaining a milk production system (Oliveira and Ferreira, 2016). However, in some countries, this phase is often neglected and receives little attention (Nor et al., 2015). The high cost of animals may explain the small number of dairy heifer studies and is likely the main reason for the lack of information regarding their nutritional requirements. In Brazil, the NRC (2001) is the main system dictating the nutritional requirements of dairy cattle and considers the nutritional requirements of dairy heifers to be similar to those of beef heifers. However, the NRC (2001) notes that the validation of these equations is practically impossible due to the small amount of data from dairy animals in the growing phase.

In Brazil, the majority of milk production is from cross-bred animals (Facó et al., 2002). The individual characteristics of specific breeds, such as the productivity of Holsteins and the hardiness, heat tolerance and parasite resistance of Gyr (Silva et al., 2011; Santana et al., 2014), make cross-breeding very important in Brazilian dairy production systems (Facó et al., 2002). Therefore, the establishment of proper nutritional requirements for these animals is necessary and consequently ensures the subsequent productive performance of the animals.

In this context, it is important to identify the growth patterns of different body components as well as the factors influencing animal growth and development. During growth, skeletal muscle tissue has the greatest influence on increases in body mass and provides a greater mass gain per unit deposited than fat or bone.

Moreover, its growth is subject to manipulation because protein storage is dynamic and adapts to different physiological conditions (Velloso, 2008).

Protein deposition is influenced by nutrition (Bergen, 1974; Lobley et al., 1980). Poor-quality diets that are unbalanced or deficient in some nutrients may promote a decrease in protein turnover rates (Garlick et al., 1975; Millward et al., 1975) and consequently reduce the rate of BW gain rates, which can result in a later first calving age than that observed in animals fed good-quality diets. Although sugarcane is considered poor-quality roughage due to the low concentration of crude protein (CP) and minerals (Leng, 1988; Rotta et al., 2014), studies have shown that a 20% increase in dietary concentrate levels can correct these deficiencies, resulting in similar performance in beef and dairy cattle as cattle fed corn silage (CS) diets (Costa et al., 2005; Rotta et al., 2014), which is one of the most widely used roughages in Brazil (Millen et al., 2009).

This is the first study estimating the nutritional requirements of Holstein × Gyr heifers. The hypothesis of this study was that a 20% increase in the concentrate level of sugarcane diets increases protein turnover in Holstein × Gyr heifers. Therefore, the objective was to estimate the energy and protein requirements for growth and to evaluate physical and chemical body composition growth [muscle tissue, bone tissue, fat tissue, CP, ether extract (EE), minerals and water] and muscle protein metabolism in Holstein × Gyr heifers.

Materials and methods

The experiment was conducted in the Animal Feedlot of the Animal Science Department of the Universidade Federal de Viçosa, Viçosa, Minas Gerais. Care procedures for the heifers during the experiment followed the protocols approved by the rules of the Ethics Committee on Farm Animal Use (CEUAP) under the protocol 95/2014.

Animal management, experimental design and diets

Twenty Holstein × Gyr heifers at an average age of 12 ± 1.0 month and with an average initial BW of 218 ± 36.5 kg were used in this study. In Brazilian dairy systems, heifers are usually raised on poor-quality pasture that generates a BW gain from 0.2 to 0.4 kg per day on average (Pereira et al., 2010; Paciullo et al., 2011), and the heifers used in this study were managed similarly prior to the trial.

The experiment was performed with a completely randomized design using a 2 × 2 factorial scheme with two roughages (CS or sugarcane) and two concentrate levels (30 or 50%) in the total DM diet. The experiment lasted 142 days with 30 days for the animals to acclimate to the location and diets and 112 days for data collection. To study the nutritional requirements and physical and chemical body composition growth (muscle tissue, bone tissue, fat tissue, CP, EE, minerals and water), the sole purpose of the experimental design was to promote a greater intake and deposition of nutrients, which were measured with regression equations.

Heifers were grouped by treatment and assigned to one of four pens. The pens had concrete floors and were approximately 45 m^2. Within the pens, feed was provided in electronic feeders (Intergado, Contagem, Minas Gerais, Brazil) to record individual animal intake. Heifers had *ad libitum* access to concrete drinkers. After 30 day of acclimation, the heifers were weighed after a 14-h fasting period [shrunk BW (SBW)] and were randomized into two groups: reference (four animals) and *ad libitum*-fed heifers (16 animals).

The four heifers in the reference group were slaughtered at the beginning of the experiment to measure the empty body weight (EBW) and to estimate the initial EBW composition of the remained experimental heifers. The slaughter procedures and body content sampling techniques used for the reference group heifers were the same as those used for heifers that remained in the experiment. The heifers designated to be fed *ad libitum* were randomly assigned to one of four experimental diets (n = 4 heifers per diet).

To evaluate the relationship between BW and SBW, the heifers were weighed over two consecutive days before (BW) and after a 14-h fasting period (SBW) at the beginning and the end of the performance trial (d-30, d-31, d-141 and d-142). However, for animal performance evaluation, only animals that were weighed after the 14-h fasting period (SBW) were considered (d-31 and d-142).

The experimental diets were calculated according to the BR CORTE (2010) recommendations to provide approximately 13.5% CP (% DM basis) and promote an average daily gain (ADG) of approximately 1.0 kg. A mixture of urea (U) + ammonium sulphate (AS; 9:1) was used to increase the CP of the roughage to 13.5% (% DM basis), and it was mixed with the roughage immediately before feeding. The same concentrate containing ground corn, soybean hulls, soybean meal, salt and mineral mixture was provided to all of the heifers at either 30 or 50% of the diet DM depending on the treatment (Table 1).

Corn silage was collected in a trench-type silo one hour before supplying the heifers, whereas fresh

sugarcane (SC, whole plant) was harvested daily and was ground (6 mm) before feeding. The total mixed ration was provided twice a day (08:00 and 16:00 hours), and the heifers had free access to clean water. The diets were adjusted daily to provide orts of approximately 5–10% of the offered total on an as-fed basis. The amounts of roughage, concentrate and supplied U + AS were recorded daily. Roughage was sampled daily and stored at −20 °C until further analysis. Samples of each roughage were combined weekly (per cent as-fed basis), dried in a forced-air oven (55 °C) for 72 h and ground through a 1-mm screen (Fortinox, Piracicaba, São Paulo, Brazil). The individual ingredients used to make the concentrate were sampled directly from the mill feed silos on the days the concentrate was mixed. These ingredients were separately analysed and used to calculate the analysed concentrate composition (Table 1).

Collection, digestibility trials and slaughtering procedures

The daily enteric methane production (CH_4) in grams per day was estimated by continuous air samples analysis from air excreted by respiration and eructation gas with the aid of electronic sensors (Greenfeed; C-Lock, Rapid City, SD, USA). For this measurement from d-111 to d-135, heifers from each treatment group had access to the Greenfeed equipment for six consecutive days. Every 4 h (six times a day), Greenfeed offered five drops of concentrate to each animal every 10 s (approximately 35 g) to attract the heifers. The air was continuously sampled, and CH_4 concentrations were determined with an infrared sensor. Air flow and gas concentrations were measured, and the machine detected the muzzle position to allow for direct measurement of CH_4 flux during each heifer's visit to the feed trough.

Table 1 Ingredients in each diet and its composition on DM basis

		Sugarcane		Corn silage	
Item	Concentrate	70:30	50:50	70:30	50:50
Proportion (%DM)					
Roughage	–	70.0*	50.0*	70.0†	50.0†
Soybean hulls	55.0	16.5	27.5	16.5	27.5
Soybean meal	4.18	1.25	2.09	1.25	2.09
Ground corn	38.8	11.6	19.4	11.6	19.4
Mineral mixture‡	1.00	0.30	0.50	0.30	0.50
Salt	1.00	0.30	0.50	0.30	0.50
Chemical composition (%)					
Organic matter	94.6	96.5	96.0	93.4	93.8
Crude protein	14.1	13.7	13.8	14.2	14.1
Ether extract	4.69	2.41	3.06	3.22	3.64
Neutral detergent fibre	31.9	42.6	39.8	45.9	42.2
Non-fibrous carbohydrates	43.9	42.7	42.9	33.5	36.3
iNDF§	3.10	14.5	11.2	11.0	8.70
Gross energy (Mcal/kg)	–	4.11	4.34	4.11	4.35

*3.85% de urea + ammonium sulphate + 96.15% sugarcane.
†2.68% urea + ammonium sulphate + 97.32% corn silage.
‡Mineral mixed composition = 2.92% of calcium, 0.07% of phosphorus, 0.21% of magnesium, 0.09% of potassium, 0.03% of sodium, 6.35% of sulphur, 0.03% of cobalt, 0.0002% of chromium, 0.33% of copper, 0.21% of iron, 0.46% of manganese, 0.78% of zinc and 0.03% of selenium.
§Indigestible neutral detergent fibre.

To evaluate the apparent total-tract digestibility of the dietary constituents and consequently the metabolizable energy (ME) intake, we performed a digestibility trial during the last week of the experimental period by collecting the total excretion of faeces and urine over the last three consecutive days (Costa e Silva et al., 2013). For this experiment, the heifers were transferred to a tie-stall system on d-136 where they stayed for 7 days to conduct the digestibility assay. At the end of each collection day, the faeces were weighed and homogenized, and a sample of approximately 300 g was taken, which was weighed and processed as described for roughage samples. Furthermore, a composite sample was performed per heifer based on the DM content of each collection day. To calculate the apparent total-tract digestibility of the dietary constituents, we considered only the DM intake for the 3 days of faeces collection.

Total urine collection was performed using disposable, sterile, two-way, number 24 Foley catheter probes with a 30-ml balloon, which were inserted directly into the bladder of the heifers and were connected to polyethylene hoses to transfer urine into plastic gallons that were kept in Styrofoam boxes with ice to reduce nitrogen loss via ammonia. At the end of each collection day, the total excreted weight and volume were quantified. The urine in the containers was homogenized, and a 10 ml sample was diluted in 0.036 N sulphuric acid (1:4 ratio) to prevent allantoin and uric acid loss. Another 20 ml sample of undiluted urine was collected to quantify the gross energy concentration and 3-methylhistidine (3MH). Subsequently, in both cases, we created a sample for each heifer based on daily urinary excretion. The urine samples were stored at −80 °C for later laboratory analysis.

After the digestibility trial, all heifers were slaughtered at the Universidade Federal de Viçosa facility. The slaughter was performed by stunning using the

captive bolt technique followed by jugular vein for exsanguination. The digestive tract of each heifer was emptied and washed, and each organ was weighed separately. The weight of the heart, lungs, liver, spleen, kidneys, internal fat, diaphragm, mesentery, tail, trachea, oesophagus and reproductive tract and the weight of the washed gastrointestinal tract were added to that of the other body parts (carcass, head, hides, hooves and blood) to determine the EBW. The rumen–reticulum, omasum, abomasum, small intestine, large intestine, internal fat, mesentery, liver, heart, kidney, lung, tongue, spleen, diaphragm, oesophagus, trachea and reproductive system were ground in an industrial cutter for 20 min, and a homogeneous sample of viscera and organs was collected. The hide of the limbs and the head of each heifer were removed, and the remaining parts were triturated using a bone grinder (TOL10 model; LUNASA, Araguari, Brazil) to collect a sample of the limbs and head. The hide was sampled in parts with two regions representing the shoulder, three regions representing the dorsal line, two representing the ventral line, two representing the rear, one representing each foot and one representing the head, approximating the entire hide. Next, the hide was manually cut into small pieces (4 cm^2). Blood samples were collected immediately after slaughter. Samples of blood, organs and viscera, head and limbs, and hide were placed in aluminium trays and freeze-dried (LP 510 model; Liotop, São Carlos, Brazil).

The carcass of each heifer was divided into two half-carcasses, which were cooled in a cold room at −4 °C for 18 h. After this time, we weighed the left half of the carcass of each heifer and separated the bones and muscle plus fat. Bones and muscle plus fat were weighed, individually ground, packed in aluminium trays and freeze-dried.

All lyophilized samples from the heifer's body were ground in a Wiley mill (Fortinox, Piracicaba, São Paulo, Brazil) using liquid N. For this procedure, the lyophilized samples were placed in plastic bags and immersed in a cryogenic tank that contained liquid N. Two samples were generated for each heifer and were labelled carcass and non-carcass for further body composition estimation. The carcass sample was the lyophilized samples of bone and muscle plus fat, and the non-carcass sample was the samples of blood, head and limbs, organs and viscera, and hide. In both cases, the samples were proportionally grouped according to heifer composition. We also removed a sample of the *Longissimus dorsi* muscle at the 11th and 12th ribs to estimate muscle protein content.

Laboratory analysis and calculations

Samples of CS, sugarcane, concentrate ingredients and faeces were analysed for DM (AOAC, 2012; method 934.01), organic matter (OM; AOAC, 2012; method 930.05), CP (AOAC, 2012; 981.10 method), EE (AOAC 2006; method 945.16) and neutral detergent fibre (NDF; Mertens et al., 2002). The NDFap (ash- and protein-free NDF) was estimated without the addition of sodium sulphite, but with the addition of alpha-amylase (Ankom Tech Corp., Fairport, NY, USA), and the CP content correction was performed as recommended by Licitra et al. (1996). The analysis of insoluble fibre content of indigestible neutral detergent (iNDF) in each diet ingredient was performed according to Valente et al. (2011). The non-fibre carbohydrates (NFC) were calculated as proposed by Detmann and Valadares Filho (2010) using the following equation: NFC = 100 − [(% CP − % CP derived from U + %U) + %NDFap + %EE + %OM]. The total digestible nutrients (TDN) of the diets were estimated by the sum of digestible nutrients, in which $TDN = CP_{digestble} + 2.25 \times EE_{digestible} + NDFap_{digestible} + NFC_{digestible}$ (NRC, 2001).

Uric acid analyses were performed using an automatic biochemical analyser (BS200E model; Mindray, Shenzhen, China). Analyses of allantoin were performed according to the colorimetric method described by Chen and Gomes (1992). The total excretion of purine derivatives was calculated by adding the quantity of allantoin and uric acid that was excreted in the urine and then multiplying the urine concentration and urine volume. Absorbed purines and the ruminal synthesis of N compounds were calculated according to Barbosa et al. (2011).

The heat of combustion was determined in the feed, faeces and urine samples with a bomb calorimeter (Ika Werke, Staufen, Germany), and the energy lost via gas was estimated by converting daily CH_4 (g) excretion to an energetic unit using the factor 13.4 kcal/g CH_4. The gross energy (GE) content of the diet was calculated using the ratio and heat of combustion for each of its constituents. The digestible energy (DE) was calculated by subtracting the faeces energy losses from the diet GE. The ME was calculated by subtracting the urinary and gas energy losses from the DE.

Intake of DM and TDN was calculated according to the amount of feed that was ingested daily and the respective concentration of each dietary ingredient. Intake was averaged every 28 days for these calculations. The metabolizable protein intake (MPI) was obtained by adding the true digestible microbial

protein (TDMP) and the digestible rumen non-degradable protein (DRNDP) intake. The TDMP was calculated using the values obtained for the microbial efficiency and consumption of TDN. We assumed that the microbial protein represented 80% of amino acids (AA) and that these AA have an 80% digestibility coefficient (NRC, 2001) according to the following equation: TDMP = Mic.efic. × TDNI × 0.64, where TDMP = true digestible microbial protein (g/day), Mic.efic. = microbial efficiency (g/kg) and TDNI = total digestible nutrients intake (kg). The DRNDP intake was estimated based on the difference between CP intake and microbial protein and multiplied by its intestinal digestibility value (80%; NRC, 2001) according to following the equation: DRNDPI = (CPI − Mic.P) × 0.80, where DRNDPI = digestible rumen non-degradable protein intake (g/day), CPI = crude protein intake (g/day) and Mic.P = microbial protein (g). We calculated the relationship between body weight (BW) and shrunk body weight (SBW), BW and EBW and between ADG and the empty body weight gain (EBWG).

Carcass and non-carcass samples were analysed for DM, CP, EE and OM to estimate the EBW chemical composition. The whole-body retained energy (RE) was calculated as the difference between the initial and final energy content (EC) in the whole body of each heifer, and the EC was obtained from the whole-body content for CP and fat using their caloric equivalent according to the equation recommended by the ARC (1980): EC = 5.6405 X + 9.3929 Y, where EC = energy content (Mcal), X = body protein (kg) and Y = body fat (kg).

To determine the net energy requirement (NE_g) for any body weight gain, a regression equation between the RE in the body and the EBWG was adjusted for a certain metabolic EBW ($EBW^{0.75}$) using the following model: $NE_g = a \times EBW^{0.75} \times EBWG^b$, where NE_g = net energy requirement for gain (Mcal/$EBW^{0.75}$ per day), $EBW^{0.75}$ = metabolic empty body weight ($kg^{0.75}$), EBWG = empty body weight gain (kg/day) and 'a' and 'b' = regression parameters.

To calculate the net protein requirements for any body weight gain (NP_g), we adjusted the following model using EBWG and RE: $NP_g = \beta 1 \times EBWG + \beta 2 \times RE$, where NP_g = net protein requirements for gain (g/day), EBWG = empty body weight gain (kg/day), RE = retained energy (Mcal/day), and $\beta 1$ and $\beta 2$ = regression parameters.

The energy (k_g) and protein (k) utilization efficiencies for gain were estimated by fitting the regression equations between the RE (Mcal/$EBW^{0.75}$ per day) and ME intake (Mcal/$EBW^{0.75}$ per day) and between the retained protein (g/$EBW^{0.75}$ per day) and MPI (g/$EBW^{0.75}$ per day), respectively, and these values were the slope coefficients of the established regression equations (BR CORTE, 2010).

Because of the variation in energy and protein intake, unfortunately, it was not possible to estimate energy and protein requirements for maintenance accurately. Because all heifers were fed *ad libitum*, they had high dry matter intake (DMI) and, consequently, high energy and protein intake. Thus, with no heifers fed near maintenance requirements, adequate maintenance values could not be obtained.

Muscle protein metabolism was evaluated by the fractional synthesis rate (FSR), the fractional degradation rate (FDR) and the fractional accretion rate (FAR) of myofibril proteins, which were estimated according to the method proposed by McCarthy et al. (1983).

The FDR was calculated as the ratio between the daily urinary excretion of 3MH and the amount of 3MH in the skeletal muscle, as described in the following equation: FDR (%/day) = [(3MH)/(MM × MC × 3.5106)] × 100, wherein FDR = fractional degradation rate of myofibrillar proteins (%/day), 3MH = urinary excretion of 3MH (μmol/day), MM = muscle mass (kg), MC = muscle protein content (g/kg) and 3.5106 = concentration of 3MH (μmol/g of protein in muscle; Nishizawa et al., 1979). The concentration of 3MH was quantified in a commercial laboratory (Laboratory test CBO, Campinas, São Paulo, Brazil) using HPLC, and the MC was estimated in samples of the *L. dorsi* muscle (AOAC, 2012; method 981.10). The FAR was estimated based on the ratio between the gain in muscle protein and muscle protein mass as follows: FAR (%/day) = (MPG/MMP) × 100, wherein FAR = fractional accretion rate of myofibrillar proteins (%/day), MMP = mean muscle protein (g) and MPG = muscle protein gain (g/day). The MPG was calculated based on the difference between the initial and final skeletal muscle protein content divided by the number of days that the heifers were in the experiment: MPG (g/day) = (fMM × fPM − iMM × iPM)/days of experiment, where MPG = muscle protein gain (g/day), fMM = final muscle mass (kg), fPM = final protein content in muscle (g), iMM = initial muscle mass (kg) and iPM = initial protein content in muscle (g). Thus, the FSR was calculated based on the sum of FDR and FAR as follows: FSR (%/day) = FDR + FAR.

We did not dissect the entire carcass of its muscle and fat. Thus, the ratio of muscle and fat in the carcass was estimated based on the composition of the section between the 9th and 11th ribs using the equations suggested by Marcondes et al. (2012) for cross-bred

females: %M carcass = 54.42 + 0.26 × %MHH − 1.28 × %VF and %F carcass = 0.47 + 0.30 × % FHH − 1.98 × %VF, where % M carcass is the muscle percentage in the carcass, %MHH is the muscle percentage in the section between the 9th and 11th ribs, %F carcass is the fat percentage in the carcass, % FHH is the fat percentage in the section between the 9th and 11th ribs and %VF is the visceral fat percentage.

Equations were generated to estimate the physical and chemical body composition based on the body composition of the heifers that were used in this study (Table 2) according to the allometric model: $C_i = a \times \text{EBW}^b$, where C_i = component '*i*' in the heifer's body, which could be muscle tissue (kg), fat tissue (g), bone tissue (kg), CP (kg), EE (g), OM (kg) or water (kg) present in the empty body, EBW = empty body weight and '*a*' and '*b*' = regression parameters.

Statistical analysis

Data on nutritional requirements and tissue deposition were analysed as linear and nonlinear models built through the REG and NLIN procedures of SAS (version 9.4; SAS Inst., Cary, NC, USA), respectively, in which the nonlinear model was adjusted with the Gauss–Newton method.

The data for protein turnover were analysed in a completely randomized 2 × 2 factorial design using the PROC MIXED method in SAS (version 9.4; SAS Inst.). Roughage, concentrate level and roughage × concentrate level interactions were the fixed effects in the model, and the heifers were the experimental units. For all the comparisons and tests, we adopted 0.05 as the critical level of probability for a type I error.

Table 2 Characterization of body composition (natural matter basis) of the heifers in the experiment used to generate estimates of physical and chemical composition across all heifers

Item	Average	Minimum	Maximum	SD
Physical composition				
Empty body weight (kg)	251	118	375	71.2
Muscle tissue (kg)	98.7	53.8	13.0	21.5
Fat tissue (kg)	35.6	3.80	70.5	19.9
Bone tissue (kg)	15.4	12.2	19.9	2.63
Chemical composition				
Crude protein (kg)	42.6	23.3	61.5	10.8
Ether extract (kg)	52.4	12.3	100	24.6
Organic matter (kg)	10.9	6.30	16.2	3.09
Water (kg)	146	76.0	199	35.0

Results

Growth of body components

The composition weight (muscle tissue, bone tissue, fat tissue, CP, EE, minerals and water) increased as the heifer EBW increased. However, this increase occurred at different rates (Table 3; r^2 = 0.99, 0.99, 0.98, 0.99, 0.99, 0.97 and 0.99 respectively). The muscle and bone tissues exhibit a similar growth pattern, and both increased less than the EBW (Fig. 1a). However, the bone tissue reached stability first, which was observed by evaluating the exponents of the equations (muscle tissue = 0.72 and bone tissue = 0.53). Adipose tissue increased more than the body, proportionally, as the heifer became heavier. The changes in chemical composition, CP, EE and OM were correlated with tissue changes in muscle, fat and bone deposition respectively (Fig. 1b). The pattern of water deposition, with water being the primary constituent of an animal's body (Table 2), was similar to that of CP deposition (water = 0.82 and CP = 0.86).

Metabolism in muscle protein

There was no interaction effect ($p > 0.05$) between the type of roughage and the level of concentrate for DM intake, BW, EBW, initial muscle protein, final muscle protein, protein muscle gain, 3MH, FDR, FSR, FAR and the FSR:FDR ratio (Table 4). Greater ($p < 0.05$) values for the daily excretion of 3MH, protein muscle gain, FSR, FDR and FAR were observed

Table 3 Equations derived from regression analysis to estimate the physical and chemical body composition using the empty body weight (kg)

Item	Equation	r^2
Physical composition*		
Muscle tissue	$\text{MT} = 1.882 \pm 0.0539 \times \text{EBW}^{0.718 \pm 0.0506}$	0.99
Fat tissue	$\text{FT} = 0.646 \pm 0.5016 \times \text{EBW}^{1.964 \pm 0.1601}$	0.98
Bone tissue	$\text{BT} = 0.853 \pm 0.3028 \times \text{EBW}^{0.526 \pm 0.0636}$	0.99
Chemical composition*		
Crude protein	$\text{CP} = 0.377 \pm 0.1267 \times \text{EBW}^{0.856 \pm 0.0597}$	0.99
Ether extract	$\text{EE} = 4.007 \pm 0.00266 \times \text{EBW}^{1.707 \pm 0.1165}$	0.99
Organic matter	$\text{OM} = 0.165 \pm 0.1488 \times \text{EBW}^{0.759 \pm 0.1607}$	0.97
Water	$\text{Water} = 1.543 \pm 0.2737 \times \text{EBW}^{0.824 \pm 0.0316}$	0.99

*MT, muscle tissue (kg); EBW, empty body weight (kg); FT, fat tissue (kg); BT, bone tissue (kg); CP, crude protein (kg); EE, ether extract (kg); OM, organic matter (kg); Water, water (kg). Equations were generated according to the allometric model: $C_i = a \times \text{EBW}^b$, wherein C_i = component '*i*' in the heifer's body, which could be muscle tissue (kg), fat tissue (g), bone tissue (kg), CP (kg), EE (g), OM (kg) or water (kg) that is present in the empty body, EBW = empty body weight, and '*a*' and '*b*' = regression parameters.

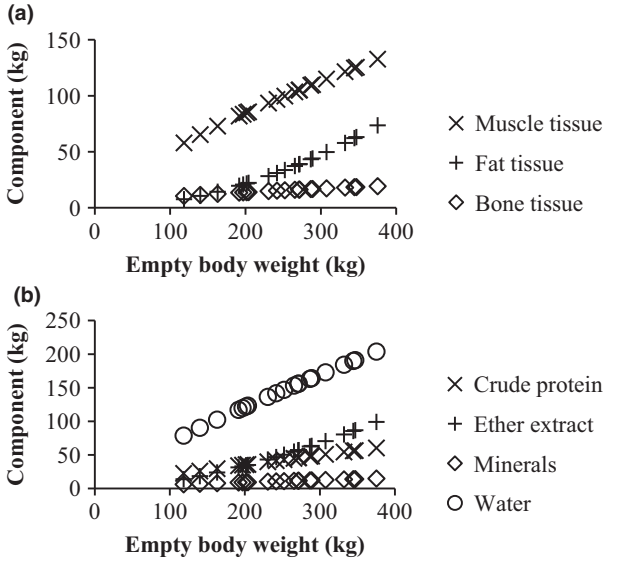

Fig. 1 Relationship between the empty body weight (EBW) and muscle tissue (MT), fat tissue (FT) or bone tissue (BT; a), and the relationship between the EBW and CP, ether extract (EE), minerals (OM) or water (b) of Holstein × Gyr heifers. MT = 1.882 ± 0.0539 × $EBW^{0.718 \pm 0.0506}$, FT = 0.646 ± 0.5016 × $EBW^{1.964 \pm 0.1601}$, BT = 0.853 ± 0.3028 × $EBW^{0.526 \pm 0.0636}$, CP = 0.377 ± 0.1267 × $EBW^{0.856 \pm 0.0597}$, EE = 4.007 ± 0.00266 × $EBW^{1.707 \pm 0.1165}$, OM = 0.165 ± 0.1488 × $EBW^{0.759 \pm 0.1607}$, Water = 1.543 ± 0.2737 × $EBW^{0.824 \pm 0.0316}$. CP = crude protein (kg); EE = ether extract (kg); OM = organic matter (kg); Water = water (kg). Equations were generated according to the allometric model: $C_i = a \times EBW^b$, wherein C_i = component '*i*' in the heifer's body, which could be muscle tissue (kg), fat tissue (g), bone tissue (kg), CP (kg), EE (g), OM (kg) or water (kg) that is present in the empty body, EBW = empty body weight, and '*a*' and '*b*' = regression parameters.

for heifers fed 50% concentrate. In addition, MPG and FAR were also influenced by the roughage offered to the heifers, with greater values ($p < 0.05$) observed for heifers that were fed with CS-based diets.

Nutritional requirements

Conversion factors

Considering that the first step to estimate cattle nutritional requirements is the conversion of SBW to EBW and that farmers usually do not fast animals to measure SBW, knowledge of the SBW:BW ratio is necessary to calculate the nutritional requirements of animals fed under different conditions. In addition, the use of EBWG for the calculation of nutrient requirements is the most highly recommended strategy when not examining factors related to feed intake and the amount of digesta in the gastrointestinal tract. Thus, the average ratio between SBW and BW, EBW and SBW, and EBWG and ADG observed in this experiment were 0.98, 0.87 and 1.07, respectively (Fig. 2a–c; $R^2 = 0.99$, $R^2 = 0.98$, $R^2 = 0.98$ respectively).

Energy and protein requirements

The net energy requirements for any weight range and BW gain can be estimated with the following equation: $NE_g = 0.0685 \times EBW^{0.75} \times EBWG^{1.095}$, in which NE_g is the net energy requirement for gain (Mcal/day), EBW is the empty body weight (kg) and EBWG is the empty body gain (kg/day).

Net protein requirements for any BW gain range were estimated from the model using EBWG and RE: $NP_g = 203.8 \times EBWG - 14.80 \times RE$, in which NP_g is the net protein requirement (g/day), EBWG is the empty body weight gain (kg/day) and RE is retained energy (Mcal/day).

To convert the NE_g and NP_g to ME and metabolizable protein (MP) requirements for BW gain, respectively, knowledge of the ME (k_g) and MP (k) utilization efficiency for BW gain is needed. According to the BR CORTE (2010), these values can be calculated with the slope of the RE regression coefficient as a function of ME intake (Fig. 2d) and retained protein as a function of MPI (Fig. 2e) respectively. The values for k_g and k were 0.41 and 0.25 respectively.

Discussion

Metabolism in muscle protein

Protein deposition is highly dependent on nutrients and is primarily influenced by the nutritional status of the animal (Jones et al., 1990). The use of poor-quality diets that are unbalanced or deficient in some nutrient decrease protein turnover rates and, depending on the severity and type of deficiency, additional muscle protein loss might occur to support the energy metabolism of the animal (Garlick et al., 1975; Millward et al., 1975). In contrast, the influence of the studied factors on FDR, FSR, FAR and MPG indicate that greater nutrient input (i.e. 50% concentrate diet) generated larger protein turnover rates, and, consequently, greater BW gains. Thus, unlike previous studies (Barnard et al., 1969; Haverberg et al., 1975; Lowell et al., 1986), which only studied the influence of dietary deficiency on protein turnover rates, this study showed that it is also important to consider how dietary manipulation can promote positive gains in animal growth.

According to Buttery (1981), changes in FSR are usually accompanied by similar changes in FDR, in agreement with the results from this study. Heifers fed SC-based diets exhibited increased FSR and FDR (53 and 54%, respectively) as concentrate in the diet increased from 30% to 50%. For heifers fed CS-based diets, the

Table 4 Protein metabolism in muscle of Holstein × Gyr heifers

	Roughage					p-Value		
	Sugarcane		Corn silage					
Variables	70:30	50:50	70:30	50:50	SEM	*R**	*C*†	*R* × *C*‡
DMI (kg/day)	6.92	9.18	8.59	10.6	0.663	0.046	0.010	0.855
BW (kg)	237	266	245	282	22.5	0.617	0.193	0.860
EBW§ (kg)	191	219	201	239	18.6	0.442	0.113	0.800
iMP¶ (kg)	13.4	13.6	13.0	13.1	1.28	0.756	0.896	0.969
fMP** (kg)	17.0	20.6	19.8	22.1	1.54	0.193	0.081	0.669
MPG†† (g/day)	36.8	58.4	56.6	73.0	3.88	0.001	<0.001	0.532
3-MH urine‡‡ (μmol/day)	698	1247	1049	1327	160.4	0.151	0.034	0.321
FDR§§ (%/day)	1.11	1.71	1.59	1.71	0.158	0.150	0.043	0.155
FSR¶¶ (%/day)	1.14	1.75	1.64	1.77	0.159	0.129	0.036	0.150
FAR*** (%/day)	0.03	0.05	0.04	0.06	0.003	0.001	0.001	0.535
FSR:FDR	1.02	1.03	1.03	1.03	0.004	0.142	0.116	0.903

*R = the main effect of roughage inclusion in the diet.
†C = the main effect of concentrate inclusion in the diet.
‡R × C = interaction between roughage and concentrate inclusion in the diet.
§EBW = empty body weight.
¶iMP = initial muscle protein.
**fMP = final muscle protein.
††MPG = muscle protein gain. MPG (g/day) = (fMM × fPM − iMM × iPM)/days of experiment, where MPG = muscle protein gain (g/day), fMM = final muscle mass (kg), fPM = final protein content in muscle (g), iMM = initial muscle mass (kg) and iPM = initial protein content in muscle (g).
‡‡3-MH urine = 3-methylhistidine concentration in urine.
§§FDR = fractional degradation rate. FDR (%/day) = [(3MH)/(MM × MC × 3.5106)] × 100, wherein FDR = fractional degradation rate of myofibrillar proteins (%/day), 3MH = urinary excretion of 3-methylhistidine (μmol/day), MM = muscle mass (kg), MC = muscle protein content (g/kg) and 3.5106 = concentration of 3-methylhistidine (μmol/g of protein in muscle; Nishizawa et al., 1979).
¶¶FSR = fractional synthesis rate. FSR (%/day) = FDR + FAR.
***FAR = fractional increase rate. FAR (%/day) = (MPG/MMP) × 100, wherein FAR = fractional accretion rate of myofibrillar proteins (%/day), MMP = mean muscle protein (g) and MPG = muscle protein gain (g/day).

increase of FSR and FDR was 8% and 7%, respectively, as the amount of concentrate increased from 30% to 50%. The accretion of muscle protein requires net balance between the amounts of synthesized and degraded protein, and minor variations in both FSR and FDR caused by the roughage response observed in heifers fed CS-based diets compared to the approximately 50% change in heifers fed SC-based diets.

Although SC is poor-quality roughage, with few nutrients (Leng, 1988), these results confirmed the hypothesis that a 20% increase in concentrate level would be effective for improving the quality of diets that contain SC as a forage source. In addition, it was noteworthy that this increase in concentrate level also enabled heifers consuming SC-based diets to reach protein turnover rates that were similar to those of heifers consuming CS-based diets, even when the diet contained 50% concentrate.

Moreover, the animal performance data that are presented in Silva et al. (2016) and the muscle protein metabolism results agree. Heifer performance was influenced by both dietary factors evaluated in this study. Greater ADG was observed for heifers that were fed CS-based diets than for heifers that were fed SC-based diets and for heifers fed diets containing 50% concentrate than for those containing 30% concentrate.

Nutritional requirements

Unfortunately, no data are available for dairy heifers; thus, our comparison parameters are derived from beef cattle.

Conversion factors

Averages of EBW:SBW and EBWG:ADG ratios were 0.87 and 1.07 respectively. The EBW:SBW ratio was below the value observed by BR CORTE (2010) and the NRC (2000), which were 0.90 and 0.89 respectively. However, according to the NRC (2000), this ratio may vary from 0.85 to 0.95. The most likely explanation for the smaller conversion factor between the two variables is that the proportion of gastrointestinal content was greater in Holstein animals than

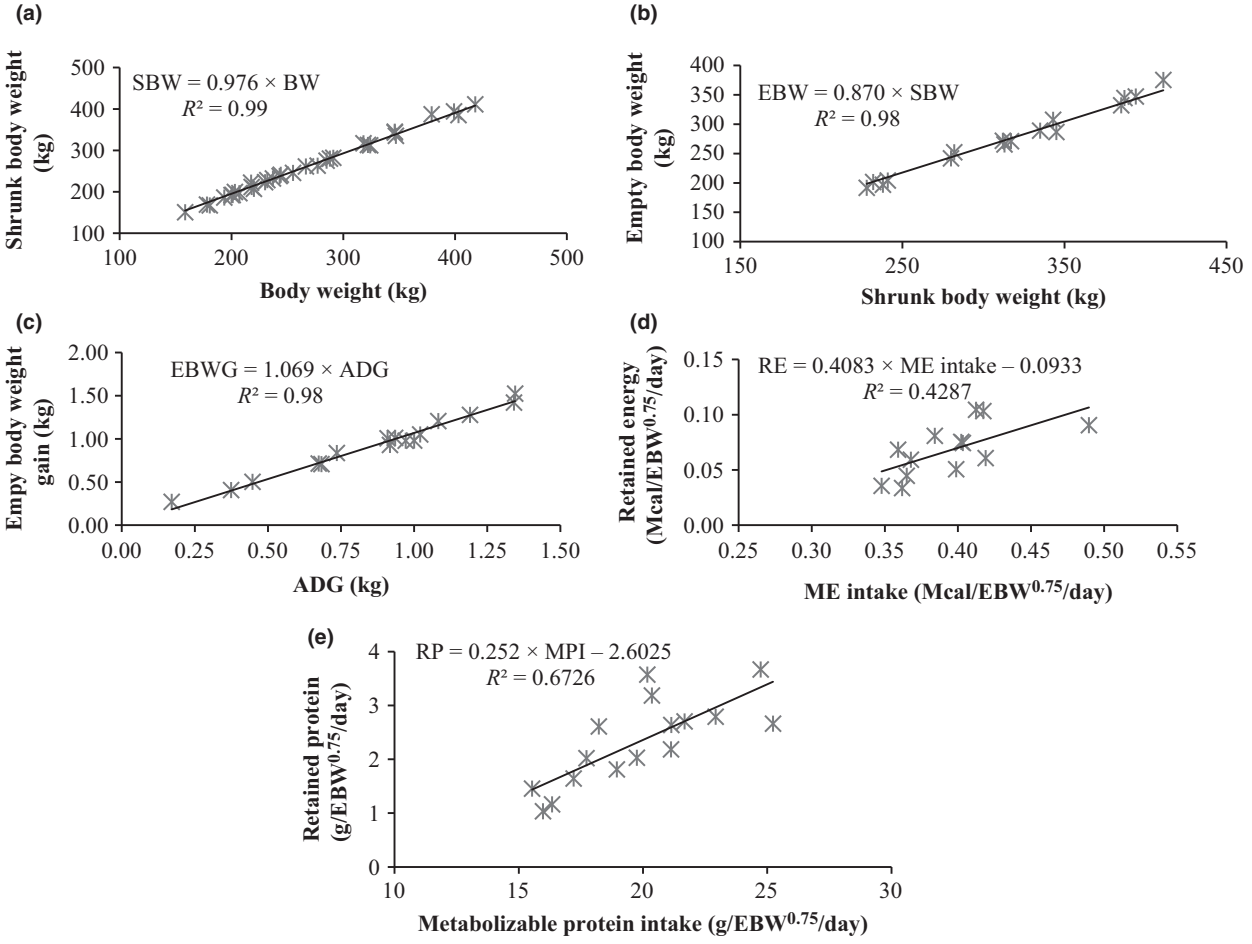

Fig. 2 Relationship between body weight (BW) and shrunk body weight (SBW; (a), relationship between the SBW and empty body weight (EBW; (b), relationship between average daily gain (ADG) and EBW gain (EBWG; (c), relationship between metabolizable energy intake (MEI) and retained energy (RE; (d) and relationship between metabolizable protein intake (MPI) and retained protein (RP; (e) of Holstein × Gyr heifers. SBW = 0.976 × BW; EBW = 0.870 × SBW; EBWG = 1.069 × ADG; RE = 0.4083 × ME intake − 0.0933; RP = 0.252 × MPI − 2.6025, where the linear models were built through the procedure REG of SAS (version 9.4; SAS Inst.).

in pure Zebu breeds and cross-bred beef cattle (Peron et al., 1993). In general, Holstein animals have a more developed gastrointestinal tract because of genetic selection that is focused on milk production, which requires increased feed consumption and is likely a characteristic of Holstein and Gyr cross-breeds (Dunklee et al., 1994).

The NRC (2000) and BR CORTE (2010) exhibited EBWG:ADG ratios of 0.95 and 0.97 respectively. These values were 11.0% and 9.63% less than the values observed in this study respectively. However, the BR CORTE (2010) reports that this relationship can also be affected by genetics. Usually, values for the EBWG:ADG ratio are less than 1, which did not occur in this study. The main explanation for the EBWG:ADG value, which was 1.07, is that these heifers exhibited greater gastrointestinal content relative to BW at the beginning than at the end of the feedlot period. Thus, a lower EBW:SBW ratio was observed at the beginning (0.77) than at the end (0.87) of the experiment, which may be due to the prior management of the heifers. Therefore, when expressing the average EBWG:ADG ratio, the value assumed for this ratio is above 1.

Energy and protein requirements

The net energy requirements for any weight range and BW gain can be estimated with the following equation: $NE_g = 0.0685 \times EBW^{0.75} \times EBWG^{1.095}$, where NE_g is the net energy requirement for gain (Mcal/day), EBW is the empty body weight (kg) and EBWG is the empty body gain (kg/day). The intercept of the equation for estimating NE_g proposed (0.0685) is approximately 11% lower than the intercept suggested by Chizzotti et al. (2008). However, the database used by Chizzotti

et al. (2008) only considered studies with pure-bred and cross-bred beef heifers, whereas dairy cross-bred cattle were used in this study.

The main system dictating the nutritional requirements of dairy cattle (NRC 2001) assumes that the nutritional requirements of dairy heifers are similar to those of beef heifers, and the equations for predicting their nutritional requirements come from the NRC (2000). Considering one animal with 217 kg of EBW and EBWG of 0.93 kg/day (the average of the heifers in this study), the NE_g and NP_g calculated by our equations and the same values calculated with NRC (2001) equations were 3.58 and 3.91 Mcal/day and 123 and 113 g/day, respectively. The values estimated for NE_g by NRC equations is approximately 9% greater than the value calculated by our suggested equation. However, the NP_g calculated with the NRC (2001) equation is approximately 8% lower than the value calculated by our suggested equation.

The composition of EBWG is the main determinant for estimating the nutritional requirements for BW gain, and it depends on the physiological maturity of the cattle, which is affected by sex and breed (Fortin et al., 1980; NRC, 1984). The differences observed between the nutritional requirements of dairy and beef heifers began because we considered a single animal sample with a single BW to compare both predicted nutritional requirements. At the same BW, beef and dairy heifers have a different chemical body composition (Fortin et al., 1980), which is likely due to different degrees of maturity, and these factors would affect the composition of the gain and, consequently, the requirements for energy and protein.

Our results suggest that Holstein × Gyr heifers have greater NP_g and lesser NE_g than the same values calculated with the NRC (2001) equations. The relationship between protein and energy content in the diet may affect mammary gland development (Whitlock et al., 2002; Albino et al., 2015). Diets with a low protein:energy ratio may increase fat pad deposition in heifer mammary glands (Albino et al., 2015). Thus, overestimating energy and underestimating protein requirements may decrease milk production at subsequent lactations.

Moreover, it is noteworthy that there is a difference with respect to the study date and conditions. The studies used to propose the NRC (2001) equations are very old and were performed under different conditions than this study. Consequently, improvements in genetic characteristics and difference in the feedstuffs used and management practices may underlie differences in nutritional requirement estimation (Kiplagat et al., 2012).

Nevertheless, it is also important to highlight that the small sample size was one of the limitations of this study. Thus, more studies are necessary to validate this result.

Conclusions

Greater quality diets provide greater rates of protein turnover, leading to greater growth rates and absolute gains in muscle protein. A 20% increase in the concentrate levels of sugarcane-based diets was sufficient to increase the nutrient supply and enhance muscle growth in Holstein × Gyr heifers.

Net nutritional requirements for energy and protein for BW gain for Holstein × Gyr heifers can be estimated using the equations $NE_g = 0.0685 \times EBW^{0.75} \times EBG^{1.095}$ and $NPL_g = 203.8 \times EBG - 14.80 \times RE$ respectively. The efficiency of utilization of ME and MP for gain is 40.8% and 25.2%, respectively.

Our results suggest that there may be differences between the nutritional requirements of beef and dairy cattle. Thus, after future validation, the equations described above may be used to calculate the nutritional requirements for the growth of Holstein × Gyr heifers instead of using the NRC (2001) recommendations regarding the nutritional requirements of dairy cattle, which are based on values from beef cattle.

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