ABSTRACT

The first studies concerning nutrient requirements for preweaned dairy calves were from the 1920s and 1930s; however, few studies were published in the following decades. We aimed to determine energy and protein requirements of preweaning Holstein and Holstein × Gyr dairy calves in a multistudy meta-regression. We used a database composed of individual measurements of 166 preweaned male calves (138 submitted to treatments and 28 used as the reference group) from 4 studies that used the methodology of comparative slaughter. Animals with less than 15/16 of Holstein genetic composition were considered crossbred Holstein × Gyr, whereas other animals were considered Holstein. Net energy requirements for maintenance (NEM) were determined by the regression between heat production and metabolizable energy intake (MEI). The metabolizable energy requirements for maintenance were calculated by the iterative method, and the efficiency of use of metabolizable energy for maintenance was obtained by NEM divided by the metabolizable energy requirements for maintenance. Net energy requirements for gain (NEG) were estimated using a regression of the retained energy (RE) as a function of empty body weight (EBW) and empty body gain (EBG). The efficiency of use of metabolizable energy for gain was estimated by the regression of RE as a function of MEI, but with partitioning the MEI into MEI from liquid feed and MEI from starter feed. Additionally, the effect of a liquid feed (milk or milk replacer) was tested on the slope of the regression. Breed did not influence any of the nutrient requirements’ estimates. The NEM was estimated as 70.2 kcal/metabolic body weight per day. The efficiency of use of metabolizable energy for maintenance observed was 66%. The NEG was estimated by the equation \[ \text{NEG} = 0.0901 \times \text{EBW}^{0.75} \times \text{EBG}^{0.9539} \].

INTRODUCTION

Knowing the nutrient requirements of animals is mandatory for correct diet balancing on a farm. Conse-
quently, an excessive supply of nutrients that leads to increased feeding costs and excretion of nitrogen to the environment is minimized (Sinclair et al., 2014), or underfeeding of animals that leads to low productive and reproductive performance and an inefficient production system (Marcondes et al., 2011b; Fonseca et al., 2012) is avoided.

Nutrient requirements vary across species, breeds, and animal category (Valadares Filho et al., 2016). In the specific case of preweaned animals, the first studies to determine the requirements of these animals were from the 1920s and 1930s (Bechdel et al., 1926; Eckles et al., 1927; Savage, 1932; Atkeson et al., 1934), and few studies were published in the following decades (Mitchell, 1947; Blaxter and Mitchell, 1948; Holmes and Davey, 1976). Those were the basis for the National Research Council (NRC) publication (NRC, 2001), especially the study by Holmes and Davey (1976). Although the NRC (2001) is used widely to assess the requirements of preweaning dairy calves, it has several limitations. For instance, the NRC (2001) suggests that newer genotypes should be used to determine requirements for calves once they have based their recommendations on data from the 1970s (Holmes and Davey, 1976). Additionally, these data were obtained with calves in temperate conditions, for animals weighing more than 100 kg, and using US management practices, which may differ from those used in other countries, especially in tropical areas, and thus could be a constraint for the unrestricted use of the NRC (Borges et al., 2007; Rodrigues et al., 2016; Silva et al., 2017). Last, the recommendations of Holmes and Davey (1976) were based on Bos taurus calves, with no inclusion of Holstein × Gyr crossbred cows, which currently represent a large portion of dairy animals in the tropics (Ruas et al., 2006; Canaza-Cayo et al., 2014).

In this sense, recent efforts were made to determine nutrient requirements for preweaning Holstein and Holstein × Gyr crossbred calves in the tropics (Rodrigues et al., 2016, 2018; Silva et al., 2017; Castro et al., 2019; Chagas et al., 2019; Jolomba et al., 2020). Consequently, we intended to determine requirements using modern genotypes and animals managed using the most recent techniques and under tropical conditions. Additionally, a multistudy approach is needed to determine requirements and breed differences in preweaning dairy calves. Therefore, our aim was to determine preweaning dairy calves’ energy and protein requirements by using individual data in a multistudy meta-regression approach (St-Pierre, 2001, 2007). Our secondary aim was to determine breed differences among those requirements.

MATERIALS AND METHODS

Approval by an ethics committee on the care and use of animals was not necessary for this study because the data were collected from previously published studies. However, each study was approved by a local committee for animal use and care.

Database

We used a database composed of individual measurements of 166 preweaned male calves (138 submitted to treatments and 28 used as a reference group) from 4 studies (Rodrigues et al., 2016; Silva et al., 2017; Chagas et al., 2019; Jolomba et al., 2020).

All studies used the methodology of comparative slaughter, and the animals designated to the reference group were slaughtered at the beginning of the experiment to estimate the body composition of the animals that remained in the experiment. Animals were classified as Holstein when their genetic composition had more than 93.75% Holstein blood (Brazilian Holstein Association). All other animals had a proportion of Holstein and Gyr varying from 50 to 93.75% and comprised the Holstein × Gyr crossbreds (Table 1).

Briefly, Rodrigues et al. (2016) used 42 Holstein calves up to 87 d old, and from these animals, 10 were designated as a reference group. The remaining animals were distributed into the following 4 treatments: 2, 4, 6, and 8 L/d of milk, with free access to starter feed and access to hay after 60 d of age. Sixteen calves were slaughtered at 59 d of age and 16 at 87 d old. Concerning the Silva et al. (2017) study, the authors used 39 crossbred Holstein × Gyr calves up to 64 d old, and 5 of these animals were used as a reference group. The remaining animals were distributed over 6 treatments consisting of 3 amounts of milk (2, 4, and 8 L/d) in combination with the presence or absence of starter feed. Calves had no access to a forage source. Chagas et al. (2019) used 43 crossbred Holstein × Gyr calves up to 60 d of age, and 7 calves were designated as a reference group. Considering the 36 animals that were kept in the study, 14 were crossbred Holstein × Gyr, and 22 were Holstein. The animals were submitted to 1 of 4 treatments that consisted of different Met and Cys levels (8.0, 8.7, 9.4, and 10.2 g/d) added to the milk replacer. Sixteen calves were slaughtered at 30 d of life, and 20 calves were slaughtered at 60 d of life. Animals had no access to starter feed or forage. The study of Jolomba et al. (2020) was carried out with 42 calves up to 60 d of life, and 6 were used as a reference group. Considering the 36 animals that were kept in the study,
16 were crossbred Holstein × Gyr, and 20 were Holstein. The treatments applied were maintenance group (fed 3 L/d of raw milk) and 4 levels of addition of milk replacer to 6 L of milk to achieve the following DM content of the liquid diet: 13.5, 16.1, 18.2, and 20.4%. Animals had free access to the starter feed.

Overall, measures of intake, digestibility, slaughter procedures, calculations of metabolizable energy intake (MEI), retained energy (RE), and retained protein (RP) were similar across the studies, and details can be accessed directly in the original publications. Regarding the calculation of metabolizable protein intake (MPI), Silva et al. (2017), Chagas et al. (2019), and Jolomba et al. (2020) estimated it as the difference between daily protein intake and true daily protein excretion. In contrast, Rodrigues et al. (2016) estimated MPI as the sum of digestible RUP and true digestible RDP, estimated by a purine derivative method. Details about MPI estimation can be accessed in the original papers.

The estimation of body energy content (BEC) was obtained for each animal from body protein and fat contents and their respective caloric equivalents of 5.7 and 9.5 (ARC, 1980) as follows:

\[
\text{BEC (Mcal)} = 5.7 \times \text{CP}_{EB} + 9.5 \times \text{EE}_{EB},
\]

where \(\text{CP}_{EB}\) = crude protein content in the empty body (kg) and \(\text{EE}_{EB}\) = ether extract content in the empty body (kg). Heat production was calculated for each animal as the difference between MEI and RE, and RE was calculated as the difference between final BEC and estimated initial BEC for each animal.

**BW and Body Gain Adjustments**

To estimate the relationship between BW and empty body weight (EBW), and between ADG and empty body weight.
body gain (EBG), linear regressions were estimated as follows:

\[ \text{EBW (kg)} = \beta_0 + \beta_1 \times \text{BW (kg)}, \]

\[ \text{EBG (kg/d)} = \beta_0 + \beta_1 \times \text{ADG (kg/d)}, \]

where \( \beta_0 \) and \( \beta_1 \) are equation parameters.

The following data were obtained for each calf: initial BW, initial EBW, final BW, and final EBW. The initial EBW was estimated by using data from the reference group of each study.

**Energy Requirements**

**Energy Requirements for Maintenance.** An exponential regression between heat production and MEI was fit to calculate the NEM. Considering that NEM represents all heat production of the animal under a state of absolute fasting, mathematically, this value can be assumed as the intercept of the model. This represents all heat production when MEI is zero as follows (Ferrell and Jenkins, 1998):

\[ \text{HP} = \beta_0 \times e^{\beta_1 \times \text{MEI}}, \]

where HP = heat production (Mcal/EBW\(^{0.75} \) per day); MEI = is expressed in Mcal/EBW\(^{0.75} \) per day; \( \beta_0 \) and \( \beta_1 \) are equation parameters.

For this study, we adopted the classical method for estimating the ME requirement for maintenance (ME\(_M\); expressed as Mcal/EBW\(^{0.75} \) per day) and the efficiency of use of metabolizable energy for maintenance (\( \text{km} \)).

For estimating ME\(_M\), an iterative calculation process was applied to the previously presented model (Eq. 4) to determine the point where the MEI and heat production are equal (i.e., the point at which there is no energy retention in the body; Chizzotti et al., 2007). Additionally, the \( \text{km} \) (expressed as \%) was obtained as the ratio between the NEM and ME\(_M\).

**Energy Requirements for Gain.** The net energy requirement for gain (NE\(_G\)) was estimated by implementing a regression of the RE as a function of EBW and EBG. Parameters were estimated using the following model proposed by Garrett (1980):

\[ \text{RE (Mcal/d)} = \beta_0 \times \text{EBW}^{0.75} \times \text{EBG}^{\beta_1}, \]

where EBW\(^{0.75} \) = average metabolic empty body weight (kg); EBG is measured in kilograms per day; \( \beta_0 \) and \( \beta_1 \) are equation parameters.

The efficiency of use of the metabolizable energy for gain (\( \text{kg} \)) is necessary to calculate the metabolizable energy requirements for gain (ME\(_G\); ME\(_G\) = NE\(_G\)/\( \text{kg} \)). The \( \text{kg} \) (expressed as \%) was estimated by the regression of the RE as a function of MEI, according to Ferrell and Jenkins (1998). To estimate the \( \text{kg} \) of liquid feed and starter feed, the MEI was partitioned into MEI from liquid feed and MEI from starter feed. Additionally, the effect of the liquid feed source (milk or milk replacer) was tested on the \( \beta_1 \) coefficient of the model as follows:

\[ \text{RE (Mcal/EBW}^{0.75} \text{ per day)} = \beta_0 + \beta_1 \times \text{MEI}_L + \beta_2 \times \text{MEI}_S, \]

where MEI\(_L\) = metabolizable energy intake from liquid feed (Mcal/EBW\(^{0.75} \) per day); MEI\(_S\) = metabolizable energy intake from starter feed (Mcal/EBW\(^{0.75} \) per day); \( \beta_0, \beta_1, \) and \( \beta_2 \) are equation parameters.

**Protein Requirements**

**Protein Requirement for Maintenance.** The metabolizable protein requirement for maintenance (MP\(_M\)) was estimated according to the recommendation of Wilkerson et al. (1993), which consists of a 2-step calculation. The first step was to fit a linear regression of MPI as a function of the ADG of the animals (Eq. 7). Subsequently, the intercept (\( \beta_0 \)) of the adjusted model was divided by the average metabolic BW of the animals used to create the model, and this result was assumed as the MP\(_M\) (Eq. 8):

\[ \text{MPI (g/d)} = \beta_0 + \beta_1 \times \text{ADG (kg/d)}, \]

\[ \text{MP} \left( \text{g/BW}^{0.75} \text{ per day} \right) = \frac{\beta_0}{\text{BW}^{a.75}}, \]

where MPI = metabolizable protein intake (g/d); ADG = average daily gain (kg/d); \( \text{BW}^{a.75} \) is the average metabolic body weight (kg); \( \beta_0 \) and \( \beta_1 \) are regression parameters.

**Protein Requirement for Gain.** The net protein requirement for gain (NP\(_G\)) was estimated by fitting a regression between RP, EBG, and RE, according to the NRC (2000). This method takes into consideration the animal performance (EBG) and the BW gain composition based on the proportion of energy retained in the gain (RE) as follows:

\[ \text{NP} \left( \text{g/d} \right) = \beta_0 + \beta_1 \times \text{EBG (kg/d)} + \beta_2 \times \text{RE (Mcal/d)}, \]
where $\beta_0$, $\beta_1$, and $\beta_2$ are equation parameters.

To calculate the metabolizable protein requirements for gain (MPG), it is necessary to estimate the efficiency of use of metabolizable protein for gain ($k$). The $k$ (expressed in %) was calculated as the regression slope between the RP and MPI, according to the following model based on Chizzotti et al. (2007), but partitioning the MPI into MPI from milk or milk replacer and MPI from starter feed. Additionally, the effect of the liquid feed source (milk or milk replacer) was tested on the $\beta_1$ coefficient of the model.

\[
\text{RP (g/EBW}^{0.75}\text{ per day)} = \beta_0 + \beta_1 \times \text{MPI}_L + \beta_2 \times \text{MPI}_S,
\]  
[10]

where $\text{MPI}_L$ = metabolizable protein intake from liquid feed (g/EBW$^{0.75}$ per day); $\text{MPI}_S$ = metabolizable protein intake from starter feed (g/EBW$^{0.75}$ per day); $\beta_0$, $\beta_1$, and $\beta_2$ are regression parameters.

### Statistical Analysis

The parameters of the linear models were tested using the MIXED procedure, and nonlinear regressions were determined with the PROC NLMIXED procedure of SAS (version 9.2, SAS Institute Inc). As the database was composed of different studies, it was necessary to quantify the variance associated with the studies using the principles of meta-analysis (St-Pierre, 2001). The random effect of study was included and tested in the intercept and slope of all models, considering the possibility of covariance. For all linear models, the following 3 covariance matrices were tested: variance components (VC), compound symmetry (CS), and unstructured. First, the unstructured matrix was tested for adjusting random effect of study, and when models did not converge or the covariance was not significant ($P > 0.05$), the matrices VC and CS were tested. The VC or CS matrix selection was based on the corrected Akaike's information criterion, and the matrix with the smallest value was chosen. For all nonlinear models, the optimization and integration were performed using the dual quasi-Newton algorithm and adaptive Gaussian quadrature, respectively (Littell et al., 2006).

The effect of the genetic group (Holstein or crossbred Holstein × Gyr) was tested on the parameters of all models, and when the parameters were equivalent ($P > 0.05$), a single equation was generated. Residuals were evaluated for normality and dispersion of standardized residuals. Individual observations with studentized residuals greater than 2.5 or below −2.5 were considered “outliers” (Pell, 2000; Tedeschi, 2006) and excluded from the database. Additionally, when Cook’s distance was greater than 1, the study was considered an “outlier” and removed from the data set for that specific analysis (Cook, 1977, 1979). For all statistical procedures, a significance level of 0.05 was adopted for fixed and random effects.

### RESULTS

#### Body Weight and Body Gain Adjustments

Breed did not influence either the intercept ($P = 0.285$) or the slope ($P = 0.647$) of the relationship between BW and EBW. Moreover, the breed also did not influence the intercept ($P = 0.432$) and slope ($P = 0.552$) of the relationship between ADG and EBG. Thus, one equation was built for each of those relationships (Figure 1). Additionally, the intercept was not significant for EBW ($P = 0.350$) and EBG ($P = 0.381$) estimations. Thus, the following equations were fit [and $R^2$ and root mean square error (RMSE) reported; for all equations, the estimated parameters are followed by the SE in subscript]:

\[
\text{EBW (kg)} = 0.936 \pm 0.017 \times \text{BW (kg)},
\]  
[11]

$R^2 = 0.953$; RMSE = 2.43

\[
\text{EBG (kg/d)} = 0.939 \pm 0.031 \times \text{ADG (kg/d)},
\]  
[12]

$R^2 = 0.933$; RMSE = 0.04.

#### Energy Requirements

**Net Energy Requirements for Maintenance.**

When estimating the NE$_M$ of young preweaning calves, the breed did not affect the intercept ($P = 0.799$) or slope ($P = 0.881$) of Equation 4. Thus, a single equation was fit for both breeds (Figure 2) as follows:

\[
\text{HP} = 0.0702 \pm 0.0037 \times \exp^{3.8559 \pm 0.2247 \times \text{MEI}},
\]  
[13]

$R^2 = 0.734$; RMSE = 0.035.

where $\exp = (\text{HP measured as Mcal/EBW}^{0.75}\text{ per day; MEI measured as Mcal/EBW}^{0.75}\text{ per day})$.

The intercept of Equation 13 was used as the NE$_M$: 0.070 Mcal/EBW$^{0.75}$ per day or 70.2 Kcal/EBW$^{0.75}$ per day.

**Metabolizable Energy Requirements for Maintenance.** Using Equation 13, we determined the ME$_M$ as the moment where HP equals MEI, and thus there is no energy left for tissue deposit. The value obtained for ME$_M$ was 0.105 Mcal/EBW$^{0.75}$ per day or 105 Kcal/EBW$^{0.75}$ per day.
EBW$^{0.75}$ per day. The $k_m$ observed, which was determined by dividing NE_M by ME_M, was 0.66 or 66%.

**Net Energy Requirements for Gain.** We did not observe a breed effect on either the intercept ($P = 0.328$) or slope ($P = 0.199$) of Equation 5 to determine NE_G. Thus, we fitted the following single model to determine NE_G for Holstein and Holstein × Gyr preweaned dairy calves:

$$\text{NEEBW}_0.75 \times \text{EBW}^{0.75} \times 0.0053 + 0.9539 \times 0.2457,$$

where $\text{EBW}_0.75$ = average metabolic empty body weight (kg); $\text{EBG}$ is measured in kilograms per day.

**Metabolizable Energy Requirements for Gain.** The MEG was determined by dividing NE_G by the $kg$, which was determined as the regression between the RE and the MEI. In this specific analysis, 2 observations from Jolomba et al. (2020) were considered outliers (studentized residue greater than $|2.5|$). Our data analysis indicated no breed effect on the intercept ($P = 0.271$), $\beta_1$ ($P = 0.189$), or $\beta_2$ ($P = 0.423$) coefficients. However, the partitioning of MEI indicated ($P < 0.001$) different partial efficiencies for milk, milk replacer, and starter feed.

$$\text{RE} = \left\{ \begin{array}{l} -0.0491_{\pm 0.0084} + 0.576_{\pm 0.036} \times \text{MEI}_L + 0.412_{\pm 0.046} \\
0.493_{\pm 0.041} \times \text{MEI}_L + 0.412_{\pm 0.046} \\
-0.0491_{\pm 0.0084} + 0.493_{\pm 0.041} \times \text{MEI}_L + 0.412_{\pm 0.046},
\end{array} \right.$$

where $\text{RE} = \text{retained energy (Mcal/EBW}^{0.75}\text{ per day)}$; $\text{MEI}_L = \text{metabolizable energy intake from liquid feed}$.
(Mcal/EBW\(^{0.75}\) per day); MEIS = metabolizable energy intake from starter feed (Mcal/EBW\(^{0.75}\) per day).

The estimated kg of starter feed was 0.412 or 41.2%. As liquid feed source effect was observed and the kg was estimated as 0.576 or 57.6% for milk and as 0.493 or 49.3% for milk replacer. This represents a 14.4% difference in the efficiency between milk and milk replacer.

**Protein Requirements**

**Protein Requirements for Maintenance.** Breed did not influence either the intercept \((P = 0.792)\) or the slope \((P = 0.256)\) of the regression between MPI and ADG in Holstein and Holstein × Gyr calves (Eq. 7). Thus, a single model was fit for both breeds (Figure 3) as follows:

\[
MPI (g/d) = 78.67 ± 6.271 + 189.71 ± 26.339 \times ADG (kg/d),
\]

\[R^2 = 0.716; RMSE = 41.041.\]

When we divided the intercept (78.67) of Equation 16 by the average metabolic EBW observed in our database (18.65 kg), we determined the MPM as 4.22 g/EBW\(^{0.75}\) per day.

**Net Protein Requirements for Gain.** We did not observe a breed effect on \(\beta_0 (P = 0.455), \beta_1 (P = 0.112), \text{ or } \beta_2 (P = 0.153)\) of Equation 9. Additionally, the intercept of this model was not significant \((P = 0.853)\). Therefore, a single equation, without intercept, was fit to determine NPG of Holstein and Holstein × Gyr preweaned dairy calves as follows:

\[
NPG (g/d) = 30.06 ± 12.181 \times EBG (kg/d) + 70.98 ± 24.734 \times RE (Mcal/d),
\]

\[R^2 = 0.898; RMSE = 12.715.\]

**Metabolizable Protein Requirements for Gain.** The MPG was determined by dividing the NPG by \(k\), and \(k\) was determined by the regression between RP and MPI (Eq. 10). For this specific model, Cook’s distance analysis indicated the study of Rodrigues et al. (2016) as an outlier, and this study was removed from the data set before determining \(k\).

We did not observe breed effects on the intercept \((P = 0.687), \beta_1 (P = 0.529), \text{ or } \beta_2 (P = 0.822)\) coefficients of Equation 10. However, the partitioning of MPI indicated \((P < 0.001)\) different partial efficiencies for milk, milk replacer, and starter feed.

\[
\begin{align*}
RP &= \left(\frac{-2.18 ± 0.557 + 0.719 ± 0.068}{-2.18 ± 0.557 + 0.719 ± 0.068} \times MPI_{L}\right) + 0.444 ± 0.085 \\
&\times MPI_{S}, \text{ MPI from milk} \\
&\times MPI_{S}, \text{ MPI from milk replacer}
\end{align*}
\]

\[R^2 = 0.761; RMSE = 1.47,\]

where \(RP = \text{retained protein (g/EBW}^{0.75} \text{ per day); MPI}_{L} = \text{metabolizable protein intake from liquid feed (g/EBW}^{0.75} \text{ per day); MPI}_{S} = \text{metabolizable protein intake from starter feed (g/EBW}^{0.75} \text{ per day).}\)

The estimated \(k\) of starter feed was 0.444 or 44.4%. Concerning the liquid feed source, the \(k\) was estimated as 0.719 or 71.9% for milk and as 0.592 or 59.2% for milk replacer. This represents a 17.6% difference in the efficiency of milk and milk replacer.

**DISCUSSION**

**Body Weight and Body Gain Adjustments**

Body weight adjustment (Eq. 11 and 12) was not affected by breed. In general, the equations presented high accuracy and precision (Figure 1), and the \(\beta_1\) was close to that previously observed in calves (Fonseca et al., 2012; Rodrigues et al., 2016; Silva et al., 2017). It is worth noting that the \(\beta_1\) of the EBW equation (Eq. 11) was greater than that observed for postweaning dairy heifers (Castro et al., 2020) and bulls (Oss et al., 2017), likely because the filling effect in calves is lower than in...
functional ruminants. The \( \beta \) of the EBG equation (Eq. 12) was also greater than that reported in postweaning animals, but the difference was considerably lower (1.38%) when compared with Equation 11 (6.25%). These results are the first indication that heavier animals (>100 kg) should not be used to estimate requirements for preweaning dairy calves, such as those used by the NRC (2001). Therefore, all further calculations presented in this study were conducted using Equations 11 and 12.

**Energy Requirements**

The \( \text{NE}_M \) is linked to basal functions such as homeothermy, blood flow, respiration, tissue synthesis, and daily physical activities of feeding and walking (Garrett et al., 1959). The most recent system to determine nutrient requirements for beef cattle (NRC, 2016) suggests that \( \text{Bos indicus} \) animals present a \( \text{NE}_M \) 10% lower than \( \text{Bos taurus} \), which would also affect the requirements of crossbred animals. Nonetheless, when using young preweaning Holstein and Holstein × Gyr dairy calves, we did not observe differences in the \( \text{NE}_M \) requirements. Other studies with adult beef animals also suggested similar requirements among purebred and crossbred animals (Chizzotti et al., 2008; Marcondes et al., 2016). No other study was observed comparing \( \text{NE}_M \) requirements between purebred and crossbred dairy animals. We suspect there might be a difference in \( \text{NE}_M \) requirements among these animals, but this difference will be clearly pronounced only in heavier animals when their full performance is seen. Nonetheless, future studies should address this topic to understand how the \( \text{NE}_M \) changes throughout an animal’s life and how breed could affect that change. For that reason, we are suggesting a single \( \text{NE}_M \) of 70.2 kcal/BW\(^{0.75} \) per day for both breeds, which is also in agreement with previously published studies (Poczopko, 1971; Rodrigues et al., 2016; Silva et al., 2017). Lastly, we should highlight that our estimates of \( \text{NE}_M \) were close to those recommended by the NRC (2001), but about a half of that recommended by the Institut National de la Recherche Agronomique (INRA, 2018; Figure 4). Nonetheless, no real discussion is presented in the INRA (2018) to explain such a high recommendation for \( \text{NE}_M \).

When converting \( \text{NE}_M \) to \( \text{ME}_M \), we observed a value of 105 kcal/BW\(^{0.75} \) per day, which is similar to that reported by Silva et al. (2017), Gerrits et al. (1996), and the NRC (2001) of 105, 107, and 104 kcal/BW\(^{0.75} \) per day, respectively. The \( \text{ME}_M \) can be calculated by dividing the \( \text{NE}_M \) by the \( \text{km} \), which has been demonstrated to be affected by breed, sex, environment, and diet metabolizability (AFRC, 1993; NRC, 2000; CSIRO, 2007). We observed a \( \text{km} \) of 0.66, which is lower than that suggested by the NRC (2001) of 0.825. However, we suspect that the value reported by the NRC (2001) is overestimated as several other studies reported a \( \text{km} \) closer to that observed in this study, such as Silva et al. (2017), Schrama et al. (1992), Arieli et al. (1995), and the INRA (2018) who reported a \( \text{km} \) of 0.70, 0.68, 0.68, and 0.725 respectively. Additionally, despite previous authors reporting a breed influence on \( \text{km} \) (AFRC, 1993; NRC, 2000; CSIRO, 2007), Holstein and Holstein × Gyr calves had a similar \( \text{km} \) in this study. As we speculated before, maybe those breed differences might appear later in their lives, when the variety of diets and environments play a more important role in the feed efficiency of dairy animals. In preweaning dairy calves, more than 90% of their MEI comes from milk or milk replacer intake, which have a high metabolizability (Silva et al., 2017) and low variability in composition.

The \( \text{NE}_G \) is the energy deposited in the body either as protein or fat (Garrett et al., 1959). Thus, it is highly affected by the level of EBG and the proportion of fat retained in the EBG (Marcondes et al., 2016). When we compared our \( \text{NE}_G \) estimates with those proposed by the INRA (2018) and used by the NRC (2001), we observed that these models overestimated the \( \text{NE}_G \) for Holstein and Holstein × Gyr dairy calves raised in tropical conditions, especially in lighter animals (Figure 4). This overestimation has been previously reported (Diaz et al., 2001; Silva et al., 2017) and is likely justified by the BW of the animals used by the INRA (2018), which surpassed 100 kg. The most widely used moment to wean dairy calves is when they double their BW (Kienitz et al., 2017). If we take into consideration that the average birth weight of calves is around 37 to 38 kg, as we have observed in our data set (Table 1), preweaning dairy calves should be a maximum of 80 to 90 kg in normal conditions, which could explain the bias in the INRA (2018) model. Additionally, as the INRA (2018) used postweaning animals, the animals used to develop their models were fed with a low quality (metabolizability) diet when compared with calves’ diets, which would increase fat deposition in detriment of protein deposition, consequently overestimating \( \text{NE}_G \) (Diaz et al., 2001; Silva et al., 2017). On the contrary, our estimates of \( \text{NE}_G \) were greater than those suggested by the NRC (2001). Likely, this is related to body gain composition because it has been shown that for animals with the same weight and ADG, Holstein × Gyr deposit more fat than Holstein animals (Valadares Filho et al., 2010), mainly because of their lower mature BW, which is around 500 kg (Marcondes et al., 2016), whereas for Holstein in Brazil, it is around 660 kg (Coelho et al., 2009).

As with \( \text{ME}_M \) and \( \text{NE}_M \), \( \text{NE}_G \) is converted to \( \text{ME}_G \) by dividing \( \text{NE}_G \) by the \( \text{kg} \). Using mixed diets, and
considering a contribution in the total DMI of 60% from milk replacer and 40% from starter feed, the NRC (2001) used a general $kg$ of 0.652, which was estimated considering a $kg$ of 0.69 for milk replacer and 0.57 for starter feed. Additionally, Gerrits et al. (1996) and Blome et al. (2003) suggested a $kg$ of 0.58, and the ARC (1980) suggested that it should be between 0.50 and 0.59. We observed a $kg$ of 0.576 for milk, 0.493 for

Figure 4. Comparison among models proposed by the NRC (2001), INRA (2018), and this study to estimate total ME requirements for calves drinking either 4 (A) or 8 (B) L/d of milk or milk replacer, and ME requirements for maintenance and gain for calves drinking either 4 (C) or 8 (D) L/d of milk or milk replacer. NRC = National Research Center; INRA = Institut National de la Recherche Agronomique.
milk replacer, and 0.412 for starter feed. The estimated $k_g$ for milk was similar to that reported by Silva et al. (2017), which was 0.574. These authors also reported a $k_g$ of 0.393 for starter feed, which was 4.6% lower than the value reported in the study.

Previous authors reported that $k_g$ could be affected by several factors such as environment, animal activity, energy retained as protein, and diet metabolizability (ARC, 1980; Signoretti et al., 1999; Marcondes et al., 2013). Additionally, Garrett and Johnson (1983) mentioned that diet ME directly affects the efficiency of use of ME, mainly $k_g$. In this sense, and considering that milk has around 4.8 Mcal/kg of DM of ME, and milk replacer and starter feed have 4.4 and 3.6 Mcal/kg of DM of ME, respectively (Silva et al., 2017; Amado et al., 2019), we believe that the metabolizability was the primary driver of the $k_g$. Briefly, the ME concentration of milk replacer is 8% lower than milk, which reflects a difference of 14.4% in the $k_g$ estimation. Concerning starter feed, the ME concentration was 25% lower than milk, which implied a difference of 28.4% in the $k_g$ estimation.

### Protein Requirements

The quantity and quality of AA absorbed in the intestine are essential for all vital processes in the body, such as synthesizing enzymes, antibodies, and protein turnover. The sum of all processes that are not linked to tissue deposition constitutes the requirements of MP$_M$ (Valadares Filho et al., 2010, 2016). We observed a MP$_M$ of 4.22 g/EBW$^{0.75}$ per day, which is greater than that observed by Rotta et al. (2016) of 3.6 g/BW$^{0.75}$ per day for growing animals. Nonetheless, we should highlight that preweaning animals have a more pronounced protein metabolic rate than adult animals, which can in turn affect the protein turnover, which could explain this difference between young and adult animals (Rodrigues et al., 2016). It is also interesting to note that the method we used to determine MP$_M$ is different from that used by the NRC (2001). The previously used version involved the factorial method proposed by Blaxter and Mitchell (1948) based on the apparent digestible protein, in which the requirements were calculated from the sum of losses in fecal and urinary N. However, as stated before, the requirements suggested by the NRC (2001) were obtained using animals with a BW greater than 100 kg, and thus nonsuckling calves, which underestimated the MP$_M$ (Figure 5). Nevertheless, the INRA (2018) estimates, also using the factorial method for estimating MP$_M$, are closer (and likely not different) than ours, maybe because they used more contemporary animals and statistical tools not available when the NRC (2001) was obtained.

The NP$_G$ is affected mainly by sex, age, body gain, and especially body gain composition (Valadares Filho et al., 2010). Thus, young animals tend to have a greater proportion of water and protein in the gain when compared with older animals, which accumulate more fat in the BW gain (Owens et al., 1993). Equation 9 has been previously used to estimate NP$_G$ in beef (Owens et al., 1993; Marcondes et al., 2011a) and dairy (Oss et al., 2017; Castro et al., 2020) animals, but we should highlight the importance of the RE coefficient ($\beta_2$) of that equation. The NP$_G$ is directly affected by body gain composition, which is considered in the model by adding the RE (NRC, 2000). When we observe estimates of NP$_G$ of adult animals, it is common to observe negative coefficients associated with the RE (Valadares Filho et al., 2010; Marcondes et al., 2011a; Valadares Filho et al., 2016; Silva et al., 2018), which means that the majority of the energy is being deposited as fat. On the other hand, when we observe positive coefficients associated with the RE, as observed in our study, most of the energy is deposited as protein (Silva et al., 2017). Additionally, we should highlight that our estimates were intermediate between the NRC (2001) and INRA (2018). According to our results, the INRA (2018) system works better for young calves, whereas the NRC (2001) works better for older calves (Figure 5).

To convert NP$_G$ to MP$_G$, we determined the efficiency of use of metabolizable protein for gain ($k$). Several factors can affect $k$, such as age, sex, and diet (Oldham, 1987; Gerrits et al., 1997). However, the AA profile of dietary feedstuffs has been mentioned as the main factor affecting $k$ (Oldham, 1987; Silva et al., 2017). Our results agree with this affirmation; our $k$ for milk was 0.719, whereas $k$ for milk replacer was 0.592, and $k$ for starter feed was 0.444. The lower 17.6% efficiency of milk replacer, when compared with milk, is probably due to its composition. Generally, milk replacer has vegetable protein sources in its composition, as well as lower CP content, which could negatively affect the use of MP (Silva et al., 2017; Amado et al., 2019).

This theory is reinforced by the differences in $k$ observed between preweaning and adult animals. In this study, milk or milk replacer composed, on average, 88% of total DM; thus, the AA profile of diets of preweaning animals has a considerably higher biological value when compared with diets for adult animals. The literature reports $k$ values of 0.40 for adult cattle (Valadares Filho et al., 2016; Silva et al., 2018), similar to our estimated value for starter feed (0.444), emphasizing that feed AA profiles appears to be the primary influencer of $k$. For preweaning calves, $k$ values higher than 0.60 have been reported, and in a few cases values higher than 0.80 were reported (NRC, 2001; Blome et al., 2003; Bartlett et al., 2006; Rodrigues et al., 2016;
Silva et al., 2017). Last, the INRA (2018) suggests an equation to estimate $k$ that ranges from 0.47 to 0.82 and is based on the concentration of truly digestible protein in the intestine (g/kg of DM); nevertheless, no mention of young animals was made in that publication.

When estimates were summarized as total ME, our recommendations were intermediate between the NRC

![Figure 5. Comparison among models proposed by the NRC (2001), INRA (2018), and this study to estimate total MP requirements for calves drinking either 4 (A) or 8 (B) L/d of milk or milk replacer, and MP requirements for maintenance and gain for calves drinking either 4 (C) or 8 (D) L/d of milk or milk replacer. NRC = National Research Center; INRA = Institut National de la Recherche Agronomique.](image-url)
(2001) and INRA (2018). This was primarily driven because of the lower recommendation for both NE_M and NE_Q by the NRC (2001) and the high NE_M requirements of the INRA (2018). The total MP requirements of the NRC (2001) were also lower than our estimates and the INRA (2018). Yet, when comparing our estimates with those of the INRA (2018), we observed a different behavior when studying animals with low (milk replacer intake = 4 L/d) or high (milk replacer intake = 8 L/d) performance. In low-performance animals, MP estimates were greater than the INRA (2018), especially after 20 d of age. In high-performance animals, the average recommendations were similar between our estimates and the INRA (2018). However, there was almost no variation in the INRA (2018) recommendations across age, which led to overestimations of total MP for young animals (<35 d old) and underestimations of MP for older preweaned calves (>40 d old). This distortion occurred mainly because the INRA (2018) recommendations for MP_Q are based solely on ADG (more specifically protein gain), and thus are not affected by the age of BW.

**Other Implications**

Although we have used a robust data set, we believe that an increase in the number of studies in this field is still necessary to increase the power of the analysis and recommendations, allowing researchers to investigate breed differences between pure and crossbred preweaned dairy calves. It is essential to highlight that we need the information of individual animals and not only the reported regressions in the papers for this kind of meta-regression. This is a limitation in creating robust data sets and may partially explain why those studies are currently so infrequently conducted. Thus, we strongly encourage researchers to continue to perform studies aiming to determine nutrient requirements for pure and crossbred preweaned dairy calves in either tropical or temperate regions. Additionally, we encourage authors to provide the original data set (with individual information) as supplementary files to support the creation of more robust data sets. Because of lack of information, data on mineral requirements could not be added to this study, and the most recent publication on mineral requirements for calves (Castro et al., 2019) also reports a lack of studies with preweaned calves.

Our models indicated differences compared with models generated in temperate climates, mainly the NRC (2001) and INRA (2018) estimations. That is probably linked to the use of heavier animals (more than 100 kg of BW) and not preweaned calves by these systems, as we have addressed above. Therefore, we highlight that all studies that composed our data set were carried out in tropical climates; therefore, we are unsure if our estimates will be totally applicable to temperate rearing conditions. Thus, future studies validating those recommendations for temperate regions are warranted.

**CONCLUSIONS**

We compared the energy and protein requirements of Holstein and Holstein × Gyr crossbred preweaned calves, and no differences were observed in energy and protein requirements. Regarding the efficiency of use of ME for gain, we observed a value of 0.576 for milk, 0.493 for milk replacer, and 0.412 for starter feed. The efficiency of use of MP for gain followed the same pattern, with values of 0.719 for milk, 0.592 for milk replacer, and 0.444 for starter feed. We also compared our estimates with those of the NRC and INRA. Our ME estimates were intermediate between those systems. Our MP estimates were greater than those suggested by the NRC, and on average, were close to those suggested by the INRA. However, the variation in the estimates of the INRA was very low, and thus did not change across ages. Therefore, we propose that the equations generated in the present study should be used to estimate energy and protein requirements for preweaned Holstein and Holstein × Gyr crossbred dairy calves raised under tropical conditions.

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