

FELIPE DE PAIVA PROVAZI

PROTEIN REQUIREMENTS FOR PREGNANCY IN DAIRY COWS

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*.

Adviser: Polyana Pizzi Rotta

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Assent:



Felipe de Paiva Provazi
Author



Polyana Pizzi Rotta
Adviser

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ABSTRACT

PROVAZI, Felipe de Paiva, M.Sc., Universidade Federal de Viçosa, December, 2019. **Protein requirements for pregnancy in dairy cows.** Adviser: Polyana Pizzi Rotta.

The data that compose the protein requirements for the pregnancy are scarce. The objective of the present study was to estimate the nutritional requirements of protein for maintenance, weight gain, and pregnancy of crossbred Holstein × Gir dairy cows. Sixty-two cows were used. We used the comparative slaughter technique to estimate the requirements for pregnancy. Six animals were used as reference group and they were slaughtered at the beginning of the experiment to estimate initial empty body weight and initial body composition. The remaining cows were divided into two groups: pregnant (n = 44) and non-pregnant (n = 12). Pregnant and non-pregnant cows received two different diets: maintenance (MA) and ad libitum (AD). Pregnant cows were slaughtered at four different days of gestation (DG): 139, 199, 241, and 268. The non-pregnant cows were slaughtered at 199, 241, and 268 days of gestation. The pregnant cows had the gravid uterus collected and weighed. The gravid uterus was dissected into fetus, placenta, uterus, and fetal fluids. The metabolizable protein for requirements of maintenance was $3.60 \text{ g/EBW}^{0.75}/\text{d}$. The adjusted equation to estimate the net protein requirements for gain (NE_g) was: $\text{NE}_g \text{ (g/day)} = 0.592554 \times (\text{EBW}_{\text{open}}^{-0.268})$. The efficiency of use of metabolizable protein for gain (k_g) was 35.30%. The equation to predict the net protein requirements for gain (NP_g) was: $\text{NP}_g \text{ (g/day)} = 0.0001767 \times \exp^{(0.02666 \times \text{DG})}$. The efficiency of use of metabolizable protein for pregnancy (k_{gest}) was 65.30%. Differences are observed when comparing our values for MP_m , NP_g , NP_{gest} , K_g and K_{gest} data with other systems and studies. These differences may be due to differences in the calculation method and breed animals used. We suggest the equations and efficiency factors proposed in this study for accurate protein requirement estimates for pregnant dairy cows.

Keywords: Fetal growth. Fetus. Gestation

RESUMO

PROVAZI, Felipe de Paiva, M.Sc., Universidade Federal de Viçosa, dezembro de 2019. **Exigências de proteína para vacas leiteiras em gestação.** Orientadora: Polyana Pizzi Rotta.

Os dados que compõem as exigências de proteína para a gestação são escassos. O objetivo do presente estudo foi estimar as exigências nutricionais de proteína para manutenção, ganho de peso e gestação de vacas mestiças leiteiras Holandês × Gir. Sessenta e duas vacas foram utilizadas para a execução do trabalho. A técnica do abate comparativo foi utilizada. Seis animais foram utilizados como grupo referência e foram abatidos no início do experimento para estimativa do peso corporal vazio inicial e composição corporal inicial. As demais vacas foram divididas em dois grupos: gestantes (n = 44) e não gestantes (n = 12). Vacas gestantes e não gestantes foram submetidas a dois regimes alimentares: manutenção (MA) e ad libitum (AD). As vacas gestantes foram abatidas em quatro dias diferentes de gestação (DG): 139, 199, 241 e 268. As vacas não gestantes foram abatidas aos 199, 241 e 268 dias de gestação. As vacas gestantes tiveram o útero grávido coletado e pesado imediatamente após serem abatidas. O útero grávido foi dissecado em feto, placenta, útero e fluidos fetais. A proteína metabolizável para as necessidades de manutenção foi 3,60 g/PCVZ^{0,75}/d. A equação ajustada para estimar as necessidades líquidas de proteína para ganho (NEg) foi: $NEg (g / dia) = 0,592554 \times (EBW_{open} - 0,268)$. A eficiência de uso da proteína metabolizável para ganho (kg) encontrada foi de 35,30%. A equação para prever as necessidades líquidas de proteína para ganho (NPg) foi: $NPg (g / dia) = 0,0001767 \times \exp(0,02666 \times DG)$. A eficiência do uso da proteína metabolizável para a gravidez (kggest) foi de 65,30%. Diferenças são observadas quando comparamos nossos valores de MPm, NPg, NPgest, Kg e Kgest com outros sistemas e estudos. Essas diferenças podem ser devidas a diferenças no método de cálculo e raças usadas. Nós sugerimos as equações e fatores de eficiência propostos neste estudo para estimativas precisas da necessidade de proteína para vacas leiteiras gestantes.

Palavras-chave: Crescimento fetal. Feto. Gestação

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INTRODUCTION

Adequate estimates of nutritional requirements allow the development of optimized and efficient diets according to the objective of the nutritionist. For example, fewer amounts of nutrients in diets can reduce N losses (Rotz, 2004). In addition, N management is a challenge to reduce excretion to the environment without negatively affecting animal performance and the efficiency of the use of this nutrient is a tool that can be used in dairy herds (Pacheco et al., 2017). Thus, knowledge of nutritional requirements and the use of systems is essential for the development of nutrition plans regardless of category.

In tropical conditions, diets formulated for dairy cattle are based on international systems such as AFRC (1993), NRC (2001), CSIRO (2007) and INRA (2018). NRC (2001) represents the most widely used system (Castro et al., 2020). However, the data and the estimates from NRC (2001) for gestation have some limitations.

This system used data only from Bell et al. (1995) for their estimates for pregnancy requirements. This study considered requirements just after 190 d of gestation and developed a quadratic equation to describe protein retention in the gravid uterus, including the variables concept weight, calf weight at birth, and days of gestation (**DG**). The weight of the calf at birth is considered a determinant factor in the requirements for pregnancy. Therefore, it is presumed that factors that affect birth weight play an important role in the requirements for pregnant cows, as bull and cow breed, heterosis occurrence, pregnant female age, environmental conditions, duration of pregnancy, occurrence of twin birth, fetal sex, and nutritional management of the pregnant cow (Ferrell, 1991).

Thus, there is clearly a need for more information about the protein requirements for gestation and the pattern of this requirement over the gestation time, due to the lack of available data and the various factors that may affect this requirement. Supply the nutritional requirements of pregnant dairy cow aims to ensure an adequate supply of nutrients for the growth and development of the fetus, ensuring to female an adequate body condition at calving, and to provide adequate nutrients for continued growth to heifers. Thus, the objective of the present study was to estimate the nutritional requirements of protein for maintenance, body gain, and gestation of dairy cows.

MATERIAL AND METHODS

This study was conducted at Universidade Federal de Viçosa (Viçosa, MG, Brazil) following the standard procedures for humane animal care and handling according to the university's guidelines (CEUAP/UFV – 11/2013).

Animals and Management

Sixty-two Holstein × Gyr cows were used, with an average initial BW of 480 ± 10.1 kg and age of 5 ± 0.5 yrs. The cows were divided into three groups: pregnant ($n = 44$), non-pregnant ($n = 12$), and baseline ($n = 6$). Baseline animals were slaughtered before starting the experiment (at day 0). Pregnant and non-pregnant cows received two different diets: maintenance (**MA**) and ad libitum (**AD**). For MA group the DMI was considered as 1.15% of BW, according to the previous study of Duarte et al. (2013). The cows were weighted every 28 d, after a 16 h fast to obtain the shrunk BW (SBW). The SBW was used for adjusted feed intake of MA treatment. Pregnant cows were slaughtered at four different DG: 139, 199, 241, and 268 d of feedlot, with 11 cows slaughtered in each DG. For non-pregnant cows, the slaughter periods were 199, 241, and 268 d of feedlot, with 4 cows slaughtered in each DG. For more information see Rotta et al. (2015 a, b, c).

Cows were housed in individual pens, equipped with individual feed bunks and an automatic water system. All cows had ad libitum access to water. The diet was based on corn silage and concentrate, with a ratio of 93:7 DM basis as a total mixed ration. Cows were fed twice daily, with 60% offered in the morning and 40% in the afternoon feeding. The amounts of corn silage, concentrate and orts were daily recorded. Corn silage and orts were daily sampled and stored at -20 °C until analyzed. The AD cows had their diets adjusted for allow approximately 5% orts daily on an as-fed basis.

Fecal Measurements, Urine and Slaughter

The study had six periods of spot fecal and urine collection, within each period of 28 d. Feces were collected during the last 5 d of each period. Fecal collections were performed at 0600, 0900, 1200, 1500, and 1800 h, being one collection per day. The fecal samples contained approximately 200 g each. They were collected by rectal stimulation or immediately after the

animal defecated. The samples were dried in a forced-air oven at 55 °C for 72 h and ground in a 1 mm knife mill (Wiley mill, A. H. Thomas). A composite sample of each animal was done at the end of each period utilizing 15 g of the dried and ground sample per day. Urine collections were performed at 0600 and 1500 h on first and fourth day of feces collection, respectively. Urine samples were taken midstream after manual stimulation of the vulva. After collection, urine samples were acidified to a pH < 4.0 with concentrated sulfuric acid and then frozen at –20 °C for further analyses of N, urea, allantoin, creatinine, and uric acid.

The slaughter was performed by a captive bolt stunner, followed by exsanguination. After exsanguination, the gravid uterus was immediately collected through section at the height of the cervix and weight. The gravid uterus was dissected into fetus, fetal membranes, uterus, and fetal fluids, and each component was ground and individually sampled. The mammary gland was also entirely sectioned and ground. Samples of the pregnant uterus and mammary gland were stored at -80 °C for further analyses.

The carcasses of each cow were divided into half carcasses and ground. The non-carcass sample was composed by the four stomachs, intestines, internal organs, head, tail, hooves, trimmings, leather, and blood. Carcass and non-carcass tissue samples were separately formed. All samples were ground and homogenized for further analyses.

Laboratorial Analyzes

Corn silage, ingredients of concentrate, and feces were analyzed for DM, OM, and N concentrations (AOAC International, 2000; method numbers 934.01 for DM, 930.05 for OM, and 981.10 for N). The NDF was estimated according Mertens et al. (2002) without the addition of sodium sulfite and with the addition of thermostable α -amylase to the detergent (Ankon Technology Corp., Fairport, NY).

Fecal excretion was estimated using indigestible NDF (**iNDF**) as internal marker. The iNDF content in samples of feces, feed, and orts were estimated in triplicate. These samples were ground in a knife mill using a 2 mm sieve. A sample of 1.5 g was weighed in each pre-weighed polyester bag with a pore size of 12 μ m and a pore area of 6% of the total surface (Saatifil PES 12/6, Saatitech S.p.A., Veniano, Como, Italy). The incubation lasted 288 h and was performed in 2 cannulated bulls fed a diet of 50% corn silage and 50% concentrate based on DM at MA level. After incubation, the bags were washed, dried at 55 °C for 48 h and weighed. Residues

were analyzed for NDF using an Ankom 200/220 Fiber Analyzer (Ankon Technology Corp.). Heat-stable α -amylase (Mertens et al., 2002) was used to estimate NDF.

Allantoin, creatinine, and uric acid were analyzed by using an HPLC (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) as previously described by Czauderna and Kowalczyk (2000), with modifications by George et al. (2006).

Samples of carcass, non-carcass, mammary gland, uterus, placenta, fetal fluids and fetus, were analyzed for DM (AOAC, 2000; method 934.01) and CP (AOAC, 2000; method 981.10).

Calculations and Estimated Equations

To estimate the excretion of purine derivatives, equations previously described by Chen, (1989), Verbic et al., (1990) and Chen et al., (1995) were used. The supply of microbial N estimated through the excretion of purine derivatives was estimated from four intermediate steps: calculation of total purine derivatives, daily excretion of DP, purines absorbed daily and, finally, supply of microbial N. More details on calculations and equations used are available in Rotta et al. (2015b).

Microbial crude protein (**MCP**) was estimated by the equation:

$$MCP = \text{microbial N} \times 6.25 \quad [1]$$

Where: MCP = microbial crude protein (g/d); Microbial N = microbial nitrogen (g/d)

The RDP requirement was considered equal to the MCP (estimated by equation 1), according to BR-CORTE (2016). The RUP intake was estimated as the difference between the CP intake and RDP.

The MP intake (**MPI**) was calculated by the following equation (NRC, 2001):

$$MPI = (RDP \times 64\% + RUP \times 80\%) \quad [2]$$

Where: MPI = metabolizable protein intake (g/d); RDP = rumen degradable protein (g/day); RUP = rumen undegradable protein (g/d); For protein originated from bacteria and protozoa was considered the value of 80% for true protein and 80% to digestibility, thus the value the 64% was used from RDP to MPI. The RUP was considered to contain 100% true protein with 80% digestibility.

The following model was used to calculate the relationship between body weight and empty body (**EBW**) weight and it was tested effect of **DG** on the model parameters:

$$EBW = \beta_0 + \beta_1 \times BW \quad [3]$$

Where: β_0 and β_1 = regression parameters; BW = Body weight.

Requirements for maintenance. The MP requirements for maintenance (MP_m , g/kg of $BW^{0.75}$) were estimated, as proposed by Wilkerson et al. (1993), from the linear regression between MPI (kg/d) and empty body gain (EBG, kg/d), with subsequent intercept division by average metabolic body weight:

$$MPI = \beta_0 + \beta_1 \times EBG \quad [5]$$

Where: MPI = Metabolizable protein intake; β_0 and β_1 = regression parameters; EBG = Empty body gain.

$$MP_m = \frac{\beta_0}{EBW^{0.75}} \quad [6]$$

Where: MP_m = Metabolizable protein for maintenance, β_0 = Intercept of equation [5] and $EBW^{0.75}$ = Average metabolic body weight the animals used in this study = 97.97 kg.

Requirements for gain. We used the EBW_{np} to estimate net protein requirements for gain. The EBW_{np} consists of discounting the portion of accumulation in gestation components (GEST) composed by uterus, fetal fluids, fetus, placenta and mammary gland, related of that is due to pregnancy. Calculated by EBW of empty animals minus the gestational component of pregnant animals, estimated according to the following equation:

$$EBW_{np} = EBW - GEST_{preg} \quad [7]$$

Where: EBW_{np} = empty body weight for pregnant animals (kg); EBW = empty body weight (kg); $GEST_{preg}$ = gestation component for pregnant cows (kg).

Net protein requirements for gain (NP_g) were estimated by the first derivative of the CP equation retained in the final EBW, as follows:

$$BCP = \beta_0 \times (EBW_f^{\beta_1}) \quad [8]$$

Where: BCP = Crude protein in final empty body weight for non-pregnant tissues (kg), EBW_f = Empty body weight final (kg), β_0 and β_1 are regression parameters. We used the final empty body weight for non-pregnant and baseline animals.

The efficiency of the metabolizable protein for gain (k_g) was calculated from the regression between the retained protein (g/ $EBW^{0.75}$ /d) and the MPI (g/ $EBW^{0.75}$ /d):

$$RP = \beta_0 + \beta_1 \times MPI \quad [9]$$

Where: RP = Retained protein, MPI = Metabolizable protein intake, β_1 admitted as k_g and β_0 and β_1 are regression parameters.

The metabolizable protein requirement for gain (MP_g , g/kg $EBW^{0.75}$ /d) was estimated by dividing NP_g by k_g .

$EBG_{corrected}$ is the empty body weight gain corrected for the gestational components gain. We used an exponential model to adjust the weight gain without accounted addition of GEST components and calculate requirements for body weight gain.

$$EBG_{corrected} = 0.008214 \times EXP^{(DG \times 0.01)} \quad [10]$$

Where: $EBG_{corrected}$ = Empty body weight gain corrected and DG = days of gestation.

Requirements for gestation. We used an exponential model with the 42 pregnant animals to estimate protein accumulation in gestational components ($GEST_{preg}$). Thus, only protein accumulation due to gestation was accounted for:

$$GEST_{preg} = 0.4228 \times EXP^{(0.01291 \times DG)} \quad [11]$$

We used the balance of GEST and the DG to estimate protein requirements for gestation. Balance of GEST was calculated as the difference between the initial and final weight of each component. To predict the initial GEST, a linear regression was estimated as a function of the final protein in the GEST and the EBW of the reference animals.

Initially, three models were selected to fit the gestation component as a function of DG: quadratic, single exponential, and double exponential.

$$Quadratic\ model = \beta_0 + \beta_1 DG + \beta_2 DG^2 \quad [12]$$

$$Simple\ exponential\ model = \beta_0 \times exp^{(\beta_1 \times DG)} \quad [13]$$

$$Double\ exponential\ model = \beta_0 \times exp^{exp^{(\beta_1 \times DG)}} \quad [14]$$

Where: DG is days of gestation and β_0 and β_1 are equation parameters.

To assess the best fit, Akaike information criterium (AIC) was used. This method demonstrates within a group of preselected models the one with the lowest Kullback-Leibler (K-L) divergence. The lowest AIC value found for a model represents the best fit.

The efficiency of protein utilization for gestation (k_{gest}) was calculated by the iterative method using the following equation:

$$\Delta = MPI - \left(MP_m + \frac{NP_g}{k_g} + \frac{NP_{gest}}{k_{gest}} \right) \quad [15]$$

Where: MPI = metabolizable protein intake (g/d), MP_m = metabolizable protein for maintenance (g/d), NP_g = net protein for gain (g/d), k_g = efficiency of use of metabolizable protein for gain (%), NP_{gest} = net protein for gestation component (g/d) and k_{gest} = efficiency of protein utilization for gestation (%).

The iteration was performed aiming at a zero deviation between MPI and the other MP requirements values. However, the other components needed to estimate MP requirements (MPI, MP_m , NP_g , k_g and NP_{gest}) were previously calculated.

The summary of all equations used in this study to estimate the protein requirements for gestation cows is listed in Table 1.

Statistical Analyses

The linear regression parameters were estimated using PROC MIXED SAS (9.4). Estimates of the parameters of non-linear regressions were adjusted through the PROC NLIN SAS (9.4). For all tests, $\alpha = 0.05$ was used as the critical probability level for type I error.

RESULTS AND DISCUSSION

Relationship between body weight and empty body weight

There was no effect of gestation days on the model intercept and the intercept did not differ from zero ($P > 0.05$). However, an effect of the DG was found for the slope of the relationship between BW and EBW. Thus, the following equations were described to estimate the EBW:

$$\text{For up to 200 DG: EBW} = \text{BW} \times 0.8737 \quad [16]$$

$$(\text{R}^2 = 0.9760)$$

$$\text{For up to 268 DG: EBW} = \text{BW} \times 0.9041 \quad [17]$$

$$(\text{R}^2 = 0.9772)$$

Where: EBW = Empty body weight and BW = Body weight.

Requirements for Maintenance

The MP_m was calculated by dividing the intercept of the equation [5] by average $EBW^{0.75}$ of cows used in the experiment (97.97 kg). Thus, the MP_m observed in this study was 3.60 g/ $EBW^{0.75}$ /d.

$$\text{MPI (kg/d)} = 356.03 \pm 11.318 + 496.40 \pm 12.364 \times \text{EBG (kg)} \quad [18]$$

$$(\text{R}^2 = 0.879, \text{MSE} = 7502.86)$$

Where: MPI = Metabolizable protein intake, kg/d and EBG = Empty body gain

The BR-CORTE (2016) suggests a maintenance metabolizable protein value of 3.93 g/ $EBW^{0.75}$ /d. The value suggested in this study is 9.16% lower than the value suggested by BR-CORTE (2016) (Figure 1B).

Comparing estimates of MP_m (g/d) for animals weighing 400 to 700 kg from NRC (2001) and our data (Figure 1B), we observed that the estimated demand for NRC (2001) data is greater than observed from our data. For calculations of maintenance estimates by NRC (2001), we considered the average DMI of ad libitum treatment of this study. This shows that NRC (2001) estimates vary little with increased body weight and DMI has a greater influence on the requirement for maintenance in this system. In NRC (2001) system the MP_m is based on the factorial system and consist of the sum of losses endogenous urinary N, scurf N (skin and hair secretions) and metabolic N fecal .

INRA (2007) suggests a value for maintenance metabolizable protein of $3.25 \text{ g/EBW}^{0.75}/\text{d}$. This value is 9.72% lower than estimated in this study. In the new version of this system some changes are suggested to compute this requirement due to level of intake or production. INRA (2018) used a factorial approach and suggests a model that considers the sum of fecal endogenous N losses, endogenous urinary N losses, and scurf N losses. This system considerer the DMI and non-digestible OM for fecal N losses and BW for urine N loss and scurf losses. Considering the average data from this study of: BW = 517 kg, DMI = 9.71 kg, and non-digestible OM = 2.33 kg and an efficiency of digestion of 67%, as suggested by this system, the MP_m would be $2.2 \text{ g/EBW}^{0.75}/\text{d}$. This value is 38% lower than that found in the present study (Figure 1B). However, using the factorial method to estimate the requirements has some limitations, such as it is not considered changes that may occur during the experimental period and variations between animals (Hauschild et al., 2009).

Requirements for Gain

The body protein content can be estimate following the equation:

$$BCP = 0.8095_{\pm 0.04705} \times (EBW_f^{0.7320_{\pm 0.00943}}) \quad [19]$$

$$(R^2 = 0.7000)$$

Where: BCP = Body crude protein, kg and EBW_f = Final empty body weight, kg.

The NP_g was calculated using the first derivative of the equation of body protein content multiplied by $EBG_{corrected}$.

BR CORTE (2016) uses a linear model that considered the weight gain of empty body and net energy for gain. The effect on the gain composition of this system is expressed through the BCS. Thus, the protein requirement for gain does not vary with body weight (figure 2B). Considering a cow of 500 kg of BW and $ECC = 5$, the net protein for gain of this system is 23%

lower than our data. However, when an ECC = 3 is compared for the same BW, the BR - CORTE requirements become 6.2% higher.

In Figure 2A we express BCP and NP_g in relation to empty body weight. We can observe the opposite pattern with increased body weight of the animal. BCP_f increases due to animal growth and more protein is deposited. However, the protein requirement for gain decreases due to the change in the type of gain.

We used the EBG_{corrected} for the final calculation of the requirement for gain (Equation 8). Thus, the adjusted equation to estimate the net protein requirement is presented:

$$NP_g = 0.8095 \times 0.732 \times (EBW_{open}^{-0.268}) \times EBG_{corrected} \quad [20]$$

$$(R^2 = 0.901, MSE = 12.43)$$

Where: NP_g = net protein requirement for gain (g/day), EBW_{open} = empty body weight final (kg), EBG_{corrected} = empty body gain corrected (kg/d).

In Figure 2B we compared estimates generated from equations suggested by our data with NRC (2001) and both exhibit the same pattern. A greater net protein requirement for gain was observed when animals had lower BW, being that the BW increases the demands decrease. However, NRC (2001) estimates show a greater reduction in net gain requirements with a greater BW, compared to our data. Using extrapolations to estimate requirements the data are not close. Therefore, our data suggest the importance of requirement estimates for each category. These observed numerical differences between our results and NRC (2001) estimates may be due to the origin of the animals used to estimate the equations. The animals used in this study had BW close to maturity at the beginning of the study, around 450 kg because we used Holstein × Gyr cows. Thus, the protein requirements for BW gain of these animals are more constant, because the fat accumulation of these animals was greater. Considering an animal fed an energy-adjusted diet, the protein ratio decreases, and the fat ratio increases in the EBW with maturity (NRC, 2016). The equations used to estimate NP_g for adult cow are the same for growing dairy heifers, considering mature size adjustments. The requirements are the same as those used for growing beef heifers. Fortin et al. (1980) indicated a difference in body composition of beef and dairy animals at the same BW. In addition, the data range used by the NRC includes animals lighter than our data, with greater protein deposition, which may explain the larger decline in requirements over time.

The efficiency of protein use for gain (k_g), estimated as the slope of the regression between the retained protein in the animal's body (CP_r) and the MPI is below:

$$CP_r = -2.0432_{\pm 0.15799} + 0.3530_{\pm 0.02126} \times MPI \quad [21]$$

($R^2 = 0.775$, $MSE=0.068$).

The estimated k_g in this study was 35.30%. The NRC (2001) suggests the equation proposed by Ainslie et al. (1993) to calculate the k_g of animals with a BW of less than 478 kg, and 28.9% for animals with BW greater than 478 kg. The data found in the literature suggest an efficiency of protein use for weight gain in the order of 74 to 80% for calves (Bartlett et al., 2006, NRC, 2001). Castro et al. (2020) suggests a 47% kg value for growing crossbred Holstein × Gyr heifers. However, our data include animals in another phase of life, close to weight to maturity. This explains the lower protein deposition efficiency for weight gain from our data. Thus, we suggest a value of 35.30% for adult Holstein × Gyr crossbred cows.

Requirements for Gestation

Requirements for gestation were calculated based on protein accumulation in GEST. After that protein accumulation was calculated, we used a simple exponential model to adjust the net protein requirement for gestation, as function of DG. The pattern between crude protein retention in GEST tissues exclusive due to gestation and **DG** are shown in Figure 3A.

This model was the most appropriate to the data, considering the AIC criterion as evaluation, as follows:

$$CP_{gest} = 0.006629_{\pm 0.00028} \times \exp^{(0.02666_{\pm 0.00016} \times DG)} \quad [22]$$

($R^2 = 0.99425$; $MSE = 2.0694552$).

Where = CP_{gest} = crude protein retained in gestation due to Gestation (kg), DG = gestation days, β_0 and β_1 = equation parameters.

The first derivative of this model was adopted as net requirement for Gestation (NP_{gest}):

$$NP_{gest} = 0.0001767 \times \exp^{(0.02666 \times DG)} \quad [23]$$

BR-CORTE (2016) uses the same methodology for the GEST components. Where only exclusive protein accumulations due to pregnancy are computed. This system also uses an exponential model to describe the requirements for pregnancy, considering the calf's weight at birth and days of gestation. The requirements of this system are slightly higher than our data at the beginning of pregnancy, but in the final third our work shows greater growth. This can be explained by the difference in breed of the animals. (figure 3B).

The current estimates of gestation requirements using NRC (2001) are based on the study from Bell et al. (1995). Data from Bell et al. (1995) come from Holstein cows with an average

BW of 714 kg. They evaluated growth rates and conceptus composition in multiparous cows slaughtered at 190 to 270 d of gestation. A quadratic regression described protein accumulation in the uterus. Calf weight and DG were considered in the protein estimation.

NRC (2001) net gestation protein estimates start at 190 d and are 49% greater in this DG than our estimates. Approximately from d 230 of DG our requirement is greater (Figure 3B). On DG 270 our requirement is 55,9% greater than NRC (2001). After this period, protein requirement of NRC appears to grow at similar rates. Our data show a high growth rate from 200 d of gestation. This large increase in requirement may be due to high protein deposition in the uterus and mammary gland. Our data differ from the NRC (2001) because we computed the increase in protein in the udder, this may explain the largest increase in late gestation coupled with fetal growth. Sguizzato (et al., 2020) using the same database as this study, found an 32,83 % increase in protein accumulation from 0 d to mid gestation and 32,98% increase in protein accumulation from mid-gestation to late gestation.

In addition, the fetus has a large participation in growth and weight at the end of gestation. Bell et al. (1995) found higher fetal growth in relation to non-fetal components in late gestation, especially in dry matter. The participation of the fetus in relation to the other components increases from 45% at 190 days to 80% at 270 days of gestation. Prior et al. (1972) found an exponential equation to describe the weight of the fetus as gestation progressed. Ferrell et al. (1976) observed great fetal growth with increasing gestation days and used an exponential equation to describe this pattern. Thus, the large growth of the fetus and mammary gland at the late gestation indicates that the exponential model presented by our data represents well the requirements for gestation.

The protein utilization efficiency for gestation (k_{gest}) was calculated by the iterative method (Equation 12). The deviation between estimated metabolizable protein intake and metabolizable protein requirements (MP_m , MP_g and MP_p) was zero when $k_{gest} = 65.8\%$. This value is close to that suggested by the NRC (2016) of 65% for beef cattle. The latest version of the NRC for dairy cattle suggests a 33% protein efficiency value during gestation. Greater estimates of protein utilization efficiency for gestation were reported for swine. Speer (1990) evaluating nitrogen and amino acid partition in swine has suggested 63.5% efficiency in protein utilization for products of gestation.

Total metabolizable protein requirements

In figure 4 we compared the sum of the metabolizable protein requirements of maintenance, weight gain and gestation of NRC (2001) with our data. Up to 190 days of gestation, the requirements of the NRC (2001) refer only to maintenance and gain. After 190 d of gestation we can observe the exponential pattern in our data due to pregnancy requirements. Although, our net requirement data for gain and pregnancy are relatively higher than NRC (2001) when we evaluated metabolizable protein, our data show a lower total metabolizable protein requirement value. This is due to the highest values of efficiency of use of metabolizable protein for pregnancy and gain.

CONCLUSION

In conclusion, our data of requirements and efficiency of use of protein for maintenance, growth, and gestation show differences in pattern and values, when compared to literature data. Due to the behavior of protein accumulation in the pregnancy components, especially in the fetus, we believe that the exponential model better describes the requirements for pregnancy. In addition, the data in the literature to estimate protein requirement for gestation are limited for dairy cows. We suggest of the use the equations described in this study for estimate MP_m , NP_g , NP_{gest} and k_{gest} and k_p for pregnant dairy cows. This data can contribute to a future database for generating accurate requirements for pregnant dairy cows.

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APPENDIX

Table 1 – Abstract of equations and estimations used in this study to estimate protein requirements for pregnancy cows.

Item	Equation	Unit	Description
Intermediate calculations			
MCP	microbial N × 6,26	g/d	Microbial crude protein

EBW	$BW \times 0.8737$	kg	Empty body weight, up to 200 GD
EBW	$BW \times 0.9041$	kg	Empty body weight, 200 – 268 GD
RDP	$MCP \times 1$	g/d	Rumen degradable protein
RUP	$CP \text{ intake} - RDP$	g/d	Rumen undegradable protein
MPI	$RDP \times 64\% + RUP \times 80\%$	g/d	Metabolizable protein intake
EBG	$EBW_f - EBW_i$	kg/d	Empty body weight
EBW_{open}	$EBW - GEST_{preg}$	kg	Empty body weight discounting a portion accumulation of GEST components due the pregnancy
$GEST_{preg}$	$0.4228 \times \exp^{(0.01291 \times DG)}$	kg	Estimated portion of accumulation of GEST tissues due the pregnancy
$GEST_{open}$	$0.000005592 \times (EBW^{2.086})$	kg	Estimated portion of GEST components that occur naturally, without influence of gestation
$EBG_{corrected}$	$0.008214 \times \exp^{(DG \times 0.01)}$	kg/d	Empty body gain discounted weight of gestation components
GEST	Uterus + Fetal fluids + Fetus + Placenta + Mammary gland	kg	Gestation component
Protein requirements			
MP_m	$3.6 \times EBW^{0.75}$	g/d	Metabolizable protein for maintenance
NP_g	$0.592554 \times (EBW_{open}^{-0.268})$	g/d	Net protein for gain
MP_g	NP_g/k_g	g/d	Metabolizable protein for gain
NP_{gest}	$0.0001767 \times \exp^{(0.02666 \times GD)}$	g/d	Net protein for pregnancy
MP_{gest}	NP_{gest}/K_{gest}	g/d	Metabolizable protein for pregnancy

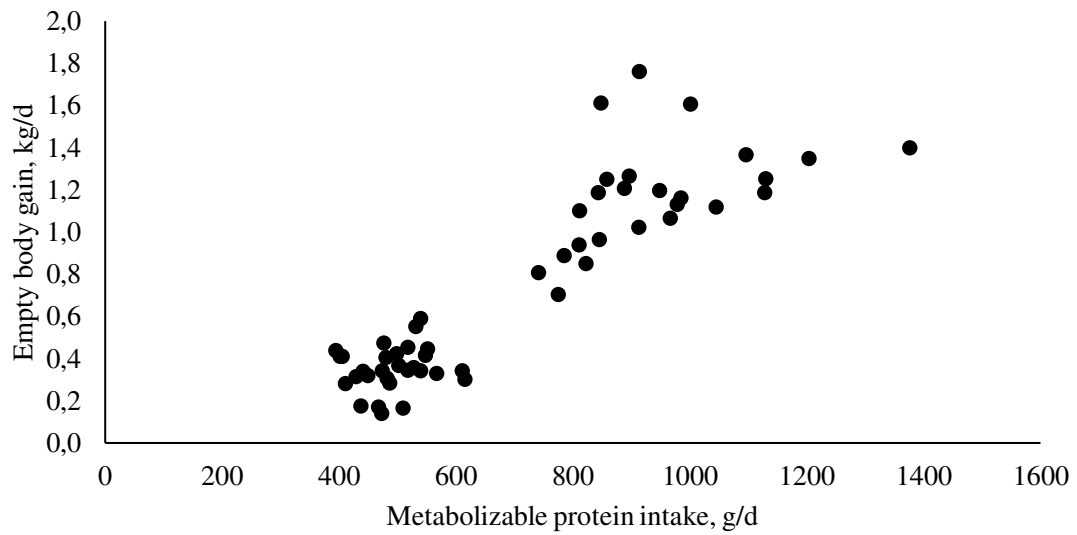


Figure 1A – Data on empty body weight gain and metabolizable protein intake.

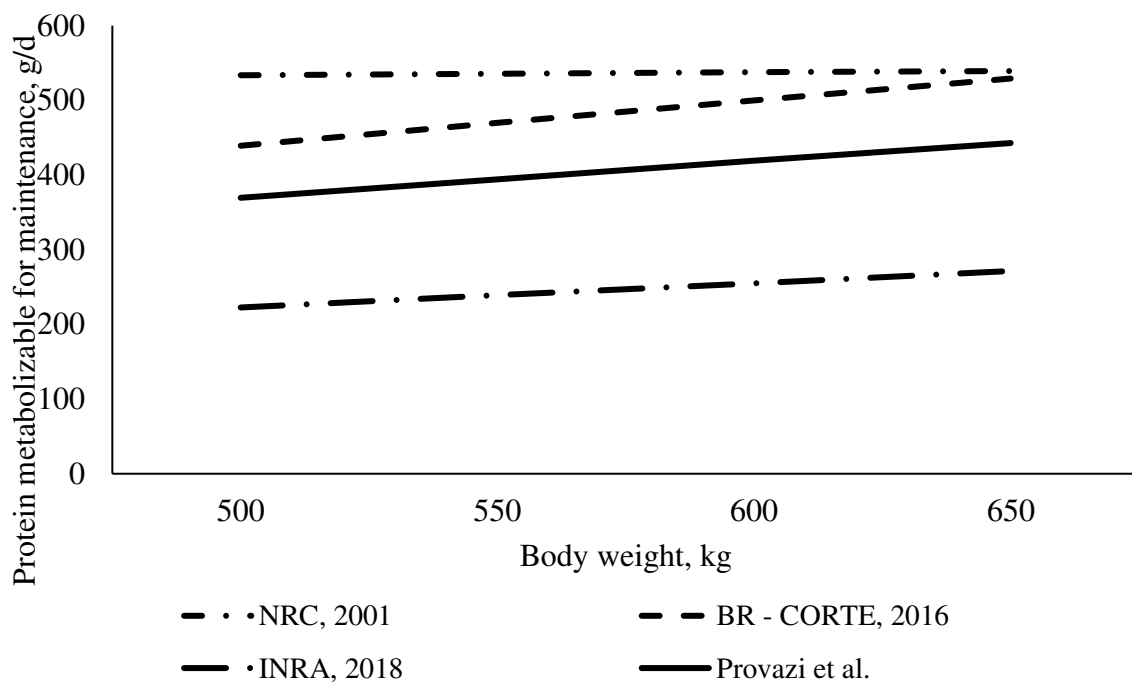


Figure 1B – Prediction equation the protein requirements for maintenance, proposed by NRC (2001), BR – CORTE (2016) and INRA (2018) compared to the model proposed by the present study ($3,6 / BW^{0,75}$).

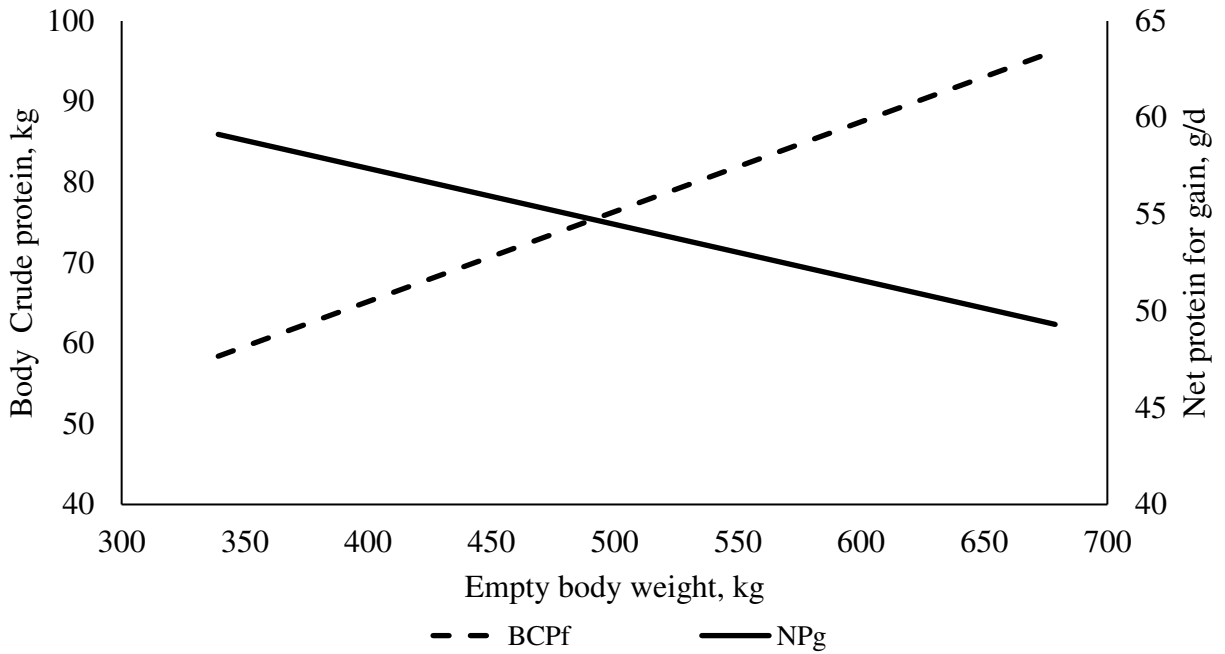


Figure 2A – Net protein requirement for body gain and crude protein in relation to empty body weight. EBG = 0.5 kg/d was used in equations. The body protein content presented in the graph were values found in the present study.

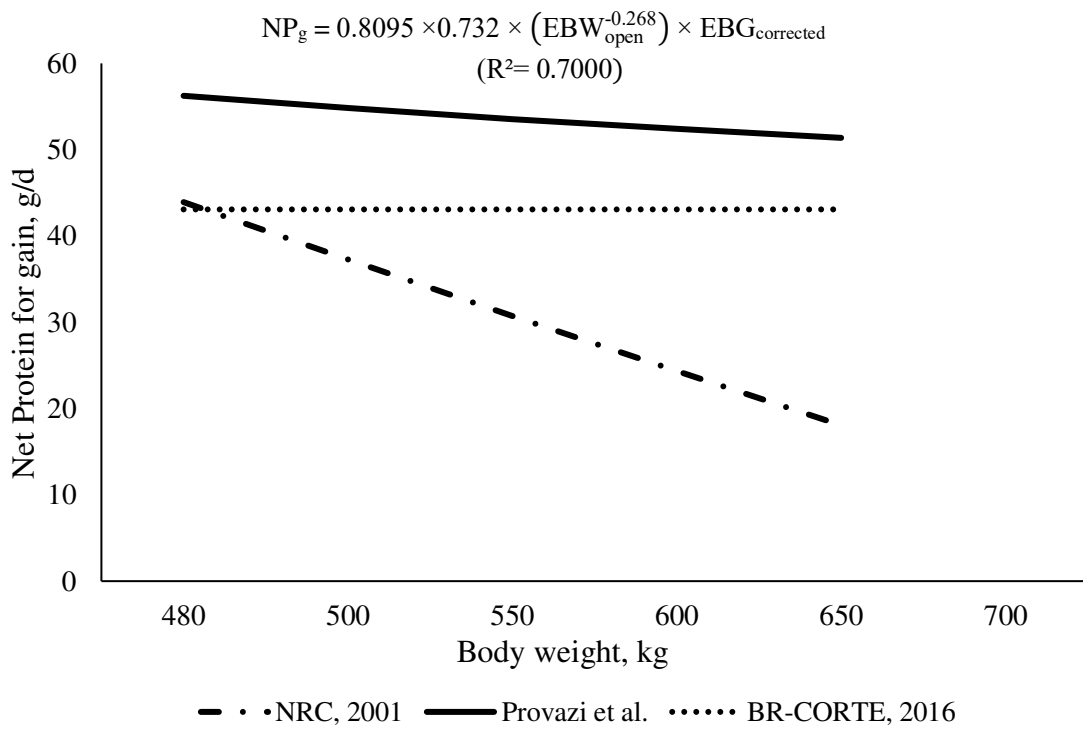


Figure 2B – Prediction equation the net protein requirements for gain, proposed by NRC (2001) and BR CORTE (2016) compared to the model proposed by the present study. EBG = 0.5 kg/d was used in equations. BR CORTE estimates BCS = 5 was considered.

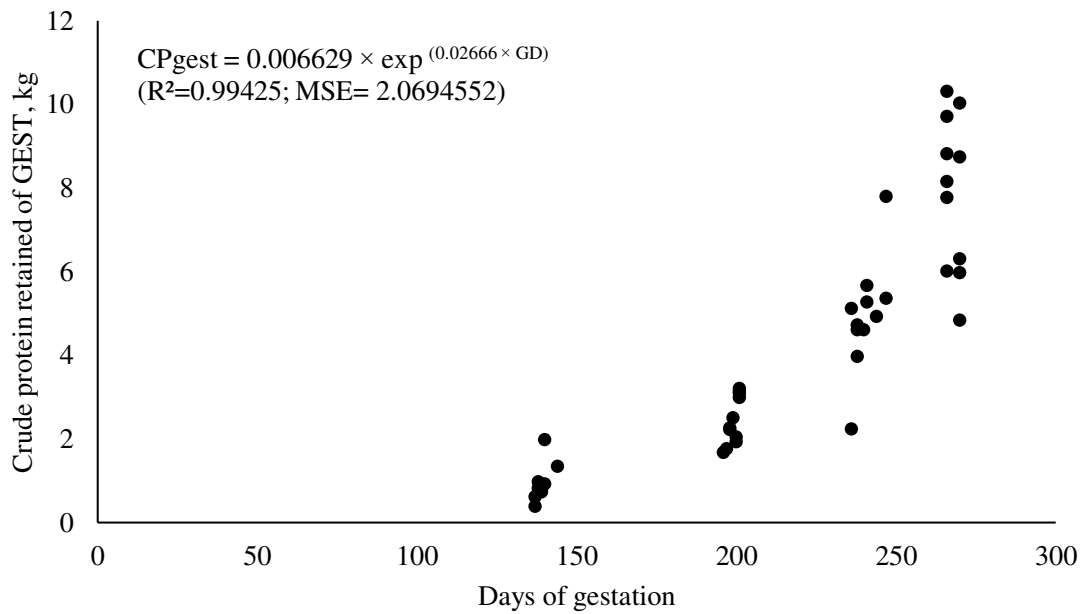


Figure 3A – Exponential relation between days of gestation and crude protein retained of GEST components.

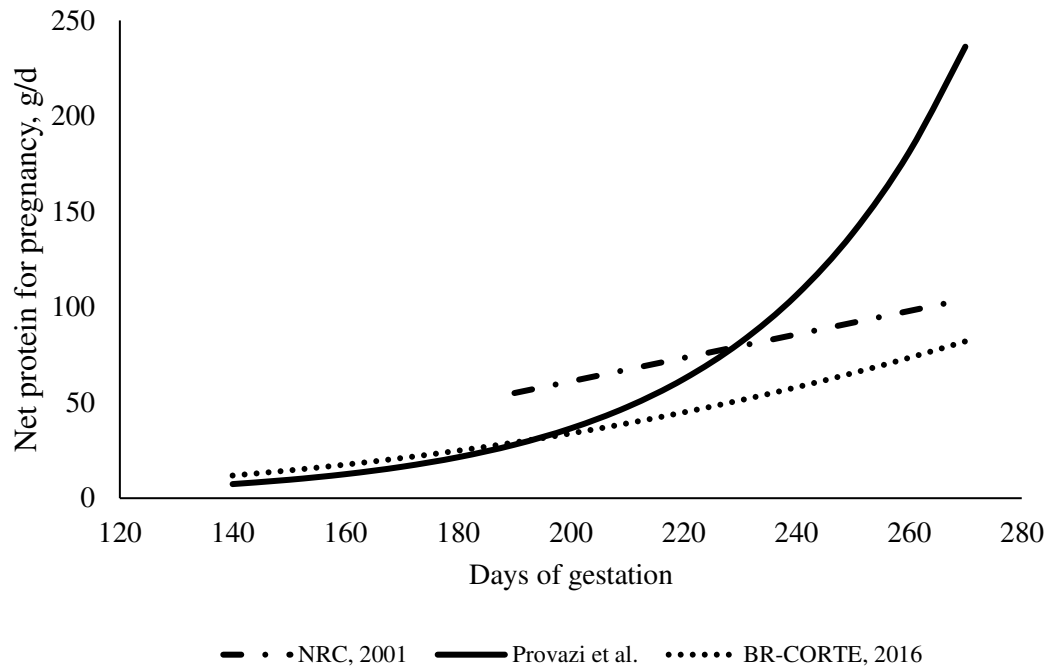


Figure 3B – Prediction equation the net protein requirements for pregnancy, proposed by NRC (2001) and BR CORTE (2016) compared to the model proposed by the present study in ration on days of pregnancy. Considered birth weight of calf = 40 kg for NRC and 32 kg for BR CORTE estimate.

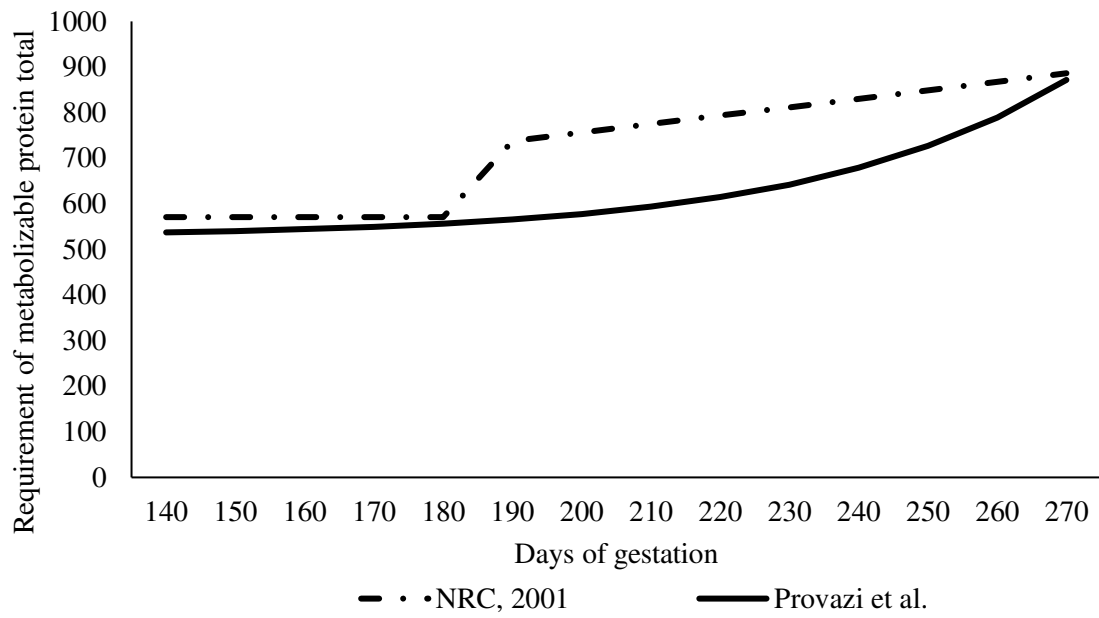


Figure 4 - Comparison between total requirements for pregnancy cows (maintenance, weight gain and pregnancy) of metabolizable protein estimated by our data and NRC (2001).