weeds increased the concentration of milk I more strongly on pea than on R supplemented diets, likely due to the inhibition of I transfer from feed to milk attributed to rapeseed glucosinolates (Trøan et al., 2018).

Conclusion and Implications
Rapeseed and pea supplements affect the mineral concentrations of milk. The pea-supplemented diets resulted in higher concentrations of Ca, K, P and I than rapeseed-supplemented diets. Seaweed supplementation can increase the I concentration of milk in geographical areas prone to I deficiency. Additional I supplementation should be considered in cow diets that contain rapeseed to reduce the risk of producing milk with low I content.

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O9 Protein requirements for pregnant dairy cows

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Keywords: Gyr; Holstein; Girolando; Pregnancy; Gestation

Introduction
The Nutrient Requirements of Dairy Cattle (NASEM, 2021) describes nutrient requirement as the daily amount of a specific nutrient necessary to meet a healthy cow's needs for maintenance, activity, growth, reproduction, and lactation without changing the body reserves. It is well known that the nutrient requirements for pregnant cows were established from experiments conducted in the 1990s or earlier (Bell et al., 1995). Given the intensive selection for milk production, research has suggested that a modern dairy cow has greater metabolic rates than before (NASEM, 2021). The eighth revised edition of NASEM (2021) used the previous edition as the starting point, but the method to estimate pregnancy requirements was modified, considering that gestation must be accounted for when the cow is between 12 and 280 days of gestation (DG). Because NASEM (2021) was just released, comparisons with NRC (2001) and INRA (2018) are still relevant. Furthermore, studies evaluating nutrient requirements for pregnant cows are scarce, and more research is warranted. Therefore, the objective of the present study was to estimate the protein requirements for maintenance, body gain, and gestation of Holstein × Gyr (HG) crossbred cows.

Material and Methods
Sixty-two Holstein × Gyr cows were used, with an average initial BW of 480 ± 10.1 kg and 5 ± 0.5 yrs of age. 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 to estimate the initial body composition of the remaining animals. Pregnant and non-pregnant cows received two diets: maintenance and ad libitum. Pregnant cows were slaughtered at 139, 199, 241, and 268 days of pregnancy. First, we used data from non-pregnant cows to determine requirements for maintenance and growth in adult cows. Requirements of metabolizable protein for maintenance (MPm; g/EBW$^{0.75}$/day) were estimated using a linear regression between the metabolizable protein intake (MPI, g/day) and average daily gain (g/day), and MPm was defined as the intercept divided by BW$^{0.75}$. Net protein requirements for gain (NPg; g/day) were estimated by the first derivative of the allometric equation between final CP in the body (kg) and the final EBW (kg). The efficiency of use of metabolizable protein (k) was calculated from the regression between the retained protein (g/EBW$^{0.75}$/day) and the MPI (g/EBW$^{0.75}$/day), and k was the slope of this regression. The MPI was estimated by summing digestible microbial protein and digestible rumen undegradable protein. Secondly, we used data from all animals to determine pregnancy requirements for adult cows. An exponential model was used to fit the protein accumulation in the gestational components in the function of DG. The first derivative of that model was considered the net requirement for pregnancy (NP$_{gest}$). The efficiency of protein utilization for gestation (k$_{gest}$) was calculated by the iterative method using
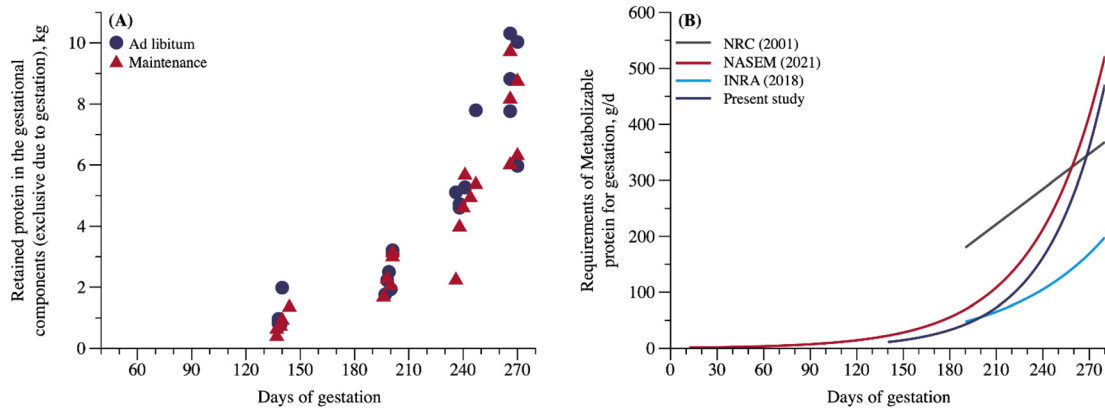
Figure 1 – Exponential relationship between days of gestation and crude protein retained in the gestational components (A); and prediction of the requirements of metabolizable protein for pregnancy proposed by NRC (2001), NASEM (2021), and INRA (2018) compared to the model proposed by the present study in days of gestation. Calculations were made considering a calf birth weight = 35 kg (B)

Figure 1. Bayesian network of volatile fatty acids concentrations (mM) parameters. Relationships among parameters are represented with edges.

the equation: $\Delta = MPI - (MPm + NPg/k_g + NPgest/k_{gest})$. The iteration was performed aiming at a zero deviation between observed MPI and MP estimated by the requirements determined herein. The linear regression parameters were estimated using PROC MIXED of SAS (version 9.4). Estimates of the parameters of non-linear regressions were adjusted using the PROC NLMIXED of SAS. Significances were declared when $P < 0.05$.

Results and Discussion
We obtained a value of 3.6 g/EBW$^{0.75}$/day for MPm. The INRA (2018) suggests 2.2 g/EBW$^{0.75}$/day for MPm, 38% lower than the present study. The estimation of NPg was calculated according to the following equation: $NPg = 0.8095 \times 0.732 \times (EBW_{open}^{-0.268}) \times EBG_{corrected}$, where EBW_{open} is the empty BW (kg) for non-pregnant animals and $EBG_{corrected}$ is the empty body gain (kg/day) corrected for the gestational component. Using the equation proposed by NRC (2001) and taking into account a cow with 450 kg BW and a 0.3 kg/day of ADG, the estimated NPg would be 43 g/day. Our equation suggests an NPg of 35 g/day (18% lower) using the same animal. The k was 0.353, which is 22% higher than NRC (2001) suggested for dairy cows with BW greater than 478 kg. The net protein requirements for gestation (NPgest) were determined as $NPgest (g/day) = 0.1767 \times \exp(0.02666 \times DG)$ (Figure 1). The efficiency of using metabolizable protein for gestation (k_{gest}) was 0.653. Overall, from 140 to 275 DG, our estimates of MPgest were 30% lower than those described by NASEM (2021), while the INRA (2018) underestimated MPgest of crossbred cows by 36%.

Conclusion and Implications
The proposed equations to estimate the protein requirements for HG pregnant cows were different from those reported by INRA (2018), NRC (2001), and NASEM (2021). We recommend using our equations to estimate protein requirements for maintenance, growth, and pregnancy of HG dairy cows.

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O10 The effect of incremental nutrient intake on energy and protein metabolism in pre-weaning dairy calves

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Keywords: Energy and nitrogen utilization; Young calf

Introduction
Despite growing interest in young dairy calf nutrition and health, nutrient requirements and recommendations are outdated. Also, these do not consider calves younger than 21 days of age, nor calves fed with high levels of whole milk or milk replacer (MR), since this was not a