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Macromineral requirements for maintenance, body weight gain, and pregnancy of dairy cows

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ABSTRACT

We aimed to predict macromineral requirements for maintenance, weight gain, and pregnancy in dairy cows. In total, 62 nonlactating cows (initial BW of 522 ± 10.1 kg [mean ± SD], initial age of 5 ± 0.5 yr, and 3 lactations) were enrolled and assigned to 3 groups: pregnant (n = 44), nonpregnant (n = 12), and baseline (n = 6). Baseline cows, which were not inseminated, were harvested at the beginning of the trial to determine the initial body composition. Both pregnant and nonpregnant groups were then divided into 2 feeding treatments: ad libitum or restricted intake at 1.15% of BW (approximating maintenance). Pregnant cows were slaughtered at 140, 200, 240, and 270 d of gestation, and nonpregnant cows were slaughtered at corresponding intervals to compare mineral accretion due to pregnancy. Total-tract digestibility was measured in six 28-d periods (d 122, 150, 178, 206, 234, and 262 of gestation) by collecting DMI, feces, and urine. The net requirements of maintenance (mg/kg of empty BW) for calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), and sulfur (S) were 13.48, 8.35, 4.06, 10.08, 45.89, and 7.82, respectively. For BW gain in pregnant cows, the models for Ca, P, Mg, K, Na, and S were set as $Net\ Ca_{gain} = 0.4168 \times EBW^{0.7115} \times EBG_c$; $Net\ P_{gain} = 0.8441 \times EBW^{0.4762} \times EBG_c$; $Net\ Mg_{gain} = 0.0492 \times EBW^{0.4391} \times EBG_c$; $Net\ K_{gain} = 0.1738 \times EBW^{0.5169} \times EBG_c$; $Net\ Na_{gain} = 0.0284 \times EBW^{0.7880} \times EBG_c$; $Net\ S_{gain} = 0.2530 \times EBW^{0.7982} \times EBG_c$, respectively; EBW = empty BW, EBG_c = empty body gain, carcass and noncarcass. Estimates of net requirements of pregnancy were adjusted as follows: $Net\ Ca_{preg} = 0.0042e^{0.0286\times GD}$; $Net\ P_{preg} = 0.0059e^{0.0253\times GD}$; $Net\ Mg_{preg} = 0.0006e^{0.0219\times GD}$; $Net\ Na_{preg} = 0.0197e^{0.0166\times GD}$; $Net\ K_{preg} = 0.0111e^{0.0176\times GD}$; $Net\ S_{preg} = 0.0106e^{0.0181\times GD}$, where GD = gestation days. Finally, we propose an innovative method to estimate the efficiency of macromineral utilization by gestational tissues. The macromineral efficiency for pregnancy (k_{preg}) for each mineral was modeled as: $Ca\ k_{preg} = 0.0004e^{0.0263\times GD}$; $P\ k_{preg} = 0.2974e^{0.0048\times GD}$; $Mg\ k_{preg} = 0.00006e^{0.0233\times GD}$; $K\ k_{preg} = 0.0003e^{0.0234\times GD}$; $Na\ k_{preg} = 0.0038e^{0.0200\times GD}$; $S\ k_{preg} = 0.0004e^{0.0199\times GD}$. These results provide valuable insights into macromineral requirements in dairy cows and offer innovative approaches to evaluating nutrient efficiency during pregnancy.

Key words: dairy cattle, mathematical modeling, minerals, nutrient requirements

INTRODUCTION

Nutrient demand during pregnancy is among the most significant homeorhetic effects influencing nutrient partitioning across the life cycle (Bauman and Bruce Currie, 1980). Feeding unbalanced diets to pregnant cows can adversely affect lactation performance, health, fetal development, and subsequent calf productivity (Reynolds and Caton, 2012; Reynolds and Vonnahme, 2017; Caton et al., 2019).

Although minerals are retained in lower proportions in the body compared with protein and fat, they serve a wide array of structural, physiological, catalytic, regulatory, and immunological functions (Valadares Filho et al., 2016; Wilson et al., 2016). Minerals are also essential for maintaining health, reproductive efficiency, and milk yield (NRC, 2001). However, their metabolism is complex, and several factors—including physiological stage, mineral source bioavailability, and interactions among different minerals in both the gastrointestinal tract and overall metabolism—can influence mineral availability and utilization (Valadares Filho et al., 2016; Tedeschi and Fox, 2018).

Traditional nutrient requirement systems consider macrominerals needs in pregnant cows only after 190 d of gestation, because fetal growth accelerated during the

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final third of pregnancy and intensifies further during the last 2 mo (Robinson, 1977). However, other components of gravid uterus, including the placenta, undergo significant development during the early stages of pregnancy, which is critical for supporting the heightened fetal growth in late gestation (Caton et al., 2019).

For certain macrominerals during pregnancy—such as sodium, potassium, and magnesium—traditional nutrient requirement systems often recommend fixed amounts until the expected calving date. However, this approach may lack precision because fetal growth follows an exponential curve, implying that macromineral deposition and requirements should match the developmental trajectory of the fetus. Moreover, sulfur partitioning remains unclear, as major nutrient requirement systems recommend a total sulfur amount without distinguishing between maintenance, growth, and pregnancy (NRC, 2001; INRA, 2018; NASEM, 2021). Hence, understanding how mineral accretion progresses in gestational components before the last third of pregnancy is critical for ensuring optimal fetal development.

Therefore, we hypothesized that macromineral accretion begins before 190 d of gestation and follows an exponential pattern. We also hypothesized that the efficiency of mineral utilization for gestational tissues varies throughout pregnancy. Hence, the objectives of this study were (1) quantify macromineral retention associated with maintenance, BW gain, and pregnancy and (2) to reevaluate the macromineral requirements by considering accretion at earlier gestational stages than traditionally assumed.

MATERIALS AND METHODS

The database used in this modeling study was derived from a single animal trial conducted by Rotta (2015a,b,c). All procedures and sampling methods were approved by the Institutional Animal Care and Use Committee of the Universidade Federal de Viçosa (CEUAP/UFV – 47/2012).

Animal Management and Diets

One hundred five multiparous cows were enrolled in a timed-artificial insemination protocol, and pregnancy was checked 60 d after insemination. Forty-four pregnant cows were allocated to a slaughter schedule of either 140, 200, 240, or 270 d of gestation. During the trial, 1 cow aborted and, therefore, was removed from the final modeling. Twelve nonpregnant cows were allocated to a slaughter schedule at 200, 240, or 270 d after insemination. Six nonpregnant cows were assigned to the baseline group, and slaughter occurred at the same time as pregnant cows were rechecked (60 d).

All cows were individually housed in 30-m^2 pens equipped with dedicated feed bunks to measure feed intake, and water was provided ad libitum. At the start of the experiment, animals weighed 522 ± 10.1 kg (mean ± SD) and averaged 5 ± 0.5 yr of age.

Both nonpregnant and pregnant cows were divided into 2 subgroups based on 2 feeding regimens to achieve distinct ADG (~0.1 kg/d [feeding regimen 1] and 1.0 kg/d [feeding regimen 2]). The trial's diet was formulated to include fetal growth and reproductive tract tissue gain as part of ADG calculation, and nutrient requirements were met following NRC (2001) guidelines. The diet consisted of corn silage and concentrate in a 93:7 ratio (roughage:concentrate) on a DM basis (Table 1). All cows were fed twice daily (0700 and 1700 h). For feeding regimen 1, feed intake was fixed at 1.15% of BW. For the feeding regimen 2, cows were offered feed ad libitum with an anticipated 5% of orts on fresh feed basis. Daily adjustments were made before the morning feeding based on the previous day's refusals. The diets were offered after the pregnancy recheck, which occurred 60 d after insemination. Before that, all animals were maintained on the same diet (Table 1) to meet their nutrient requirements according to NRC (2001).

Table 1. Ingredients of diet fed both pregnant and nonpregnant cow groups

Item	Amount
Ingredient (g/kg DM)	
Corn silage	930.0
Cottonseed meal	50.0
Limestone	5.0
Salt	5.0
Urea	9.0
Ammonium sulfate	1.0
Mineral mix[1]	0.2
Chemical composition	
DM (g/kg)	376.0
OM (g/kg DM)	929.0
CP (g/kg DM)	111.0
NDF (g/kg DM)	497.0
Ether extract (g/kg DM)	37.0
NFC (g/kg DM)	284.0
Calcium (g/kg DM)	3.9
Phosphorus (g/kg DM)	1.9
Magnesium (g/kg DM)	1.6
Potassium (g/kg DM)	11.7
Sodium (g/kg DM)	2.2
Sulfur (g/kg DM)	2.2

[1]Mineral mix composition: calcium = 29.2 g/kg (calcium carbonate source); phosphorus = 0.7 g/kg (dicalcium phosphate source); magnesium = 2.1 g/kg (magnesium oxide source); potassium = 0.89 g/kg (potassium chloride source); sodium = 0.3 g/kg (sodium chloride source); sulfur = 63.5 g/kg (cobalt and zinc sulfate); cobalt = 348 mg/kg (cobalt sulfate); chromium = 2.6 mg/kg (chromium chelated source); copper = 3.3 mg/kg (copper chelated source); iron = 2.1 mg/kg (iron sulfate source); manganese = 4.7 mg/kg (manganese chelated source); zinc = 7.8 mg/kg (zinc sulfate source); selenium = 318.0 mg/kg (selenium chelated source).

Intake, Urine, and Fecal Excretion Measurements

Six 28-d sampling periods (122, 150, 178, 206, 234, and 262 d of gestation) were used to estimate the apparent digestibility of DM and nutrients. Both feed and orts samples were collected over the final 7 d of each period (2 d before fecal collection and 5 d during fecal collection) dried, and proportionally composited by period. Fecal samples were obtained via a spot collection method to estimate total fecal output following Sampaio et al. (2011). Briefly, feces were collected from each cow at 0600, 0900, 1200, 1500, and 1800 h during the last 5 d of every 28-d period. Approximately 200 g of feces was collected by rectal stimulation or as cows defecated, dried in a forced-air oven at 55°C for 72 h, then ground to pass through a 1-mm screen (Wiley Mill, A. H. Thomas) and composited by animal for further analysis.

Fecal excretion was estimated using iNDF as an internal marker, calculated by dividing daily iNDF intake (g/d) by the iNDF concentration (g/g) in fecal grab samples, following Sampaio et al. (2011). All feed and fecal samples were then incubated in situ in the rumen of 2 cannulated cows for 288 h. The cows were fed a 50:50 forage-to-concentrate diet at a maintenance level.

Urine samples were collected on d 1 and 4 of each 28-d sampling period at 0600 and 1500 h. Samples were obtained via vulva stimulation and acidified with sulfuric acid to prevent NH_3 volatilization, then frozen at −20°C until analysis. Daily urine excretion was calculated using the equation from Pacheco et al. (2009), with a urinary creatinine excretion rate set at 0.9 mmol/kg of $BW^{0.75}$ for this study. Creatinine concentrations were measured by HPLC (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) following the method described by George et al. (2006).

Comparative Harvesting Method and Sampling

The harvest procedure was conducted to quantify the mineral content of the body composition, thereby enabling the estimation of net requirements (Lofgreen and Garrett, 1968). The baseline group was harvested at the beginning of the experiment to determine initial BW and macromineral concentrations in body components. The cows in the baseline group were never inseminated and were harvested on d 1 of the study, which corresponded to 60 d after insemination, when pregnancy check was conducted. Pregnant cows were harvested at 140, 200, 240, and 270 d of gestation and nonpregnant cows were harvested at 200, 240, and 270 d of the feeding trial. Nonpregnant cows were not harvested at 140 d due to the limited number of animals in the trial, and because 140 d was considered too early to detect meaningful differences in pregnancy-related components.

Cows were fasted for 16 h before harvest to obtain shrunk body weight (**SBW**). The harvest was performed using a captive bolt stunner followed by exsanguination. Subsequently, noncarcass components (gastrointestinal tract, organs, head, tail, hooves, trimmings, hide, and blood) were separated, weighed, ground using an electric meat grinder (item no. 3 CV CAF 82 E-TI TR 220V, Bauru, São Paulo, Brazil), and then homogenized to create a composite sample for macromineral analyses. The contents of each gastrointestinal compartment were removed and weighed. The carcass was split into 2 halves, and the left half was weighed and sampled for mineral content. Only the left half of the carcass was sampled, as standard practice. This approach assumes that both halves are similar in composition, with comparable proportions of bone, fat, and muscle, as well as similar chemical composition. In pregnant cows, the entire reproductive tract was collected and dissected into placenta, uterus, fetus, and fetal fluids; the mammary gland was also sampled to account for pregnancy-related macromineral requirements. In nonpregnant cows, the uterus and mammary gland were collected and analyzed for mineral content. These data were used in model calculations to isolate pregnancy-associated reproductive components from those of nonpregnant animals.

Laboratory Analyses

Corn silage, ration ingredients, orts, feces, urine, and body components (carcass, noncarcass tissues, fetus, placenta, mammary gland, fetal fluids, and uterus) were analyzed for DM using method 934.01 (AOAC International, 1995). All samples were subsequently analyzed for macrominerals (Ca, P, Mg, K, Na, and S). Calcium and Mg were quantified by atomic absorption spectrometry (method 968.08; AOAC International, 2000), whereas Na and K were measured by flame emission spectrometry (method 985.35; AOAC International, 2000). Phosphorus was determined colorimetrically (method 965.17; AOAC International, 2000), following Detmann et al. (2012). Sulfur was processed by acid digestion and analyzed via inductively coupled plasma-atomic emission spectroscopy (method 999.11; AOAC International, 1999).

Calculations

Relationship Between EBW and SBW. Empty body weight (**EBW**) was calculated by a summative equation of weights of carcass, noncarcass, mammary gland, and uterus as described by Sguizzato et al. (2020a) and Marcondes et al. (2023), given that these data were collected in the same dataset. Also, a simple linear regression was set between EBW and SBW to compare this study with the requirements systems and other studies. The intercept for gestation days (**GD**) did not differ from zero ($P > 0.05$). However, a significant GD effect was observed for the slope of the regression relating to SBW and EBW, indicating that before and after 200 GD, the slopes were different ($P < 0.05$). Consequently, EBW was estimated using the following equations:

$$\text{if GD lower than 200 d, } EBW = 0.8776_{\pm 0.0053} \times SBW, \quad [1]$$

$$\text{if GD greater than 200 d, } EBW = 0.9018_{\pm 0.0048} \times SBW. \quad [2]$$

Net Maintenance Requirement. The net mineral requirement for maintenance of nonpregnant and pregnant cows was determined as the amount of retained mineral in the body (Valadares Filho et al., 2016), which is estimated as the difference between mineral intake and the mineral output in urine and feces. The true mineral retention coefficient (β_1) was determined by simple linear regression as proposed by Lofgreen and Garrett (1968) using Equation [3]:

$$RM = \beta_0 + \beta_1 \times MC + \varepsilon_i, \quad [3]$$

where RM is the retained mineral; MC is mineral intake (g/d); β_0 is the net requirement for mineral maintenance (g/d); β_1 is the true retention coefficient; and ε_i is the residuals.

Net Mineral Requirement for BW Gain. To estimate the net mineral requirement for BW gain, an allometric model (ARC, 1980) was applied to relate the mineral content in carcass and noncarcass components of nonpregnant cows to EBW (Equation [4]). The net requirement for BW gain was obtained by taking the first derivative of Equation [4], resulting in Equation [5]. At this stage, a correction was applied in empty body gain (**EBG**) to account for growth of gestational components, yielding a corrected EBG (**EBG$_c$**) as described by Marcondes et al. (2023). The EBG$_c$ represents carcass and noncarcass weight gain, plus the additional mass of gestational components associated with overall BW gain. This correction factor helps prevent underestimation of net requirements for weight gain and overestimation of net requirements for pregnancy in cows (Marcondes et al., 2023).

$$RM_{gain} = \beta_0 \times EBW^{\beta_1} + \varepsilon_i, \quad [4]$$

where RM_{gain} is retained macromineral (g) in carcass and noncarcass tissues; EBW is empty body weight (kg); β_0 is the intercept; β_1 is the slope; ε_i is the residuals.

$$Net\ Requirement_{gain} = \beta_0 \times \beta_1 \times EBW^{\beta_1 - 1} \times EBG_c, \quad [5]$$

where $Net\ Requirement_{gain}$ is the net macromineral requirement for BW gain (g/d); EBW is empty body weight (kg); β_0 is the net requirement for the maintenance of macrominerals; β_1 is the slope; EBG_c is the corrected empty body gain (kg/d).

Dietary Mineral Requirements for Nonpregnant Cows. For nonpregnant cows, dietary macromineral requirements were determined by summing the net requirements for maintenance and gain and dividing by the retention coefficient (Equation [6]).

$$Dietary\ requirements = \frac{Net\,requirement_{Maintenance} + Net\,requirement_{Gain}}{Retention\,Coefficient}, \quad [6]$$

where $Dietary\ Requirements_M$ is a demand by the cow for any macromineral in the diet.

Net Mineral Requirement for Pregnancy. After establishing net requirements for maintenance and BW gain, the net macromineral requirement for pregnancy was calculated as the difference between total retained macromineral in pregnant cows and the sum of dietary macrominerals allocated to maintenance and BW gain (Equation [7]).

$$Available\,mineral_{preg} = Mineral\,ingested - \frac{Net\,req._{maint.} + Net\,req._{gain}}{Retention\,coefficient} \quad [7]$$

Mineral Retention and Requirements Over Gestation. Macromineral retention for pregnancy was determined from the body macromineral composition of pregnant cows (uterus, placenta, fetus, fetal fluids, mammary gland) minus that of non-pregnant cows at each time point, thereby isolating macromineral accretion directly attributable to pregnancy. The true macromineral retention over gestation was fitted to a simple exponential model (Equation [8]) following Sguizzato et al. (2020a):

$$RM_{preg} = \beta_0 \times e^{\beta_1 \times DP} + \varepsilon_i, \quad [8]$$

where RM_{preg} is the retained macromineral for pregnancy (g); DP is the days of pregnancy; β_0 is the intercept; β_1 is the slope; ε_i is the residuals.

Equation [8] also provided the initial day at which macromineral requirements diverged between pregnant and nonpregnant cows, as determined by the lower confidence limit of macromineral retention in gestational components (Sguizzato et al., 2020a).

The net macromineral requirement for pregnancy was then obtained from the first derivative of Equation [8]:

$$Net\,Requirement_{preg} = \beta_0 \times \beta_1 \times e^{\beta_1 \times DP}, \quad [9]$$

where $Net\ Requirement_{preg}$ is the net macromineral requirement for pregnancy (g/d); DP is the days of pregnancy; β_0 is the intercept; β_1 is the slope.

Efficiency of Mineral Utilization. Efficiency of macromineral utilization for pregnancy was determined using an interactive approach:

$$\Delta = MI - \left(\frac{NetMM_{maint.} + NetMM_{gain}}{Retention\,Coefficient} + \frac{Net\,MM_{preg}}{k_{preg}}\right), \quad [10]$$

where MI is macromineral intake (g/d); $NetMM_{maint}$ is the net requirement for maintenance (g/d); $NetMM_{gain}$ is the net mineral requirement for BW gain (g/d); $NetMM_{preg}$ is the net macromineral requirement for pregnancy (g/d); k_{preg} corresponds to the efficiency of use for macromineral for pregnancy.

Time-Specific Efficiency of Macromineral Uptake. Regarding the efficiency of macromineral uptake by gestational components, we developed a new approach to capture the variation in retention efficiency throughout pregnancy. Numerous factors can affect nutrient absorption and utilization in cattle, including diet composition, macromineral concentration, and physiological stage (NASEM, 2021). Consequently, using a fixed efficiency of macromineral utilization for gestational components, as employed in most nutrient requirement systems, is not appropriate because fetal growth follows an exponential pattern.

In this study, the efficiency of macromineral utilization for gestational components was computed at 4 time points (140, 200, 240, and 270 d of pregnancy) using an interactive method similar to the conventional k_{preg}. These time-specific efficiencies were then fitted to an exponential function to predict efficiency on any given day of pregnancy. To more precisely identify the inflection point, a horizontal shift transformation (*days of pregnancy* [*d*] × 0.747) was applied to the exponential model, based on the second derivative of the exponential model of k_{preg}. The multiplier 0.747 marks the onset of altered concavity in the exponential curve for k_{preg} and helps correct for potential bias due to the limited number of sampling points in this trial.

Dietary Requirements of Pregnant Cows. The dietary requirements of pregnant cows for each macromineral were calculated by the sum of the net requirement for maintenance, BW gain divided by the true retention coefficient, and net requirement for pregnancy divided by the efficiency of use of a given macromineral for pregnancy:

$$Dietary\ requirement(g/d) = \left(\frac{NetMM_{maint.} + NetMM_{gain}}{Retention\,Coefficient}\right) + \left(\frac{Net\,MM_{preg}}{k_{preg}}\right), \quad [11]$$

where $NetMM_{maint}$ is the net macromineral requirement for maintenance (g/d); $NetMM_{gain}$ is the net macromineral requirement for gain (g/d); $NetMM_{preg}$ is the net macromineral requirement for pregnancy (g/d); k_{preg} corresponds to the macromineral efficiency for pregnancy.

Statistical Procedures

The maintenance requirement models for all macrominerals were tested using the PROC MIXED procedure in SAS software (version 9.4; SAS Institute Inc., Cary, NC) to evaluate differences in the β_0 and β_1 parameters between pregnant and nonpregnant cows. Macromineral retention parameters for weight gain and pregnancy were estimated by NLIN procedures in SAS (version 9.4), and differences between pregnant and nonpregnant cows were tested with the PROC NLMIXED procedure. A significance level of $P \leq 0.05$ was used as the threshold for type I error in all tests.

RESULTS

Net Requirements of Macrominerals for Maintenance

In this study, the average BW of the cows was 522.1 kg (±10.10 kg) and the DMI used to calculate net macromineral requirements for maintenance was 8.70 kg (±2.87 kg).

Macromineral intake, excretion, and retention in pregnant and nonpregnant cows for all macrominerals are summarized in Table 2. The net maintenance requirements for macrominerals were estimated using the mineral balance approach, which the intercept (β_0) in the linear regression between macromineral intake and retention represents the net requirement for maintenance (Figure 1). Differences between pregnant and nonpregnant cows for maintenance were not found in the study for all macrominerals ($P > 0.05$). The estimated net maintenance requirements for Ca, P, Mg, K, Na, and S were 13.48, 8.35, 4.06, 45.89, 10.08, and 7.82 mg/kg of EBW, respectively (Table 3; Figure 1). Additionally, the regression slope (β_1) indicates the true retention coefficient for each mineral (Table 3; Figures 2 and 3)

Table 2. Means of mineral intake,[1] excretion,[2] and retention[3] to estimate macromineral balance to predict requirements for maintenance

Mineral (g/d)	Nonpregnant cows								Pregnant cows							
	Intake	SD	Feces	SD	Urine	SD	Retention	SD	Intake	SD	Feces	SD	Urine	SD	Retention	SD
Calcium	46.3	13.14	20.7	6.61	1.0	0.54	24.1	7.71	48.1	16.36	19.9	7.37	1.2	0.77	27.1	10.3
Phosphorus	19.4	5.60	15.0	4.51	0.2	0.07	4.3	1.87	20.2	6.93	14.6	5.07	0.1	0.04	5.5	2.82
Magnesium	14.1	3.96	6.4	2.17	3.9	1.11	3.7	1.67	14.8	5.00	6.5	2.33	3.8	1.08	4.4	2.58
Potassium	51.3	14.56	8.7	2.67	38.4	7.96	4.3	10.5	53.7	18.34	9.3	4.37	35.8	9.94	8.5	12.23
Sodium	18.3	5.25	10.0	4.38	5.4	1.74	3.0	2.58	19.0	6.50	9.3	4.31	6.0	2.55	3.7	3.60
Sulfur	6.8	1.95	2.5	0.67	1.9	0.63	2.4	1.66	7.1	2.43	2.5	0.85	2.0	0.56	2.6	1.80

[1]Intake was for any given macromineral was calculated as: *micromineral daily intake (g/d) = Total DMI × [Macromineral]$_{Diet}$*.

[2]Fecal and urine excretion for any given macromineral were calculated as: *micromineral daily intake (g/d) = Total daily estimated excretion × [Macromineral]$_{excretion}$*.

[3]Macromineral retention was calculated as: *micromineral retention (g/d) = Macromineral intake − (Macromineral excretion by urine + Macromineral excretion by feces)*.

The mineral balance analysis revealed significant urinary excretion for all macrominerals (Table 2). Among the minerals assessed, Ca exhibited the lowest urinary excretion, accounting for ~9.50% of its total excretion. In contrast, urinary K excretion comprised 81.6% of total K excretion. Considering urinary excretion in the overall mineral balance, the estimated retention coefficients for Ca, P, Mg, K, Na and S were 65.2%, 74.0%, 40.6%, 56.4%, 42.7%, and 85.0%, respectively (Table 3).

Net Requirement of Macrominerals for BW Gain

All net requirement predictions for weight gain were derived from the first derivative equation of macromineral retention in carcass and noncarcass components, as presented in Table 4. The equations used to estimate net macromineral requirements for BW gain are as follows:

$$\text{Ca: } NetCa_{gain} = 0.4168 \times EBW^{0.7115} \times EBG_c,$$

$$\text{P: } NetP_{gain} = 0.8441 \times EBW^{0.4762} \times EBG_c,$$

$$\text{Mg: } Net\ Mg_{gain} = 0.0492 \times EBW^{0.4391} \times EBG_c,$$

$$\text{K: } Net\ K_{gain} = 0.1738 \times EBW^{0.5169} \times EBG_c,$$

$$\text{Na: } Net\ Na_{gain} = 0.0284 \times EBW^{0.7880} \times EBG_c, \text{ and}$$

$$\text{S: } Net\ S_{gain} = 0.2530 \times EBW^{0.7982} \times EBG_c.$$

For a pregnant cow weighing 500 kg with an EBG_c of 0.1 kg/d, the estimated net macrominerals requirements for BW gain are 3.00 g/d for Ca, 1.45 g/d for P, 0.07 g/d for Mg, 0.38 g/d for K, 0.32 g/d for Na, and 3.10 g/d for S.

Gestational Components and Pattern of Macromineral Retention

For all macrominerals, the available portion for pregnancy was determined by subtracting the dietary requirements for maintenance and weight gain from total mineral intake. The retention of macrominerals in gestational components followed an exponential pattern throughout pregnancy (Table 5). The fetus and mammary gland were the primary gestational components retaining Ca and P, with considerable accretion occurring during mid gestation (Table 6). Sodium and K were predominantly found in fetal fluids, whereas the mammary gland exhibited significant S accretion during mid gestation. The highest macromineral retention was observed in the fetus, particularly during the last third of gestation (Table 6).

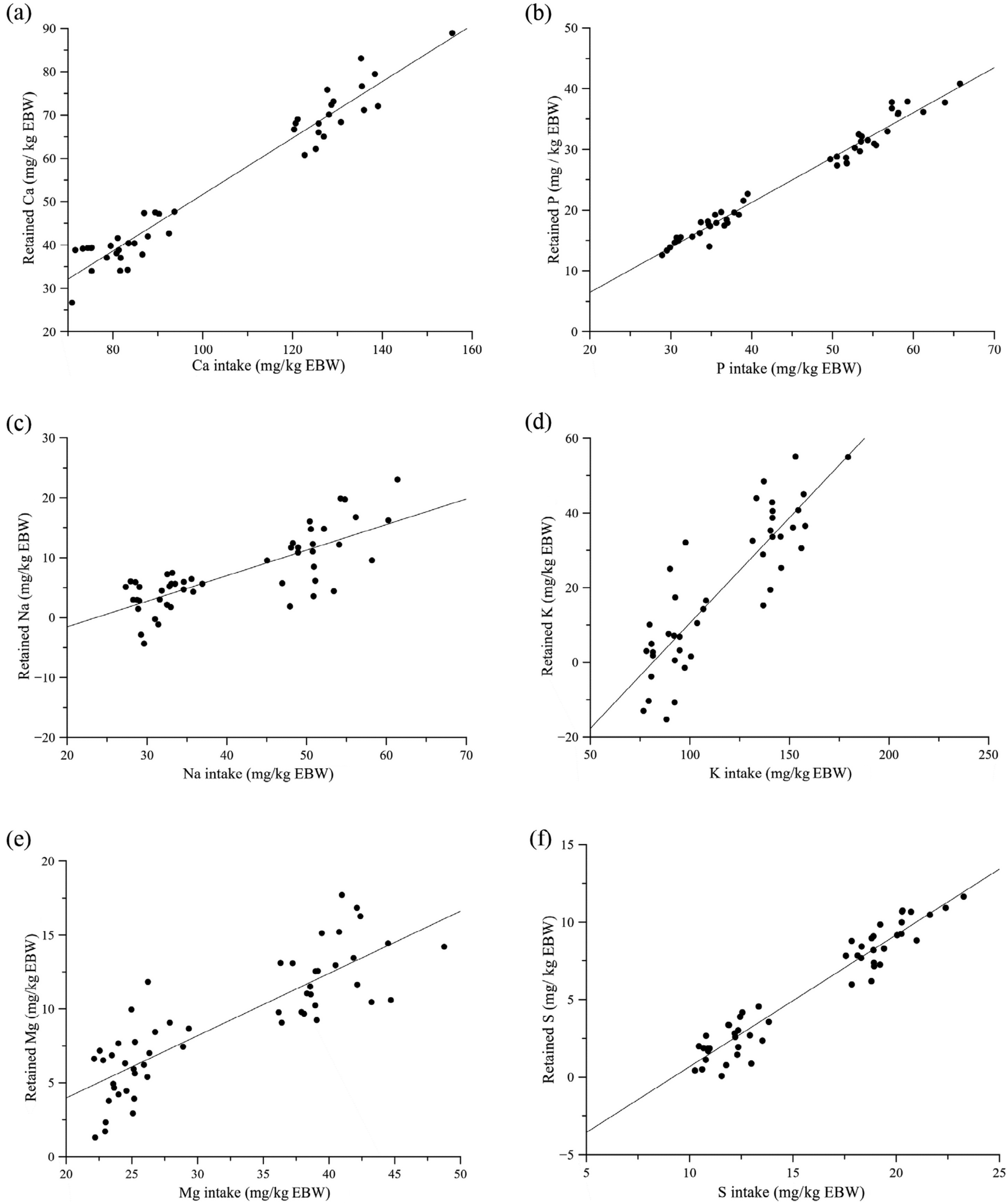

Figure 1. Linear models were developed to predict the net maintenance requirements for (a) calcium, (b) phosphorus, (c) sodium, (d) potassium, (e) magnesium, and (f) sulfur based on intake and excretion data. For all macrominerals, the *x*-axis represents intake expressed as a proportion of empty body weight (EBW). The *y*-axis indicates macromineral retention, calculated as follows: retention = intake − fecal excretion − urinary excretion, and also expressed relative to EBW.

Table 3. Estimation of parameters (β_0 and β_1) by mineral retention (mg/kg EBW) and mineral intake (mg/kg EBW) to predict net requirements of macrominerals for maintenance

Mineral	β_0	SEM	P-value	β_1	SEM	P-value	RMSE[1]	R^2
Calcium	−13.4837	2.47930	0.9128	0.6516	0.02337	0.9671	14.47	0.95
Phosphorus	−8.3534	0.83190	0.0553	0.7402	0.01807	0.0780	1.84	0.97
Magnesium	−4.0584	1.18310	0.6626	0.4055	0.03577	0.8683	4.24	0.72
Potassium	−45.8889	6.12810	0.2217	0.5641	0.05106	0.4078	93.54	0.75
Sodium	−10.0751	2.16810	0.6488	0.4267	0.05102	0.8197	14.04	0.60
Sulfur	−7.8202	0.56590	0.1149	0.8498	0.03487	0.1149	0.96	0.93

[1]RMSE = root mean square error.

Net Macromineral Requirements for Pregnancy

The net macromineral requirements for pregnancy were determined from the first derivative of the mineral retention model (Table 7). The predictive equations are as follows:

$$\text{Ca: } Net\ Ca_{preg} = 0.0042e^{0.0286\times GD} \times (Expected\ CBW/35),$$

$$\text{P: } Net\ P_{preg} = 0.0059e^{0.0253\times GD} \times (Expected\ CBW/35),$$

$$\text{Mg: } Net\ Mg_{preg} = 0.0007e^{0.0219\times GD} \times (Expected\ CBW/35),$$

$$\text{K: } Net\ K_{preg} = 0.011e^{0.0176\times GD} \times (Expected\ CBW/35),$$

$$\text{N): } Net\ Na_{preg} = 0.0197e^{0.0166\times GD} \times (Expected\ CBW/35),$$

and

$$\text{S: } Net\ S_{preg} = 0.0106e^{0.0181\times GD} \times (Expected\ CBW/35).$$

Macromineral retention efficiency during pregnancy was determined by using exponential regression models for each macromineral, based on the changes observed at different time points when cows were harvested (Table 7; Figure 5). Residual analysis was performed to evaluate the accuracy of this approach for estimating k_{preg} for each macromineral. The residual distribution was more homogeneous using this dynamic method compared with a fixed efficiency model, indicating that the proposed method improves predictive accuracy (Figure 6). Phosphorus was the only macromineral that did not exhibit a satisfactory fit to an exponential model. Phosphorus was the only macromineral that did not exhibit a satisfactory fit to an exponential model. This limitation may be due to the availability of multiple endogenous sources of P in the body, such as bone reabsorption and recycling through bloodstream and saliva. Additional data collection may be necessary to refine the predictive model for P utilization during pregnancy.

Initial Point of Macromineral Requirements for Pregnancy

The estimation of the initial onset of macromineral requirements for pregnancy is illustrated in Supplemental Figure S1 (see Notes). Each macromineral exhibited a distinct gestational day at which demand by gestational components began. All macrominerals showed initiation of demand before the final third of pregnancy in dairy cows. The initial onset of macromineral requirements for pregnancy in this study were 150 d for Ca, 92 d for P, 41 d for Na, 31 d for K; and 34 d for Mg.

DISCUSSION

Net Macromineral Requirements for Maintenance

Ca Requirements. In Figure 2, all maintenance models from this study were compared with traditional nutrient requirements systems. The net Ca requirement for maintenance in this study was 13.5 mg/kg of EBW, which is lower than the recommendations by NRC (2001; 15.4 mg/kg of BW) and INRA (2018; 15.0 mg/kg BW per day). However, it was not very different from the values recommended by those systems. Using the equation from NASEM (2021), the predicted Ca requirement for maintenance was 18.5 mg/kg BW, which represents the highest Ca requirement among all systems. The equation from NASEM (2021) considers DMI as a factor, as metabolic fecal excretion of Ca increases proportionally with intake.

Although differences exist in the predictions based on BW, the total daily Ca intake for maintenance does not vary drastically. For a 500-kg cow, this study estimated a requirement of 6.72 g/d, whereas NRC (2001) recommended 7.7 g/d, and NASEM (2021) suggested 9.2 g/d. The larger difference between this study and NASEM (2021) is likely due to the latter predicting nearly double the DMI for heifers and dry cows compared with NRC (2001). Because maintenance requirements are closely linked to feed intake (Suttle, 2010), using the NRC (2001) DMI equation in this study likely resulted in lower estimates compared with NASEM (2021).

P Requirements. The net P requirement for maintenance was estimated at 8.3 mg/kg EBW, which is lower than values reported by ARC (1980; 12.0 mg/kg BW), NRC (2001; 16.0 mg/kg BW), and NASEM (2021; 17.0 mg/kg BW. The lower prediction in this study may be attributed to the use of mature cows, which exhibit

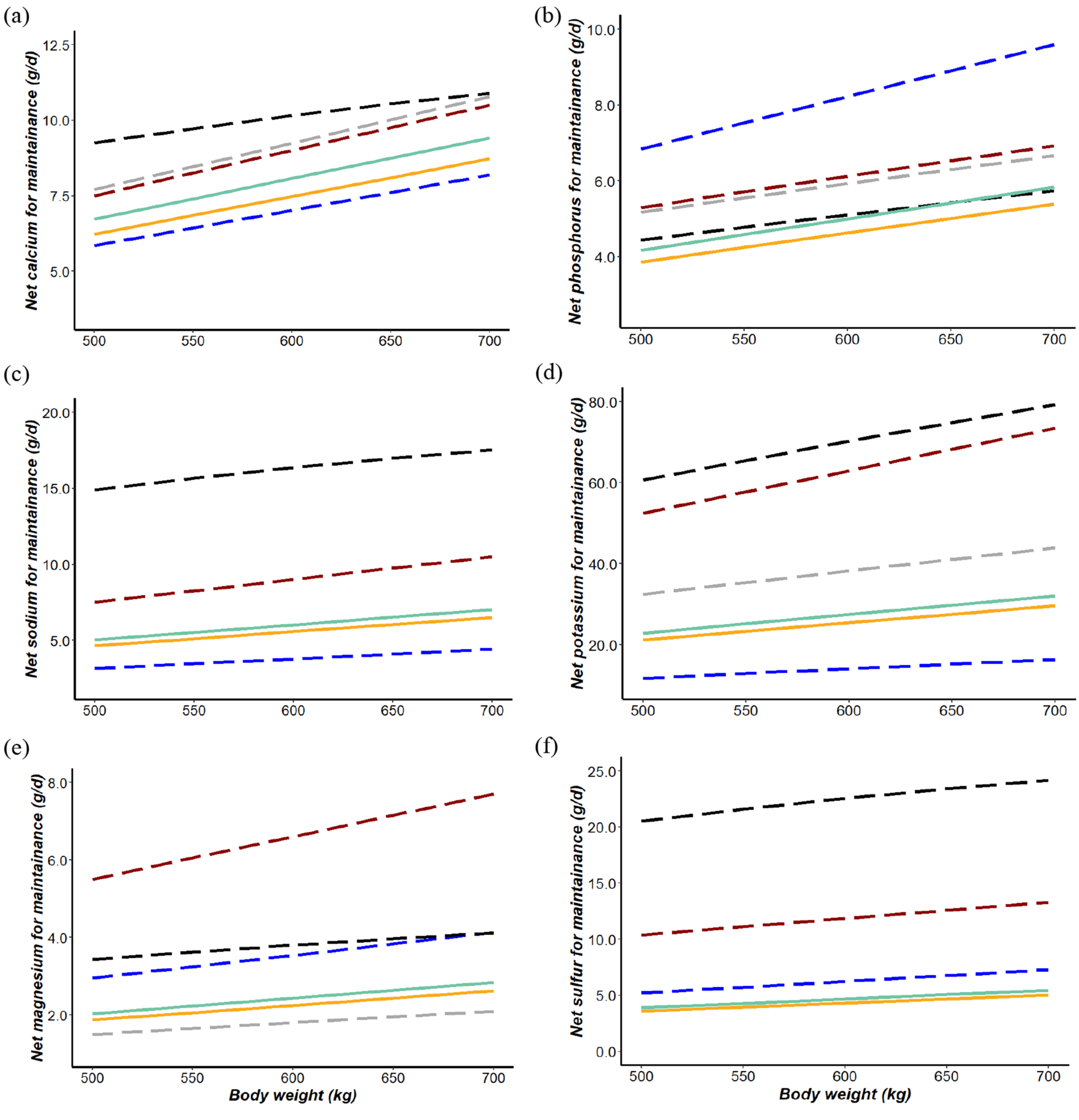

Figure 2. Comparison of predicted net macromineral maintenance requirements (g/d) for nonpregnant (green solid line) and pregnant cows (yellow solid line) with estimates from traditional nutrient requirement systems: NRC (2001; gray dashed line), BR-CORTE (Valadares Filho et al., 2016; blue dashed line), INRA (2018; red dashed line), and NASEM (2021; black dashed line). Predictions are shown for (a) calcium, (b) phosphorus, (c) sodium, (d) potassium, (e) magnesium, and (f) sulfur.

minimal variation in bone metabolism (Shahin and Berg, 1985). Additionally, the Holstein × Gyr crossbred cows in this trial are smaller than Holstein cows, which are predominantly used in traditional requirement systems. House and Bell (1993) noted that cows used in the NASEM (2021) dataset had completed only an average

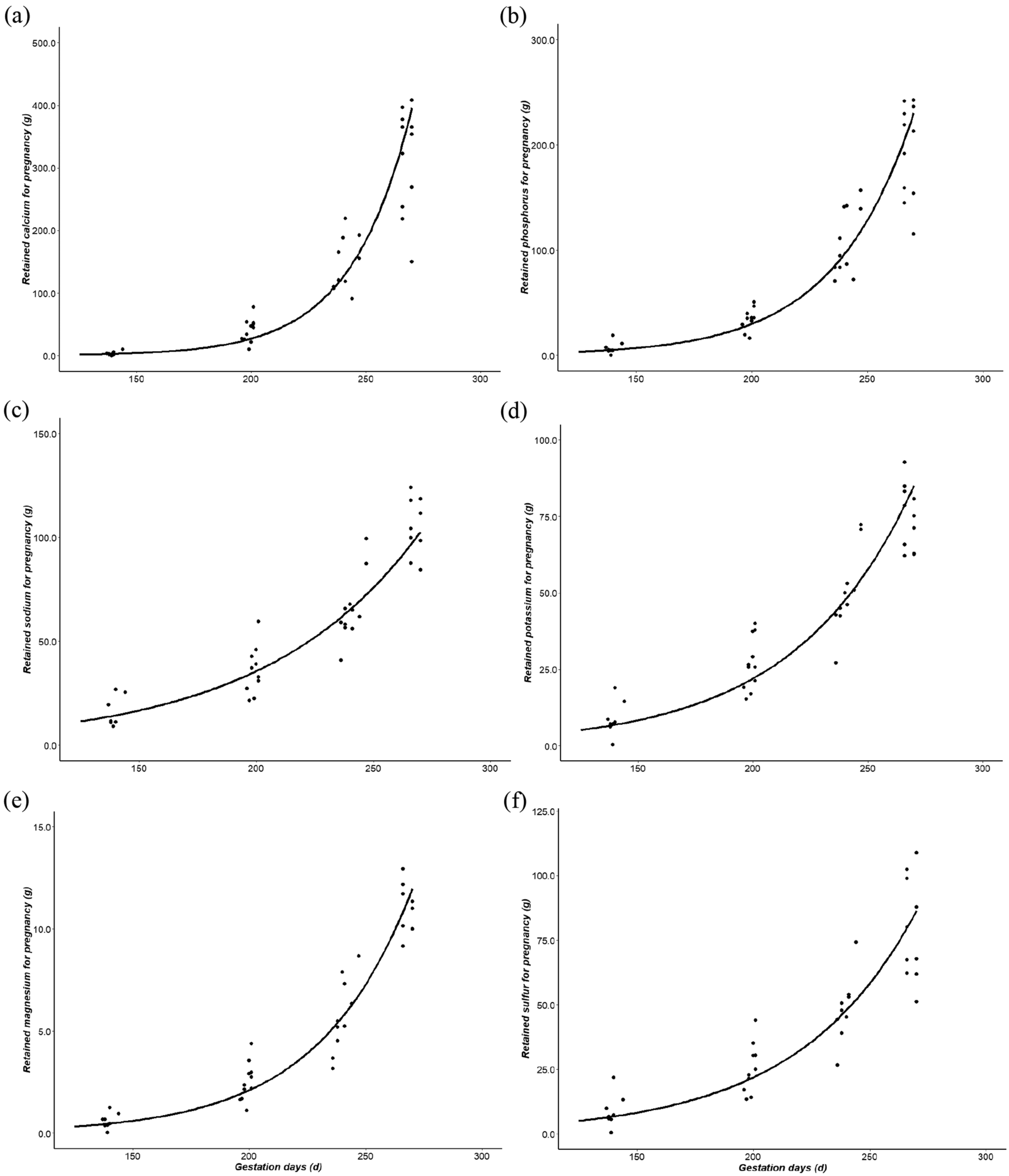

Figure 3. Retention of each macromineral in gestational components across pregnancy for (a) calcium, (b) phosphorus, (c) sodium, (d) potassium, (e) magnesium, and (f) sulfur. The black dots represent individual cow data for macromineral content in gestational components, and the curve represents the fitted exponential regression model describing macromineral retention throughout pregnancy.

Table 4. Estimation of parameters (β_0 and β_1) by mineral retention (g of a given macromineral/kg of EBW) in carcass and noncarcass to predict net requirements for weight gain

Mineral	β_0	SEM	*P*-value	β_1	SEM	*P*-value	R^2
Calcium	0.2435	0.1248	0.1322	1.7115	0.0829	0.0015	0.81
Phosphorus	0.5718	0.4905	0.3373	1.4762	0.1393	0.0076	0.95
Magnesium	0.0342	0.3683	0.3804	1.4391	1.9053	0.0099	0.90
Potassium	0.1146	0.3835	0.0713	1.5169	0.5421	0.0009	0.97
Sodium	0.0159	0.0941	0.4405	1.7880	0.9591	0.0089	0.94
Sulfur	0.1407	0.0856	0.1938	1.7982	0.0982	0.0022	0.85

of 2.7 ± 0.3 lactations before breeding, indicating they had not yet reached full maturity, which may explain the higher P requirement estimates.

Mg Requirements. The estimated net Mg requirement for maintenance was 4.06 mg/kg EBW, aligning closely with NRC (2001; 3 mg/kg BW) and NASEM (2021; 6.3 mg/kg BW). This similarity suggests that the Mg maintenance requirements in this study are consistent with established nutrient systems.

K Requirements. The net K requirement for maintenance was 45.9 mg/kg EBW, which is similar to NRC (2001; 38.0 mg/kg BW). The differences between this study and NRC (2001) may be explained by breed and climatic variations. NRC (2001) was developed using data from dairy herds in temperate climates, whereas this study was conducted in a warmer environment. Because K excretion via sweating and urine is significant (Underwood and Suttle, 1999; Suttle, 2010), higher temperatures increase K losses through sweat. Furthermore, *Bos indicus* cattle excrete more K via sweating than *Bos taurus* (Johnson, 1970). The use of *Bos indicus* × *Bos taurus* crossbred cows in this study may explain the higher net K requirement observed.

Na Requirements. The net Na requirement for maintenance was estimated at 10.1 mg/kg EBW, which is higher than the ARC (1980) recommendation of 6.8 mg/kg BW but lower than NRC (2001; 15.0 mg/kg BW). NASEM (2021) estimated Na requirements based on predicted DMI, leading to a daily Na recommendation ~3 times higher than this study. For a 500-kg cow, this study estimated 5.02 g/d of Na, whereas NASEM (2021) recommended 15.0 g/d. NASEM (2021) set an empirical equation for mature cows to prevent Na deficiency and milk yield reduction, which may explain the higher recommended intake. No signs of Na deficiency were observed in this study, likely because all cows were nonlactating. The drastic increase in Na requirement when cows begin lactation was a key factor behind the higher NASEM (2021) recommendation compared with NRC (2001).

S Requirements. The net S requirement for maintenance was estimated at 7.82 mg/kg EBW, which is lower than the BR-CORTE (Brazilian system for nutrient requirements; Valadares Filho et al., 2016) recommendation of 10.4 mg/kg BW. Limited studies have evaluated S requirements in dairy cattle, with most data coming from beef cattle trials. Using a comparative slaughter approach, Costa e Silva et al. (2015) estimated an S requirement of 9.4 mg/kg BW for young Nellore cattle, a value higher than that observed in this study. The lower S requirement estimated for mature cows aligns with reduced nutrient deposition in older animals, supporting the lower maintenance estimates found in this study.

Absorption and Retention Coefficient

Unlike energy and protein, the availability of minerals for metabolism is determined based on retention or

Table 5. Macromineral retention regression throughout pregnancy

Item	Retention for pregnancy[1] (grams)	SEM		*P-value*			
		B_0	β_1	B_0	β_1	RMSE[2]	R^2
Calcium	$0.1488e^{0.0285 \times GD} \times (Expected\ CBW/35)$	0.1487	0.0037	0.5017	0.0849	50.10	0.77
Phosphorus	$0.2324e^{0.0253 \times GD} \times (Expected\ CBW/35)$	0.1511	0.0024	0.3401	0.0635	24.50	0.80
Magnesium	$0.03065e^{0.0219 \times GD} \times (Expected\ CBW/35)$	0.0147	0.0018	0.3152	0.0597	1.19	0.87
Sodium	$1.1842e^{0.0166 \times GD} \times (Expected\ CBW/35)$	1.2827	0.0009	0.1640	0.0482	13.80	0.75
Potassium	$0.6332e^{0.0176 \times GD} \times (Expected\ CBW/35)$	0.3985	0.0023	0.3665	0.0897	1.57	0.85
Sulfur	$0.5837e^{0.0181 \times GD} \times (Expected\ CBW/35)$	0.4641	0.0029	0.4344	0.1039	13.68	0.73

[1] $Mineral\,retention_{preg} = \beta_0 e^{\beta_1 \times GD}$. The total retention of each micromineral for pregnancy is given in grams. GD = gestation days, CBW = calf body weight in kg.

[2] RSME = root square mean error.

Table 6. Descriptive statistics of macromineral content in each gestational component

	Days of pregnancy							
	140		200		240		270	
Mineral retention	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Calcium (g)								
Mammary gland	2.35	3.846	2.00	1.172	6.08	6.629	6.97	5.853
Uterus	0.28	0.106	0.95	1.427	0.94	0.421	1.34	0.525
Placenta	0.08	0.018	0.20	0.072	0.35	0.142	0.42	0.098
Fetal fluids	0.43	0.271	0.64	0.423	0.47	0.111	1.36	1.355
Fetus	6.31	1.492	66.2	26.66	196.00	53.96	389	96.02
Phosphorus (g)								
Mammary gland	12.7	9.336	18.00	8.253	23.3	15.14	24.9	14.86
Uterus	2.26	0.496	4.55	0.669	7.64	1.708	9.15	2.458
Placenta	0.37	0.174	1.32	0.763	1.99	0.909	2.86	1.228
Fetal fluids	0.14	0.069	0.23	0.135	0.20	0.067	0.47	0.471
Fetus	4.38	0.886	38.60	11.62	118.00	30.69	211.00	46.0
Magnesium (g)								
Mammary gland	0.98	0.451	1.37	0.588	2.12	1.624	2.66	1.302
Uterus	0.21	0.063	0.47	0.112	1.01	0.2478	1.18	0.412
Placenta	0.05	0.014	0.15	0.074	0.34	0.144	0.32	0.117
Fetal fluids	0.31	0.422	0.41	0.385	0.31	0.246	0.63	0.563
Fetus	0.23	0.031	2.03	0.576	4.80	1.470	8.34	1.610
Potassium (g)								
Mammary gland	16.8	8.348	20.4	7.929	22.5	8.518	27.4	10.440
Uterus	3.85	0.482	8.34	1.318	12.8	2.382	15.0	3.099
Placenta	0.59	0.131	1.75	0.936	2.25	0.899	3.67	1.361
Fetal fluids	1.62	1.796	2.65	2.569	1.71	1.165	4.80	5.751
Fetus	2.17	0.498	14.0	2.310	30.6	7.746	44.9	11.710
Sodium (g)								
Mammary gland	22.4	10.93	27.2	8.787	29.9	10.6	37.0	15.030
Uterus	3.56	0.518	6.65	1.029	10.6	2.39	12.6	2.330
Placenta	1.11	0.309	2.23	1.008	3.09	0.709	5.36	1.732
Fetal fluids	10.7	4.422	15.7	8.743	13.4	4.482	35.1	30.150
Fetus	2.85	1.166	15.3	4.426	35.2	9.217	52.3	8.658
Sulfur (g)								
Mammary gland	19.8	9.547	26.1	9.905	35.4	20.42	38.4	21.710
Uterus	2.92	0.358	6.89	2.983	10.6	1.727	11.1	2.708
Placenta	0.42	0.120	1.32	0.793	1.90	0.662	2.77	1.042
Fetal fluids	0.35	0.268	0.61	0.251	0.58	0.231	1.06	0.806
Fetus	1.46	0.304	10.4	3.084	29.9	8.075	49.5	12.59

absorption coefficients. Traditional nutrient requirement systems estimate mineral availability using absorption coefficients, which account for mineral excretion only through feces. However, certain minerals, such as K, are excreted in high concentrations via urine. For instance, NRC (2001) assumes 100% K absorption, but most K excretion occurs through urine (Ward, 1966). In this study, macromineral availability for metabolism was estimated using retention coefficients, providing a more accurate representation of mineral losses via urine, which notably impacts Na and K requirement predictions.

Table 7. Net requirement of macrominerals for pregnancy, and efficiencies of mineral utilization for pregnancy

Macromineral	Net requirement$_{preg}$[1] (g/d)	Efficiency of mineral retention by gestational components k_{preg}[2]
Calcium	$0.0042e^{0.0286\times GD}$	$0.0004e^{0.0263\times GD}$
Phosphorus	$0.0059e^{0.0253\times GD}$	$0.2974e^{0.0048\times GD}$
Magnesium	$0.0006e^{0.0219\times GD}$	$0.00006e^{0.0233\times GD}$
Sodium	$0.0197e^{0.0166\times GD}$	$0.0003e^{0.0230\times GD}$
Potassium	$0.0111e^{0.0176\times GD}$	$0.0003e^{0.0234\times GD}$
Sulfur	$0.0106e^{0.0181\times GD}$	$0.0004e^{0.0199\times GD}$

[1]First derivative equation from mineral retention equation. GD = gestation days.

[2]k_{preg} = efficiency of macromineral retention throughout pregnancy.

Retention Coefficients for Macrominerals

Calcium. The retention coefficient for Ca in this study was 65% (Table 3). NRC (2001) and AFRC (1991) estimate Ca absorption coefficients at 70% and 68%, respectively. NASEM (2021) recommends a range of 30% to 60%, depending on dietary composition. BR-CORTE (Valadares Filho et al., 2016) recommends a Ca retention coefficient of 56.8%. Because urinary Ca excretion remains relatively constant (Goff and Horst, 1993), the similarity between absorption and retention coefficients

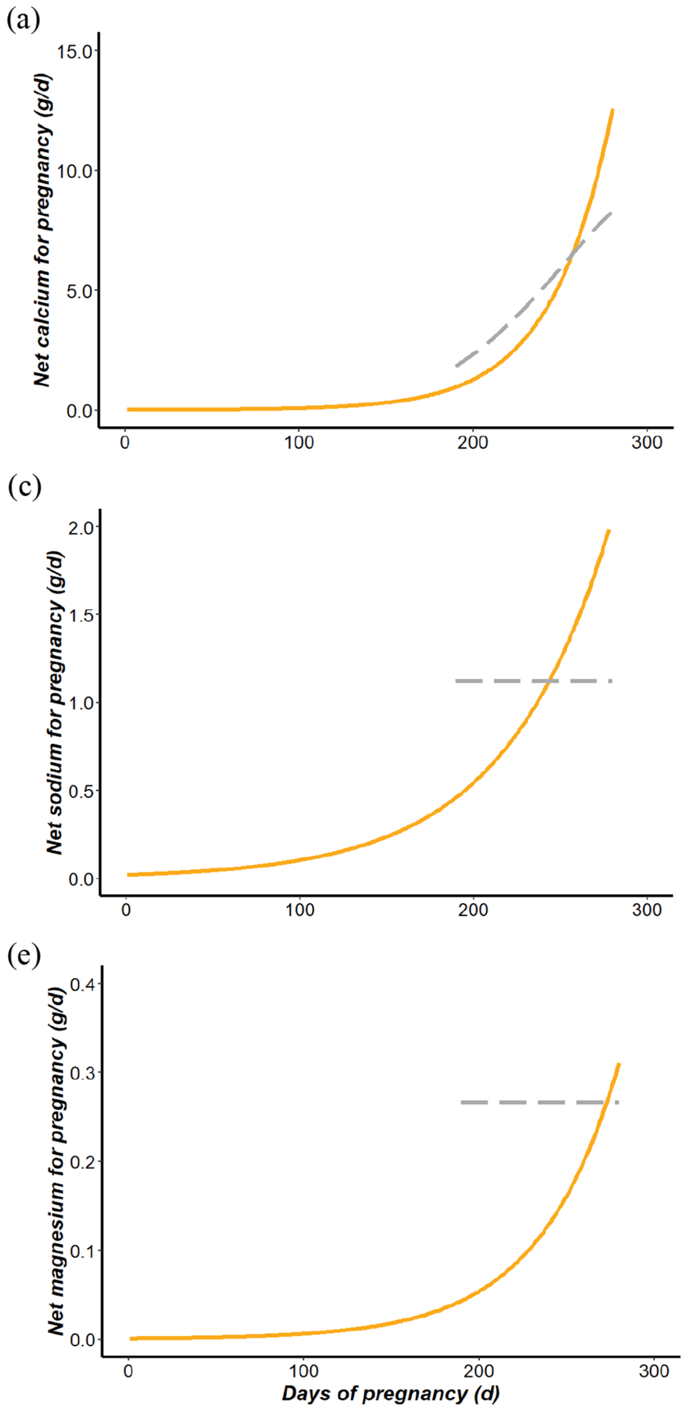

Figure 4. Predicted macromineral requirements throughout pregnancy using the approaches from this study (yellow line) for (a) calcium, (b) phosphorus, (c) sodium, (d) potassium, (e) magnesium, and (f) sulfur, compared with NRC (2001) recommendations (gray line).

observed in this study can be attributed to the low variability of urinary Ca excretion.

Phosphorus. The retention coefficient for P in this study was 74.2% (Table 3), higher than the 67.8% reported by BR-CORTE (Valadares Filho et al., 2016). ARC (1980) and AFRC (1991) estimate P absorption coefficients at 60% and 58%, respectively. NRC (2001) and NASEM (2021) calculate P absorption based on individual feedstuffs, with an average efficiency of 80%. As with Ca, fecal excretion is the primary route of P loss in ruminants. Variations in P requirements may be influenced by dietary P concentration and P recycling,

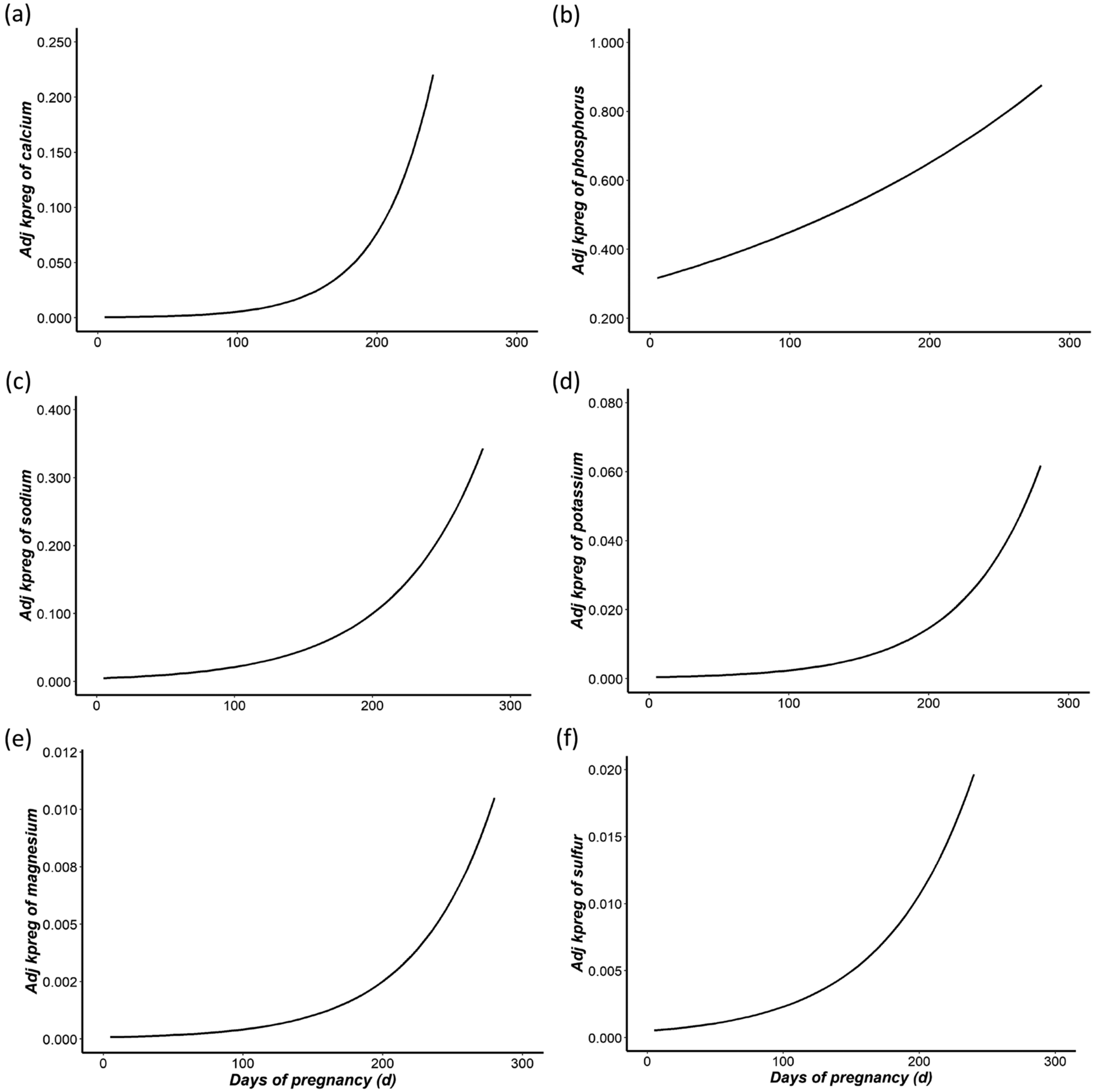

Figure 5. Efficiency of macromineral retention in gestational components (k_{preg}) for (a) calcium, (b) phosphorus, (c) sodium, (d) potassium, (e) magnesium, and (f) sulfur. The *y*-axis represents the adjusted efficiency of macromineral retention ($Adj\ k_{preg}$), plotted against days of pregnancy (*x*-axis).

which can lead to under- or overestimation of P needs (Suttle, 2010). Additionally, dicalcium phosphate, the primary P source in this study, has a high gastrointestinal absorption rate, potentially explaining differences in P coefficients across systems.

Magnesium. The retention coefficient for Mg was 40%. BR-CORTE (Valadares Filho et al., 2016) estimated a Mg retention coefficient of 35.5%, whereas NRC (2001) reported an absorption coefficient of 17%. The higher Mg retention coefficient observed in this study

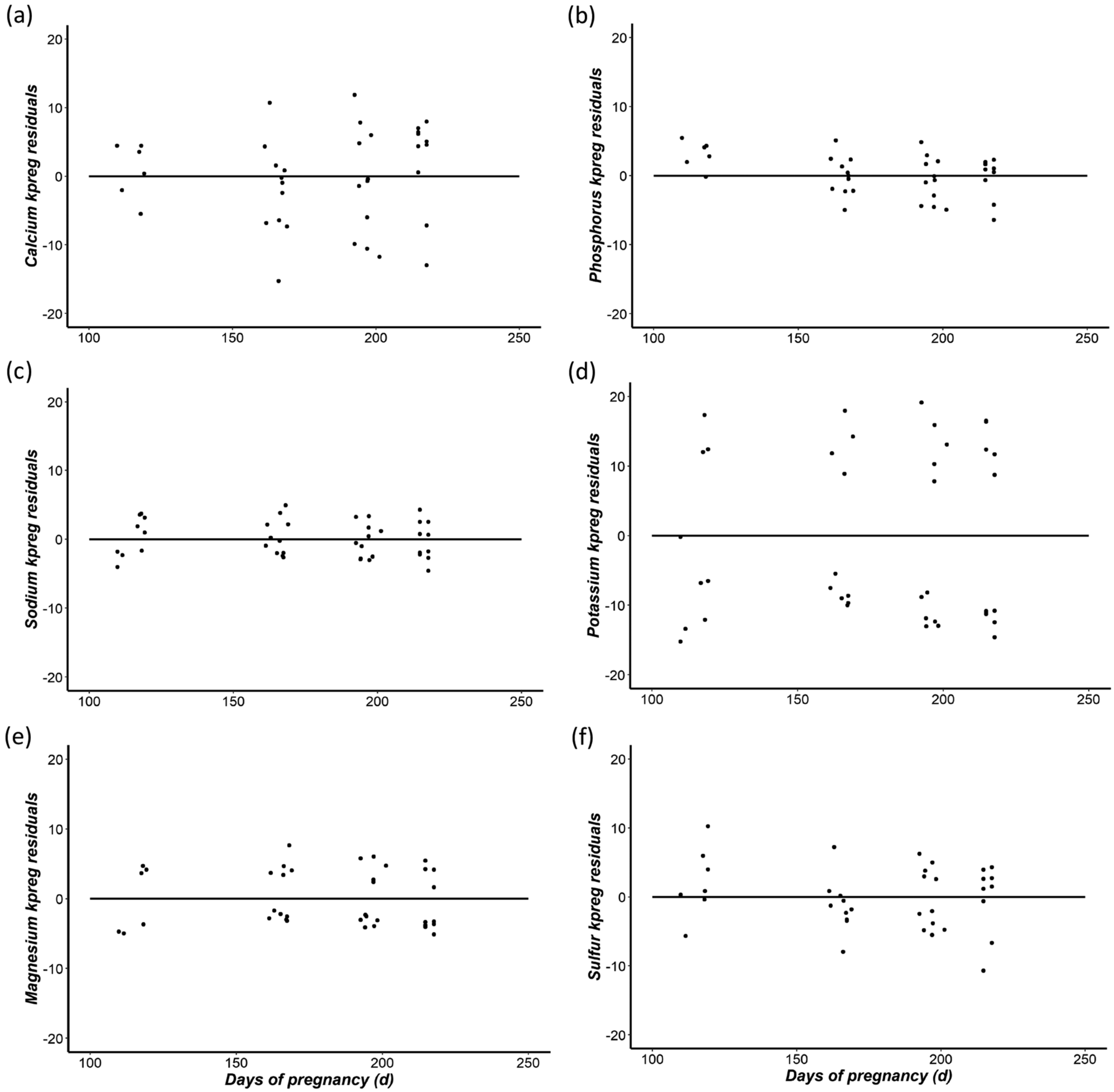

Figure 6. Residuals of k_{preg} of (a) calcium, (b) phosphorus, (c) magnesium, (d) potassium, (e) sodium, and (f) sulfur. Residuals were calculated as observed values − predicted values. Residual plots assessed the homoscedasticity of the exponential regression of efficiency of macromineral uptake by gestational components (k_{preg}).

may be attributed to climate conditions. Castro et al. (2019) found similar results when comparing Holstein and Holstein × Gyr calves. Magnesium balance is affected by warm climates (Sanchez et al., 1994), suggesting an adaptive response in cattle raised in tropical environments. Additionally, K intake negatively affects Mg absorption (Fisher et al., 1994). Because dietary K levels in this study were lower than current dairy cattle recommendations, this may have contributed to the higher Mg retention coefficient.

Potassium. The retention coefficient for K in this study was 56.4%, exceeding the 48.4% recommended by

BR-CORTE (Valadares Filho et al., 2016). NRC (2001) assumes a K absorption coefficient of 90%, whereas NASEM (2021) considers 100% absorption. As previously discussed, urinary excretion is the primary route for K loss, explaining the differences between retention coefficients in this study and those proposed by NRC (2001) and NASEM (2021).

Sodium. The retention coefficient for Na in this study was 56.4% (Table 3), which is higher than BR-CORTE (Valadares Filho et al., 2016) but significantly lower than NRC (2001; 90%) and NASEM (2021; 100%). Similar to K, Na is primarily excreted via urine. Table 2 demonstrates that over one-third of Na excretion occurs through urine, highlighting how absorption coefficients overestimate retention capacity in cows.

Sulfur. Few studies have investigated S retention in cattle. In this study, the retention coefficient for S was 85.0%, higher than the values reported by Costa e Silva et al. (2015) and BR-CORTE (Valadares Filho et al., 2016), which recommend 77.1% and 67.3% for beef cattle, respectively. The higher S retention observed in this study may be related to pregnancy, as previous studies focused on beef cattle. As pregnancy progresses, fetal S uptake increases, reflecting the increasing demand for S from the diet (House and Bell, 1994). It is important to note that retained S in this study represents S incorporated into organic molecules (e.g., AA such as methionine, cysteine, homocysteine, and taurine; vitamins such as biotin and thiamine; and enzymes), rather than free sulfur retained in tissues.

Net Macromineral Requirements for BW Gain

All macromineral models for weight gain were adjusted using allometric models (Table 4). All macrominerals exhibited a positive slope in the regression equations, indicating that mineral content increases proportionally with body mass. This trend is suitable for the animals in this study, which had already reached mature weight (age, ~5 yr old; average BW, ~520 kg). As a result, body mass and macromineral retention increased proportionally.

To compare net requirements for weight gain between this study and traditional nutrient requirement systems, a standard cow weighing 500 kg with a target ADG equals to 0.1 kg/d was used. Under this scenario, the net requirements for BW gain for Ca, P, Mg, Na, K, and S were estimated as follows: 2.9, 1.4, 0.07, 0.31, 0.37, and 2.98 g/d, respectively.

Compared with the net requirements for BW gain proposed by NRC (2001), which are still recommended by NASEM (2021), the values estimated in this study were higher for all macrominerals. The primary reason for this increase is likely the new adjustment for EBG_c proposed by Marcondes et al. (2023). This adjustment accounts for the gestational components that experience mass increases related to ADG as part of BW gain rather than tissue accretion for pregnancy. By attributing these components to carcass and noncarcass weight gain, the model increases the estimated mineral content associated with BW gain, leading to higher macromineral requirements

Macromineral Deposition in Gestational Components

Calcium and Phosphorus. The fetus and mammary gland were the primary sites of Ca and P retention, with significant amounts observed during mid gestation (Table 6). Ferrell et al. (1982) reported that fetal Ca and P accretion begins early and increases significantly during mid pregnancy in crossbred beef heifers, supporting the findings of this study. Additionally, exponential models were found to best describe mineral deposition in the gravid uterus, consistent with previous studies (House and Bell, 1993; Meschy, 2007; Sguizzato et al., 2020a).

Magnesium, Potassium, and Sodium. House and Bell (1993) applied linear regressions to estimate fetal Mg, K, and Na content, which was appropriate for their study, as they only evaluated the final third of pregnancy. However, Ferrell et al. (1982) found that an exponential model was better suited for estimating conceptus mineral content from 100 d of pregnancy to parturition. The findings of this study support an exponential pattern for Mg, K, and Na retention, aligning with Ferrell et al. (1982).

Sulfur. House and Bell (1994) proposed an exponential model for S accretion beginning at 190 d of gestation. Unlike their study, which did not include the mammary gland as a gestational component, this study found that the mammary gland retained a significant proportion of S during mid gestation. At 100 d of pregnancy, 79.36% of S in pregnancy-associated tissues was retained in the mammary gland (Table 6). The fetus also exhibited substantial S retention at 240 and 270 d of pregnancy, increasing from 29.9 to 49.5 g (Table 6), which is consistent with the exponential pattern reported by House and Bell (1994).

These findings reinforce the importance of considering the mammary gland as a gestational component when estimating macromineral requirements for pregnancy. Additionally, the exponential retention patterns observed in this study align with previous research on mineral deposition during pregnancy, further validating the modeling approach used.

Net Macromineral Requirements of Pregnancy

Comparing the models proposed by NRC (2001), INRA (2018), and this study from 190 through 262 d of gestation, NRC (2001) and INRA (2018) predicted higher Ca requirements for pregnancy. However, beyond 262 d of pregnancy, this study predicted higher Ca demand until calving (Figure 4). This increased Ca demand may be due to colostrum synthesis in the mammary gland, which was not included as a gestational component in the models by House and Bell (1993).

The higher Ca predictions by INRA (2018) compared with this study may be attributed to breed differences used in model development. INRA (2018) does not adjust for calf size at birth across different breeds common in warm climates, such as Girolando crossbreds (Sguizzato et al., 2020b). NRC (2001) estimated Ca requirements based on a newborn weight of 46 kg (House and Bell, 1993), whereas the average calf birth weight in this study was 33 kg, which is typical for Holstein × Gyr crossbreds (Silva et al., 2017; Azevedo et al., 2019; Azevedo et al., 2024; Sguizzato et al., 2020a).

Both NRC (2001) and INRA (2018) used exponential models to estimate P requirements during pregnancy, similar to the approach in this study. Differences in predicted P requirements are largely due to variations in newborn weight, as Holstein × Gyr calves (~35 kg) are lighter than Holstein calves, which dominate the NRC (2001) and INRA (2018) datasets. Including the mammary gland as a gestational component in this study may explain the similarities in P requirement predictions beyond 190 d of gestation compared with NRC (2001) and INRA (2018).

Macromineral Models for Na, K, Mg, and S

For Na, K, and Mg, NRC (2001), INRA (2018), and NASEM (2021) recommend fixed values for pregnancy. However, this study found that these minerals follow an exponential pattern, as observed for Ca and P. Assigning a constant Na, K, and Mg accretion rate throughout pregnancy does not align with the mineral retention trends observed in this study. The exponential models proposed here for Na and K agree with findings by Ferrell et al. (1982). For Mg, NRC (2001) recommended a fixed requirement due to concerns about hypomagnesemia. Although this study did not assess the risk of hypomagnesemia (as all animals were harvested), future research should evaluate whether exponential models accurately predict Mg needs without increasing the risk of metabolic disorders.

For S, both NRC (2001) and INRA (2018) recommend a fixed requirement of 2 g/kg DMI/d. However, using fixed values may limit understanding of nutrient partitioning and the efficiency of S utilization and excretion. This study is the first to determine S requirements for maintenance, BW gain, and pregnancy in pregnant dairy cows. The findings provide insights into the dynamics of S needs throughout pregnancy. For example, comparing 2 pregnant cows—one at 150 d of gestation and another at 250 d—the net S requirement for pregnancy in the latter was 6 times greater than in the former, emphasizing the necessity of dynamic models for S accretion during pregnancy.

Initial Point of Requirements of Each Macromineral for Pregnancy

It is important to note that macromineral demands begin before 190 d of gestation, which has traditionally been considered the initiation of macromineral requirements by conventional nutrient requirement systems (INRA, 2018; NASEM, 2021). In fact, Ca and P demands start in the early second third of pregnancy, whereas Na, K, Mg, and S begin in the first third of pregnancy (Supplemental Figure S1).

Although extensive studies in literature describe the intensification of fetal growth in the last third of pregnancy as the primary driver of nutrient retention in the gravid uterus, other tissues, such as mammary gland, also accumulate significant amounts of macrominerals. For energy and protein requirements, Sguizzato et al. (2020a) reported that energy demand for pregnancy begins at 70 d of gestation, whereas Marcondes et al. (2023) described that protein demand starts at 140 d of pregnancy. These findings suggest that considering 190 d as the onset of macromineral requirements in dairy cows for diet formulation may be too late.

Although macromineral requirements at these early stages are minimal compared with lactation and maintenance, incorporating them into diet formulation could allow for more precise nutritional adjustments. However, the initial points proposed in this study should be interpreted with caution, as all macrominerals except for Ca exhibited an onset of requirement before the earliest time point of harvesting in this trial (140 d of pregnancy). Therefore, further studies investigating macromineral requirements in early and mid pregnancy are necessary to more precisely define the onset of macromineral demands in pregnant dairy cows.

Efficiency of Use of Macromineral for Pregnancy

Using the comparative harvesting method in this study to predict macromineral requirements for pregnant cows, we estimated retention efficiency at different time points throughout pregnancy (Table 7 and Figure 5). As is well known, nutrient requirements change due to various factors such as breed, age, and physiological status (NRC, 1995). Similarly, retention efficiency for nutrients follows the same trend (CSIRO, 2007; Marcondes et al., 2010).

The adjusted k_{preg} provided a better fit for our data compared with a fixed k_{preg}. The distribution of residual components was more homogeneous, with lower variability when an adjusted efficiency model was applied throughout pregnancy (Figure 6). The adjusted k_{preg} for all macrominerals followed an exponential model, which aligns with the exponential equations commonly used to describe pregnancy, mirroring the growth pattern of the fetus in the uterus.

This is the first study evaluating the variation in retention efficiency of macrominerals throughout pregnancy. Many factors influence mineral availability in metabolism, such as hormonal activity (e.g., parathyroid hormone regulation in Ca metabolism) and interactive effects between minerals, such as Na and K. In this study, only the retention of macrominerals in body tissues was calculated. Future research should investigate the dynamic changes in efficiency of use and macromineral availability in response to physiological changes, enabling more precise predictions of macromineral requirements during pregnancy.

Prediction of Dietary Macromineral Requirements During Pregnancy

All macrominerals were used to predict dietary mineral requirements, which were calculated as the sum of the net requirements for maintenance and BW gain divided by the true retention coefficient, plus the net requirements from pregnancy divided by k_{preg} at the i_{th} day of pregnancy. To illustrate, consider 2 pregnant cows, both weighing 500 kg and having the same EBG_c of 0.1 kg/d.

The total absorption requirements of macrominerals for a cow at 150 d of pregnancy are 23.8 g/d for Ca, 5.5 g/d for P, 10.9 g/d for Mg, 7.82 g/d for Na, 35.8 g/d for K, and 26.8 g/d for S. For a cow at 250 d of pregnancy, the total absorption requirements increase to 27.5 g/d for Ca, 8.4 g/d for P, 9.8 g/d for Mg, 7.0 g/d for Na, 29.3 g/d for K, and 23.5 g/d for S. As expected, macromineral demand increases as pregnancy progresses. However, the difference in absorption requirements between the 2 cows is not substantial because as macromineral demand increases, the efficiency of uptake by gestational components also improves. For some minerals, such as Na, K, and S, the absorption requirements decrease in late pregnancy. This occurs because the efficiency of mineral uptake by gestational components increases at a greater rate than the mineral demand, thereby reducing dietary mineral requirements.

Using the equations from NRC (2001), the estimated total absorption requirements of macrominerals at 150 d of pregnancy are 8.8 g/d for Ca, 5.8 g/d for P, 1.5 g/d for Mg, 7.6 g/d for Na, 32.6 g/d for K, and 10.4 g/d for S. At 250 d of pregnancy, NRC (2001) estimates 14.7 g/d for Ca, 9.4 g/d for P, 1.9 g/d for Mg, 8.8 g/d for Na, 33.6 g/d for K, and 10.4 g/d for S.

Using the NASEM (2021) equations, the estimated total absorption requirements at 150 d of pregnancy are 10.3 g/d for Ca, 11.2 g/d for P, 3.5 g/d for Mg, 15.0 g/d for Na, 60.9 g/d for K, and 20.5 g/d for S. For 250 d of pregnancy, NASEM (2021) estimates 16.2 g/d for Ca, 14.8 g/d for P, 3.8 g/d for Mg, 16.1 g/d for Na, 61.7 g/d for K, and 20.5 g/d for S.

Differences in Estimates and Implications

For Ca, Mg, and S, the estimates in this study are higher than those recommended by NRC (2001) and NASEM (2021) at both time points. This difference is primarily due to higher net macromineral requirements for BW gain; accounting for requirements before 190 d of pregnancy; the inclusion of efficiency of macromineral uptake by gestational components.

Although the recommended levels for these minerals are higher than those in traditional nutrient requirement systems, the intake levels suggested in this study do not exceed the tolerable intake limits for ruminants (NRC, 2005). For P, Na, and K, the estimates in this study align closely with NRC (2001) recommendations. This similarity is likely because the diet formulation used in this trial was based on NRC (2001) equations.

NASEM (2021) estimates higher requirements for P, K, and Na, largely due to its higher recommended DMI for dry cows. Because net macromineral requirements for maintenance are estimated as a function of DMI, this results in higher maintenance and total absorption requirements in the NASEM (2021) model. These findings highlight the importance of incorporating dynamic macromineral efficiency adjustments and considering physiological changes throughout pregnancy when estimating dietary mineral requirements for pregnant dairy cows.

Considerations on This Study

Several points should be carefully considered in interpreting the findings of this study. First, this study was conducted with only 2 levels of DMI due to limitations in the number of available animals. Consequently, DMI had to be included as a parameter in the models. Future studies with a broader range of DMI levels would help refine the predictions and enhance their applicability.

Second, the micro- and macromineral sources remained consistent throughout the study, meaning that interactions among different mineral sources were not evaluated. This is a crucial limitation because the bioavailability of certain minerals is highly dependent on their source and the presence of other minerals and vitamins. For instance, calcium absorption is closely linked to vitamin

D availability, and potassium and magnesium interact in metabolic pathways. Future research exploring these interactions and their impacts on mineral requirements at the animal level would provide deeper insights. Additionally, maintenance requirements for macrominerals were assumed to be fixed throughout pregnancy. However, as physiological status changes with gestation, macromineral requirements for maintenance may also fluctuate. This study did not assess such variations, highlighting the need for further research to determine how maintenance mineral needs evolve as pregnancy progresses.

A third key consideration is the estimation of total daily urine and fecal excretion based on spot sampling. As noted earlier, we employed established models from the literature to estimate total daily excretion using spot sampling procedures. Ideally, direct measurement of excretion would have been performed to avoid potential cumulative errors associated with model-based estimations. However, logistical constraints prevented total collections in this trial, making spot sampling the most practical approach for both urine and feces. Furthermore, it is important to acknowledge that iNDF was used as an internal marker to estimate fecal excretion, which may have introduced additional bias.

CONCLUSIONS

This study is the first in the literature to evaluate the influence of pregnancy on macromineral requirements for maintenance and BW gain, providing enhanced accuracy in predicting macromineral utilization by pregnant dairy cows. Although the last third of gestation represents the primary stage for nutrient retention in the gravid uterus and mammary gland, it is crucial to consider macromineral demands earlier in pregnancy to prevent deficiencies that may affect both the fetus and the cow. Our findings indicate an exponential increase in the demand for all macrominerals starting at 150 d of pregnancy. Additionally, the retention efficiency of all macrominerals also follows an exponential increase as the pregnancy progresses, Therefore, the k_{preg} values proposed in this study for macrominerals provide a more precise approach to feeding pregnant dairy cows, ensuring optimal mineral utilization throughout gestation.

NOTES

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Nonstandard abbreviations used: CBW = calf BW; EBG = empty body gain; EBG_c = empty body gain carcass and noncarcass ; EBW = empty BW; GD = gestation days; kpreg = micromineral efficiency of pregnancy; RMSE = root mean square error; SBW = shrunk BW.

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