CARLOS HENRIQUE PAIVA CAMISA NOVA

MACROMINERAL REQUIREMENTS FOR MAINTENANCE, BODY WEIGHT GAIN AND PREGNANCY OF DAIRY COWS

Dissertation submitted to the Graduate Program in Animal Science in Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*

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BIOGRAPHY

Carlos Henrique Paiva Camisa Nova, son of Antonio Carlos Fernandes Camisa Nova and Meire dos Santos Paiva Camisa Nova, was born in São Paulo, SP – Brazil.

He started Animal Science major in 2011 and obtained bachelor's in animal science in 2018 at Universidade Estadual do Norte Fluminense – Darcy Ribeiro, Campos dos Goytacazes – RJ, Brazil.

On August of 2014, He was granted with a scholarship to study abroad for one year and six months in University of Wisconsin – Madison, USA sponsored by the improvement of Higher Education Personnel – CAPES.

In March of 2019, he was approved to take Master position in Graduate Program in Animal Science focused on Dairy Cattle Nutrition and Production, supervised by Dr. Polyana Pizzi Rotta

On February 22nd of 2021, he submitted his dissertation to the committee to obtain the *Master Scientiae* degree in Animal Science.

ABSTRACT

CAMISA NOVA, Carlos Henrique Paiva, M. Sc., Universidade Federal de Viçosa, February, 2021. **Macromineral requirements for maintenance, body weight gain and pregnancy of daiy cows.** Supervisor: Polyana Pizzi Rotta.

Macrominerals are essential to maintain healthy, high reproductive efficiency and performance of dairy cattle. Based on it, understanding the macromineral requirements are crucial to formulate diets to meet the needs of cows accurately. However, studies to predict macromineral requirements for pregnant dairy cows are scarce. Thus, the goal of this study was to set approaches to predict the macromineral requirements of pregnant crossbred Holstein x Gyr cows. 62 Holstein-Gyr crossbred cows were enrolled in this experiment. The cows were sorted into 3 different groups: pregnant, open, and reference cows. Two subgroups were formed both for pregnant and open cow groups, where, the first subgroup was submitted to *ad libitum* feed management and the second subgroup was submitted to the maintenance nutritional management (feed intake equals 1.15% of body weight). The pregnant cows were harvested at four different days of gestation: 140, 200, 240, and 270 days, as well as the open cows, were harvested at day 200, 240, and 270 of gestation of the pregnant group. Seven periods of 28 days were accomplished for total tract digestibility (122, 150, 178, 206, 234, and 262 days of gestation). Net requirements for maintenance, growth, and gestation were estimated as well as the retention coefficient and efficiency to use macrominerals for gestation. The net requirement for calcium, phosphorus, sodium, potassium, and magnesium were 13.4837, 8.3534, 10.0751, 45.8889, and 4.4436 mg/kg of empty body weight (EBW), respectively. Additionally, the retained coefficient for calcium, phosphorus, sodium, potassium, and magnesium were 0.6516, 0.7402, 0.4267, 0.5641 and 0.4211, respectively. The net requirement for gain showed statistical differences between pregnant and open cows for calcium and phosphorus. On the other hand, sodium, potassium, and magnesium did not present differences for gain between pregnant and non-pregnant cows. For calcium, the predicting models of gain for non-pregnant cows and pregnant cows were $Ca\ (g/d) = 0.2818 \times EBW^{0.6202} \times EBG$ and $Ca\ (g/d) = 3.1319 \times EBW^{0.1484} \times EBG$, respectively. For phosphorus, the models of gain for open and pregnant cows were $P\left(\frac{g}{d}\right) = 0.7669 \times EBW^{0.3843} \times EBG$ and $P\ (g/d) = 2.4456 \times EBW^{0.1435} \times EBG$, respectively. The predicting models of gain for sodium were $Na\ (g/d) = 0.0201 \times EBW^{0.7219} \times EBG$ and $Na\ (g/d) = 0.1597 \times EBW^{0.3185} \times EBG$ for open and pregnant cows, respectively. For potassium, the models of potassium for open cows were $K\ (g/d) = 0.2178 \times EBW^{0.3514} \times$

EBG and for pregnant cows was $K\ (g/d) = 0.7269 \times EBW^{0.1191} \times EBG$. About magnesium requirement for gain, the model for open cows was $Mg\ (g/d) = 0.0508 \times EBW^{0.4163} \times EBG$ and for pregnant cows was $Mg\ (g/d) = 0.6170 \times EBW^{-0.0700} \times EBG$. Predictions for gain for sulfur for non-pregnant and pregnant cows were $Net\ S_{gain} = 0.1708 \times EBW^{0.5185}$, and $Net\ S_{gain} = 1.2422 \times EBW^{0.1333}$, respectively. For gestation, the predicting model for calcium was $Ca\ req_{preg}\ (g/d) = 0.0125\ e^{0.02489 \times DP}$. For phosphorus, the model was $P\ req_{preg}\ (g/d) = 0.0207\ e^{0.02049 \times DP}$. On sodium the equation was $Na\ req_{preg}\ (g/d) = 0.0588\ e^{0.0115 \times DP}$. For potassium, the model for pregnancy was $K\ req_{preg}\ (g/d) = 0.0439\ e^{(0.0119 \times DP)}$. The magnesium model for pregnancy was $Mg\ req_{preg}\ (g/d) = 0.0021\ e^{(0.01748 \times DP)}$ and sulfur for pregnancy was $S\ req_{preg}\ (g/d) = 0.0263\ e^{(0.0148 \times DP)}$.

Keyword: Girolando, Pregnancy; Dairy cattle; Nutrient requirements; Mathematical modeling.

RESUMO

CAMISA NOVA, Carlos Henrique Paiva, M. Sc., Universidade Federal de Viçosa, fevereiro de 2021. **Exigências de macrominerais para mantença, ganho de peso e gestação para vacas leiteiras**. Orientador: Polyana Pizzi Rotta.

Os macrominerais (Ca, P, Mg, Na, K, and S) são essenciais para manter a saúde, alta eficiência reprodutiva e produção de leite em vacas leiteiras. Com isso, conhecer as exigências de macrominerais é essencial para formular dietas que atendam as necessidades das vacas. No entanto, predicoes de macrominerais ao longo da gestação de vacas leiteiras são escassos. Assim, o objetivo deste estudo foi estabelecer as necessidades macrominerais de vacas mestiças Holandês x Gir ao longo da gestação. 62 vacas mestiças Holstein × Gyr foram utilizadas neste experimento. As vacas foram divididas em 3 grupos diferentes: vacas prenhes, vazias e referência. Foram formados dois subgrupos para vacas gestantes e vacas vazias, sendo o primeiro subgrupo submetido ao manejo alimentar *ad libitum* e o segundo ao manejo nutricional de manutenção (consumo de ração igual a 1,15% do peso corporal). As vacas gestantes foram abatidas ao longo de quatro tempos de gestação distintos: 140, 200, 240 e 270 dias, assim como as vacas vazias foram abatidas nos dias 200, 240 e 270 de gestação do grupo gestante. Sete períodos de 28 dias foram realizados para digestibilidade total do trato (122, 150, 178, 206, 234 e 262 dias de gestação). As necessidades líquidas de manutenção, crescimento e gestação foram estimadas, bem como o coeficiente de retenção e a eficiência do uso de macrominerais para a gestação. As necessidades líquidas de cálcio, fósforo, sódio, potássio, magnésio e enxofre foram 13,48, 8,35, 10,08, 45,89, 4,44 e 7.82 mg / kg de peso corporal vazio (PCVZ), respectivamente. Além disso, o coeficiente retido para cálcio, fósforo, sódio, potássio e magnésio foram 65%, 74%, 43%, 56%, 42% e 85%, respectivamente. A exigência líquida de ganho mostrou diferenças estatísticas entre vacas gestantes e vacas abertas para cálcio e fósforo. No entanto, sódio, potássio e magnésio não apresentaram diferenças para ganho entre vacas gestantes e não gestantes. Para o cálcio, os modelos de previsão de ganho para vacas não gestantes e vacas gestantes foram: $Ca\ (g/d) = 0.2818 \times EBW^{0.6202} \times EBG$ and $Ca\ (g/d) = 3.1319 \times EBW^{0.1484} \times EBG$, respectivamente. Para o fósforo, os modelos de ganho para vacas abertas e prenhes foram $P\ (g/d) = 0.7669 \times EBW^{0.3843} \times EBG$ and $P\ (g/d) = 2.4456 \times EBW^{0.1435} \times EBG$, respectivamente. As estimativas de sódio para ganho de peso foram $Na\ (g/d) = 0.0201 \times EBW^{0.7219} \times EBG$ e $Na\ (g/d) = 0.1597 \times EBW^{0.3185} \times EBG$ para vacas vazias e prenhas, respectivamente. Para potássio, a predição para vacas vazias foi $K\ (g/d) =$

$0.2178 \times EBW^{0.3514} \times EBG$ enquanto para vacas prenhas: $K\ (g/d) = 0.7269 \times EBW^{0.1191} \times EBG$. Para magnésio, vacas vazias foi: $Net\ Mg_{gain} = 0.0508 \times EBW^{0.4163}$ e para vacas prenhas $Net\ Mg_{gain} = 0.6170 \times EBW^{-0.0700}$ Enxofre para ganho de vacas vazias foi $Net\ S_{gain} = 0.1708 \times EBW^{0.5185}$, e para vacas prenhas $Net\ S_{gain} = 1.2422 \times EBW^{0.1333}$. Para gestação, as seguintes predições foram proposta: Ca $Ca\ req_{preg}\ (g/d) = 0.0125\ e^{(0.02489 \times DP)}$; P $P\ req_{preg}\ (g/d) = 0.0207\ e^{(0.02049 \times DP)}$; Na $Na\ req_{preg}\ (g/d) = 0.0588\ e^{(0.0115 \times Days\ of\ pregnancy)}$, K foi $K\ req_{preg}\ (g/d) = 0.0439\ e^{(0.0119 \times Days\ of\ pregnancy)}$, predição para magnésio foi $Mg\ req_{preg}\ (g/d) = 0.0021\ e^{(0.01748 \times Days\ of\ pregnancy)}$ e S $S\ req_{preg}\ (g/d) = 0.0263\ e^{(0.0148 \times Days\ of\ pregnancy)}$.

Palavras-chave: girolando; gestação; gado leiteiro; exigências nutricionais; modelos matemáticos.

SUMMARY

LITERATURE REVIEW

Macrominerals: metabolic and physiological functions

Minerals have been described with five major functions in the body: structural, physiological, catalytic, regulatory, and immunological (Suttle, 2010; Valadares Filho et al., 2016; Wilson et al., 2016) Macrominerals refer to inorganic compounds required by animals in relatively large quantity when compared to other inorganic compounds, all of them is required by animals in grams per day (NRC, 2001). Each macromineral plays a specific role in the metabolism, which is involved closed with health status and metabolic efficiency of utilization of nutrients by the tissues. Each macromineral are described based on their function below.

Calcium

Calcium is the major mineral found in the body composition mainly in the skeleton where contains 99% of all calcium found in the farm animal species. Thus, only 1% of the body calcium is readily available in the extracellular fluids for metabolic functions such as nerve conduction, muscle contraction, and cell signaling. Additionally, calcium is more emphasized for dairy cow research due to the high calcium content in the milk (Weiss, 2017).

Based on the crucial importance of calcium in the metabolism, the serum calcium concentration is support by a complex and many pathways where they kept the calcium concentration in the blood nearly 9.0 through 10.0 mg/dL for dairy cows (Goff, 2018). The main calcium source of the cattle body comes from the diet and bones. Calcium absorption in the digestive tract can be both passive and active transport. However, passive absorption occurs only when the concentration ratio between digestive content and the apical plasma membrane of the enterocytes is greater than 6 mM (Bronner, 1987). According to Goff and Horst (1993), passive absorption only occurs in calves fed milk, and cows submitted to calcium drenches at post-calving to prevent metabolic disorders.

The active calcium absorption through the intestine is the main pathway to the calcium reaches the blood. There is a specific hormone to regulate the calcium absorption called 1,25 – dihydroxy vitamin D, and this hormone is derived from Vitamin D where it is submitted into two processes in the liver and kidneys to become the active hormone in the metabolism (NRC, 2005). The activation process of vitamin D is controlled by other hormones called parathyroid hormone, which is secreted by the parathyroid gland and it is the key regulator for serum calcium homeostasis and works in the kidneys to increase the 1,25 – dihydroxyvitamin D synthesis. The 1,25 – dihydroxyvitamin D increases the synthesis of calcium transport protein in the erythrocytes to enhance the transcellular calcium uptake by the intestine cell (Goff, 2018).

Furthermore, the parathyroid hormone is an activator of the reabsorption of the calcium storage in the bone when the calcium absorption from the digestive content is not at a satisfactory amount to keep adequate serum calcium. It is evident at the post-calving period when the calcium requirement for milk synthesis in the mammary gland is high and the dry matter intake is still low to supply calcium demand by cow. Consequently, the potential occurrence of milk fever increases.

During pregnancy, a great amount of calcium is required to produce milk and keep the fetal growth in the uterus (Pitkin, 1975). In pregnant cows, there is an intensification of calcium mobilization to the fetus in the last third of the gestation period. The mechanism to keep homeostasis in the metabolism might be not just dependent on 1,25 – dihydroxyvitamin D, some studies have hypothesized that calcitriol is an important component to increase calcium uptake from the intestine by increasing calcium receptors in the erythrocyte (Halloran and DeLuca, 1980; Kovacs, 2001).

Phosphorus

Most of the phosphorus content in the body is retained in the skeleton and teeth in phosphate anion, 80% of the phosphorus are found in those body components, and the other 20% are contained in body fluids and soft tissues (NRC, 2001). In quantity, phosphorus is the second most abundant mineral in the body, and it is involved in many physiological roles in the metabolism, such as: energy utilization by AMP, ADP and ATP, protein synthesis, and fatty acid transportation. Additionally, structural functions are described for phosphorus, for example, cellular membranes are composed of phospholipids and the bone matrix is dependent on phosphorus to be synthesized (Suttle, 2010).

Ruminal microorganisms need phosphorus supply to grow because it has associated function in different microbe metabolism as degrading cellulose, protein synthesis, and structural component synthesis (NRC, 2001). The sources of phosphorus to the rumen come from the diet and salivary recycling. Phytate is available to the ruminants because microorganisms in the rumen have enzymes to degrade phytate-bound phosphates and it becomes free to be uptake by the animal.

The great amount of phosphorus absorption in cattle occurs in the small intestine. Specifically, it is a more evident occurrence in the duodenum and jejunum (Care et al., 1980; Scott et al., 1985). The absorption may happen by two different processes: passive and active mechanisms. Passive absorption is the main process in the erythrocytes. When a minimal concentration of phosphorus occurs in the small intestine, active transport is done via a cotransport with sodium in the apical membrane (Goff, 2018).

Phosphorus is a great concern for environmental pollution. Thus, a variety of opportunities is to develop studies to improve phosphorus utilization by animals. Understanding the recycling process and absorption of different sources of phosphorus in the diet could increase the efficiency of use by animals, so decreasing phosphorus excretion in the soil (Wu et al., 2001).

Studies about phosphorus during gestation is limited, and more knowledge is interesting to understand its importance. Some studies showed that phosphorus supplementation in the last third of gestation is important because this period is the greatest development of the body fetus (NRC, 2005). However, the levels of inclusion should be considerer to avoid decreasing magnesium and calcium absorption, which increases the occurrence of hypomagnesemia and milk fever in the post-calving period (Reinhardt and Conrad, 1980; Schonewille et al., 1994).

Magnesium

Since 1930, the effects on the health of using magnesium in the diet have been described. Because of magnesium deficiency in grazing cows, convulsions were observed, which was identified as a disorder called grass tetany (Sjollema and Seekles, 1930; Suttle, 2010)

Magnesium is a major intracellular cation. It plays many functions in several physiological processes and metabolic pathways in the organism. It is involved in oxidative phosphorylation on

ATP formation, pyruvate oxidation, synthesis of Succinyl CoA, β-oxidation of fatty acids, pentose monophosphate transketolase reaction (Shils, 1997). Further, magnesium has function on Na^+/K^+ pump, bone formation, nerve conduction, muscle contraction, ribonucleotide folding phospholipid linkage in the cell membrane (Ebel and Günther, 1980; Shils, 1997; Suttle, 2010).

Rumen microorganisms have a demand for magnesium for cellular functions and growth. Magnesium deficiency in the rumen reduces the efficiency of fiber degradability via bacterial fermentation (Underwood and Suttle, 1999; Suttle, 2010).

A close relationship with other minerals is evident. Low intake of magnesium could increase toxicoses risk by aluminum (Nielsen et al., 1988). Also, a high intake of magnesium increases the risk of milk fever occurrence at post-calving because as magnesium it is a cation, the parathyroid hormone signals for calcium resorption from the bone is low(NRC, 2005; Goff, 2018).

The absorption of magnesium changes through the life of a ruminant. Before the complete development of the gastrointestinal tract, magnesium absorption occurs mainly in the small intestine. Over time, rumen and reticulum become the key locations of magnesium absorption (Gäbel and Martens, 1986). The active process is the main role of magnesium absorption, and passive absorption occurs when the ionized magnesium concentration is greater in the intestinal lumen than in the bloodstream (Cragle, 1973; Goff, 2018).

The kidneys are the main organs related to the control of magnesium concentration in the blood. They increase the excretion of magnesium via urine when the intake is greater than the animal demand. However, when the intake is required by cattle in low quantity, there is not endocrinological control to mobilize magnesium from the bone, or to increase the absorption in the gastrointestinal tract, thus the animal should be fed with a daily amount of magnesium in the diet (NRC, 2001, 2005).

Sodium

Sodium is the main ion in the extracellular fluid, and it has an important function on osmotic homeostasis because it is intimately involved with water in the body. Researchers have shown the association of water and sodium by increasing the sodium intake, consequently, the water intake increases as well (Suttle and Field, 1967). The Na^+/K^+ pump is the key factor to keep the osmotic condition in the metabolism, where it spends energy to move sodium ions from the inner to the outer part of the cell. Additionally, sodium gradient is important to keep constant the nutrient Na^+-associated transportation to the cytoplasm via gradient of concentration of sodium, such as glucose and amino acids.

Because of low sodium concentration in the diet in natural habitat, ruminants, and other mammals have developed efficient mechanisms to absorb sodium. Passive diffusion of sodium does not occur easily because of the high sodium concentration in the interstitial space; thus, the passive transport does not flow naturally through the paracellular region. Therefore, the major sodium transportation occurs via active transport. In ruminants, sodium absorption occurs via several pathways, and some of them just occur in specific sections of gastrointestinal tracts, such as Na^+/AA symporters in duodenum and jejunum; Na^+ channels on colon epithelium; Na^+/HCO_3 cotransporter in ileum and colon; Cl^-/OH^- linkage to sodium in the rumen, small and large intestine cells, and Na^+/H^+ exchange in the entire of the gastrointestinal tract (Goff, 2018).

Hormones play an important regulatory function of osmolality. The increased formation of angiotensins by renin associated to the vasopressin can active aldosterone activity, which controls the water balance and sodium excretion via the kidneys, controlling the reabsorption in the distal tubule (Suttle, 2010). Additionally, ruminants may conserve sodium via the salivary exchange of sodium for potassium ions (Blair-West et al., 1963).

Signals of sodium deficiency are not evident because they could happen because of many other reasons. However, some conditions increase the risk of sodium deficiency, for example, infections in the gastrointestinal tract could cause diarrhea, which increases the sodium concentration in the feces. Other examples are the sodium losses via sweating when the animal is submitted to hot and dry climates, and diets with low-sodium ingredients. In summary, sodium could be in deficit to animals by dietary and climatic causes, and disease occurrences.

Potassium

In animal metabolism, potassium has been studied since the early 19th century (Suttle, 2010). It is the third most abundant macromineral in the body tissues, it is found in high concentration in soft tissues and muscles. As well known, potassium is the main intracellular ion presented, and it is fundamental to create both chemical and electrical gradient to guarantee a range of mechanisms in the cell(NRC, 2005).

Although potassium is required quantitatively more than other minerals by cattle, its supply by forages is enough to meet the animals' demand in most cases. Additionally, the absorption is high throughout the gastrointestinal tract. Therefore, concerns on potassium are more related to excessive intake than deficiency because it has a high capacity to interact with other minerals, such as calcium, magnesium, sodium, and chloride.

Potassium has an importance on a variety of functions to keep homeostasis, such as osmotic pressure, acid-base balance, water preservation, membrane potential, neural impulse, and muscle contraction (Ward, 1966; Goff, 2018).

Simple paracellular diffusion the main mechanism involved in potassium absorption. The high concentration of potassium in the diet and a low concentration in the extracellular fluid. Thus, the potassium flow from the gastrointestinal lumen through the bloodstream is favorable by the gradient. It occurs mainly at the end of the small intestine because the water removing before this section increases the potassium concentration in the chime. Besides, some transcellular transport of potassium may occur in the rumen, abomasum, small, and large intestine, but this mechanism does not absorb too much potassium. Kidneys play an important role in potassium regulation. By signaling from aldosterone, where it stimulates the reabsorption of sodium via an exchange of potassium (Gleeson, 1992; Kronshage and Leonhard-Marek, 2009; Goff, 2018).

Sulfur

Many organic compounds in the body have sulfur in their composition, such as amino acids, vitamins, specific hormones, heparin, fibrinogen, cartilage, etc. Sulfur is essential to microbial nutrition in the rumen for the synthesis of sulfur-containing molecules, which is the main sulfur substrate for the absorption by the animal in the intestine. In other words, sulfur is important for microbial growth in the rumen, then the microbes are the main source of essential organic sulfur-compounds for the host animal, such as methionine, cysteine, biotin (Underwood and Suttle, 1999; NRC, 2001, 2005; Valadares Filho et al., 2016; Goff, 2018).

Because of the microorganism in the rumen, sulfur is converted into sulfide by specific bacteria who use sulfur as an electron acceptor in the metabolic processes. Other groups of bacteria could uptake available sulfides to produce proteins or it might be metabolized by the animal, converting sulfide into sulfate in the liver. Otherwise, hydrogen sulfide or sulfur dioxide can be formed and released from the rumen by eructation (Dougherty et al., 1965; Gould et al., 1997; Loneragan et al., 1998; NRC, 2005).

Sulfur-containing compounds can be absorbed in the small intestine wall efficiently. Sulfide and sulfate are the main sulfur-compounds absorbed by the ruminants (Bouchard and Conrad, 1973). The transport of sulfate to intracellular fluid is accomplished in the basolateral membrane, where 2 chloride ions or carbonic acid are exchanged for sulfate (Markovich, 2001).

Sulfide has a high potential to decrease the absorption of other minerals, such as copper and selenium. Furthermore, hydrogen sulfide gas has a high potential to cause toxicity to the animal. This gas is not absorbed in the rumen, and the eructation is the main way to release this toxic gas. However, some research showed that the animal can inhale hydrogen sulfide after eructation, which causes the intoxication of animals. Specific lesions in the brain and failure of the central nervous system are the signals of hydrogen sulfide toxicity (Coghlin, 1944; McAllister et al., 1997; NRC, 2005).

The excretion of sulfur is mainly accomplished by urine. The catabolism of sulfur amino acids and other sulfur-molecules in the body releases are oxidized to sulfate, which reaches the kidneys to be excreted (NRC, 2005; Suttle, 2010).

Sulfur deprivation does not have specific signs in the ruminants. Reduction of feed intake, decreasing fiber degradation, spending more time ruminating, and low body weight gain might be changes caused by sulfur deficiency (Kennedy, 1974; Hegarty et al., 1994; NRC, 2001; Goff, 2018).

Physiological adaptation of cows throughout pregnancy and fetus development

Both cows and fetuses are affected by pregnancy *status.* Studies have described a variety of changes throughout the gestation period. In cows, dry matter intake is the main described change during the pregnancy. Especially at the last third of pregnancy, where there is an exponential growth rate of the fetus, associated with many hormonal modifications, mainly by the high concentration of estrogen, affect the DMI (Forbes, 1986; NRC, 2001; Bertoni and Trevisi, 2013). According to (Rotta et al., 2015b) , the dry matter intake decreases approximately 40% from 150 through 262 days of gestation. Thus, diets for pregnant cows should be formulated carefully, it means a more concentrated ration, to supply cow and fetus demand for nutrients.

Several studies showed many issues involved in reduced or excessive intake of nutrients by pregnant cows and serious losses both to cow and fetus. Sen et al. (2016) studied the differences of gestation components when cows are fed below and over nutritional demand at periconception. The trial described effects on gravid uterus weight, fetal weight, fetal membrane weights, total placentome numbers.

One of the first steps in the gestation process is the development of embryo-uteroplacental circulation, this process is involved closely in fetal growth in the first half of pregnancy (Funston et al., 2010). Undernutrition at early pregnancy reduces the cotyledonary weights and surface areas of placentome (Long et al., 2009), which could impair the nutrient flux to the fetus. Persistent undernutrition of pregnant cows through the second-third of gestation could reduce muscle mass, and changes in the ovarian structure, which might increase the culling risk for replacement heifers (Long et al., 2012). Zhu et al. (2007) did not find differences in body weight in newborns when maternal nutrition was restricted in the first trimester of gestation. However, the liver and pancreas of newborns are affected to metabolize nutrients. When glucose homeostasis is not kept in cow's blood, the fetus is restricted to glucose uptake, as a result, the fetus' endocrine system could change its sensibility, increasing the risk of insulin resistance and lipid accumulation (Symonds et al., 2010). Changes in endocrine responses of the liver and pancreas could be impactful consequences on the capacity to metabolize body fat after parturition and the occurrence of metabolic disorders (Schoonmaker, 2014).

On the other hand, observed some differences in gene expression and placenta size when cows are feeding over the nutritional demand. High nutrient intake by pregnant Holstein-Gyr crossbred cows presented smaller placenta, cotyledon, caruncle, and placentae related to empty body weight. Additionally, high intake decreased the expression of *IGFR1* and *IGFR2*, indicating effects on nutrient uptake by the fetus from the cow's blood. Those results are in agreement with is described by Caton et al. (2007), where they found that over-nutrition affected placental and fetal development.

Moreover, Weller et al. (2016) found observed modifications in the reproductive system both in male and female fetuses when pregnant cows are fed over the nutrient demand. In female fetuses, the gene expression of follicle-stimulating hormone (FSH) is increased when cows are feeding over the requirement. Consequently, antral follicles number increased, and greater cellular apoptosis was identified in those fetuses. Based on those studies, the nutrient requirements of pregnant cows must be calculated carefully to ensure correct body condition score for cows, and fetus growth adequately.

Based on how the balanced nutrient intake by cows is important both for cows and fetus, some considerations pregnancy period should be studied closely. Although, the last third of the gestation period is the most impactful on fetal development, where exponential growth is more evident (Ferrell et al., 1976). Information about initial and mid-pregnancy is scarce. Recently, some studies on energy and protein requirements throughout pregnancy have been published. Sguizzato et al. (2020) and Provazi (unpublished) have studied the energy and protein requirements of

Holstein - Gyr crossbred pregnant cows. Both studies have shown some differences about the beginning of energy and protein requirements for pregnancy when they are compared to the recommendation described by NRC (2001). Sguizzato et al. (2020) claim the initial point of energy requirement for pregnant cows is 70 days. For protein requirement of pregnancy, Provazi (unpublished) did not mention an initial point during pregnancy for protein requirement, but this study pointed to the differences in protein deposition mainly in the mammary gland.

For other nutrients, more information is needed to get further about the nutrient requirement of pregnant dairy cows. (NRC, 2001) considers the initial point of mineral requirements for gestation from 190 days. However, this recommendation is based only on one study, and the deposition of minerals in the fetus starts before the last third of gestation (Ramberg Jr., 1995). Therefore, more studies are needed to provide accurate predictions for pregnant dairy cows.

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CHAPTER 1: Macromineral requirements for maintenance and gain of pregnant and non-pregnant Holstein vs Gyr cows

Interpretative summary: By Camisa-Nova et al. Nutrient requirement approaches are essential to predict animal demand for any essential nutrient for metabolism. Macrominerals are inorganic compounds involved to many physiological and biochemical process in the body, where disbalanced diets may cause several economic productive losses for dairy facilities. Additionally, excessive excretion of minerals, such as: phosphorus may result in a~~;~~ great potential of soil contamination. Therefore, mathematical models to estimate macromineral demand are important to avoid pollution associated with dairy facility systems and formulate diet as precise as possible to support cow`s demand for major minerals.

Running Head: Macromineral requirements for dairy cows

Macromineral requirements for maintenance and gain of pregnant and non-pregnant Holstein vs Gyr cows

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ABSTRACT

This present study aimed to set approaches to predict the macromineral requirements for maintenance and weight gain of pregnant and non-pregnant Holstein x Gyr cows. Sixty-two cows were enrolled in this experiment. Randomly, animals were divided into 3 different groups: pregnant, open, and reference cows. Two subgroups were formed both for pregnant cows and open cow groups, where the first subgroup was submitted to *ad libitum* feed management and the second subgroup was submitted to the maintenance nutritional management (feed intake equals 1.15% of body weight). Pregnant cows were submitted to comparative slaughter throughout four different days of gestation: 140, 200, 240, and 270 days, as well as the open cows, were slaughtered at day 200, 240, and 270 of gestation of the pregnant group. Seven periods of 28 days were accomplished for total tract digestibility (122, 150, 178, 206, 234, and 262 days of gestation). Macrominerals were analyzed by specific methods as each one needs to be quantified. The net requirement for calcium, phosphorus, sodium, potassium, and magnesium were 13.48, 8.35, 10.06, 45.89, and 4.44 mg/kg of empty body weight (EBW), respectively. Additionally, the retained coefficient for calcium, phosphorus, sodium, potassium, and magnesium were 0.6516, 0.7402, 0.4267, 0.5641 and 0.4211, respectively. The net requirement for gain showed statistical differences between pregnant and open cows for calcium and phosphorus. On the other hand, sodium, potassium, and magnesium did not present differences for gain between pregnant and non-pregnant cows. However, body mass was different for pregnant and non-pregnant cows. Consequently, dietary requirements for pregnant and non-pregnant cows should be calculated separately. Further, retention coefficient should be considered for all minerals to estimate true retention.

Key words: dairy cattle, mathematical modeling, minerals, nutrient requirement, pregnancy

INTRODUCTION

The importance of inorganic compounds for the organisms has been demonstrated since the 17th century (Goldwhite and Adams, 1970). From 1919, research related to mineral requirements for dairy cattle has been performed to meet the correct amount to feed the cows. However, studies on mineral requirements do not advance as quickly as other nutrients have been explored. Weiss

(2017) enhanced that mineral research is limiting because of several factors related to: (1) The measurement by current methods is difficult, and (2) the trials are much expensive because it needs long experimental periods and a greater number of animals.

Several traditional nutrient requirement systems (AFRC, 1991; NRC, 2001; CSIRO, 2007; INRA et al., 2018) are available to predict nutrient demand of dairy cattle. However, these systems were developed based on dataset with high predominance of the *Bos taurus*, what is a limitation for tropical regions where the predominance of *Bos indicus* and *Bos indicus* × *Bos taurus* crossbred cattle are high. Consequently, several scientific studies focusing on the efficiency and sustainability of milk production under tropical climate have increased significantly in recent years. (Miranda and Freitas, 2009; Oosting et al., 2014; Tedeschi et al., 2017; Hernández-Castellano et al., 2019).

Minerals are essential to maintain healthy, high reproductive efficiency and milk yield (NRC, 2001). However, the mineral requirement is influenced by several factors, such as: physiological stage, bioavailability of the mineral source, and interaction among the minerals (NASEM, 2016; Valadares Filho et al., 2016; Tedeschi and Fox, 2018).

Additionally, the dataset to estimate the mineral requirements throughout the pregnancy of dairy cows is limited, and it is a critical stage where nutritional failures may influence straightly health status and performance on both fetus and cows. Several studies have illustrated the deleterious effect on fetal development both feed restriction and over-nutrition during pregnancy of cows(Long et al., 2009; Rotta et al., 2015a; b; c; Weller et al., 2016). Moreover, deleterious effects caused by disbalanced nutrient intake by cows could also affect lactation because it would increase the risk of metabolic diseases in the post-calving period (Dann, 2017).

Therefore, the present study aimed to estimate the macromineral requirements for maintenance and gain of crossbred Holstein × Gyr cows. Then, dietary requirements were proposed for pregnant and non-pregnant cows.

MATERIAL AND METHODS

The The trial was conducted at Universidade Federal de Viçosa, MG, Brazil. Procedures and sampling executed in this study were evaluated and approved by the animal care and handling committee (CEUAP/UFV – 47/2012).

The experiment was arranged with sixty-two Holstein × Gyr cows, where cows were grouped into 3 different groups randomly: baseline group (n=6), non-pregnant (n=12), and pregnant (n=44). One cow aborted during the study, and it was removed from the experiment. Consequently, only 43 cows were submitted to statistical modeling.

Each animal in the trial was kept in individual pens (30 m²), where an individual feed bunker and automatic water system were used to control feed and water intake. All cows were fed twice a day with a diet based on corn silage and concentrate at a ratio of 93:7 adjusted on a DM basis (Table 1). The diet formulation was performed according to the recommendation provided by NRC (2001). Feed intake was adjusted before the first feeding daily.

Feeding management was set to result in 5% of orts based on fresh feed. Both pregnant and non-pregnant cows were divided into two subgroups, where: the first subgroup was fed *ad libitum*, and the second subgroup was fed restrictedly to ensure a low ADG, about 0.1 kg. Thus, the feed intake for this subgroup was set at 1.15% of BW.

Comparative Slaughter Method and Sampling

Slaughter procedure is applied to quantify the body composition to estimate net requirements. The baseline group was slaughtered at the beginning of the experiment to estimate the initial concentration of each body component. Both non-pregnant and pregnant cows were slaughtered according to the gestation time. The animals included in the pregnant group were slaughtered at 140, 200, 240, and 270 d of gestation and the non-pregnant group were slaughtered at: 200, 240, and 270 d of feedlot. Non-pregnant cows were not slaughtered at d-140 of gestation because it was considered an early gestation time for differences on pregnancy components.

Cows were fasted 16 h before the slaughter procedures to obtain the shrunk body weight (**SBW**) from each animal. The slaughter of cows was executed via captive bolt stunner followed by exsanguination. Then, the carcass was divided into two parts, and the left section was sampled to measure mineral content. Non-carcass components (gastrointestinal tract, organs, head, tail, hooves, trimmings, leather, and blood) were ground, mixed, and homogenized to obtain a composite sample to be submitted for quantification of micromineral composition. The gastrointestinal content was removed to get the compartment structures of each compartment of the gastrointestinal tract.

Digestibility Estimation

Six periods of feces and urine samplings were accomplished to estimate apparent digestibility, each sampling period was adjusted to 28 d (122, 150, 178, 206, 234, and 262 d of gestation). The fecal sample collection was performed by the spot collection method, where fecal samples from all cows were taken during the last 5 d of each 28-d sampling period at 0600, 0900, 1200, 1500, and 1800 h. Samples were dried, ground, and homogenized to get composite fecal samples by each animal by each collection for further laboratory analysis.

Urine samples were collected on d-1 and 4 of each 28-d sampling period at 0600 and 1500 h. The urine samples were collected via stimulation of the vulva and diluted into sulfuric acid to avoid NH_3 volatilization. Then, urine samples were frozen at -20ºC for further analyses of creatinine.

Laboratory Analyses

Corn silage, concentrate ingredients, feces, urine, and the cow body components (carcass, non-carcass, fetus, placenta, mammary gland, fetal fluids, and uterus) were submitted to DM (method 934.01, AOAC, 2000) and macromineral (Ca, P, Mg, K, Na, and S) analyses.

Fecal excretion was estimated by iNDF as an internal marker. All feed and fecal samples were incubated in the rumen throughout 288 h in two cannulated bull supplied a diet based on a 50:50 ratio at maintenance level. Daily urine excretion was quantified based on the creatinine concentration in the urine samples. The creatinine excretion was estimated for equation (1) as described by Costa e Silva et al. (2013):

$$CE = 0.0345 \times BW^{0.9491} \qquad [1]$$

where CE is the daily creatinine excretion and BW means body weight (kg).

Macrominerals were analyzed by specific methods as each one needs to be measured. Calcium and magnesium were estimated by the lanthanum dilution method (Korn et al., 2013). Sodium and potassium were analyzed by flame emission spectrometry procedure (reference). Phosphorous was analyzed according to the method described by Detmann et al. (2012), realized by the colorimetric reaction.

Calculations

The net requirement for maintenance was calculated based on the difference between the input (mineral intake) and output (mineral content in the urine and feces) to get the retained mineral in the body. Then, a linear regression applying for intake and retained mineral was set up to estimate the truly retained coefficient and the net requirement for maintenance by equation 2:

$$RM = \beta_0 + \beta_1 \times MC + \varepsilon_i \qquad [2]$$

where: RM is the retained mineral; MC is the mineral intake by the animal; β_0 is the net requirement for the maintenance of macrominerals; β_1 is the true retention coefficient; ε_i residual effect.

For growth, a non-linear regression was set as a function of mineral content in the carcass and non-carcass components related to the EBW (equation 3). Then, the net requirement for growth was obtained by the sum of EBG, and the first derivative of equation 3 (Equation 4).

$$RM_{growth} = \beta_0 \times EBW^{\beta_1} \quad [3]$$

where: RM_{growth} (g) is the retained mineral in the carcass and non-carcass compounds; EBW (kg) is the empty body weight; β_0 is the intercept; β_1 is the regression slope; ε_i residual effect.

$$Net\ Requirement_{growth} = \beta_0 \times \beta_1 \times EBW^{\beta_1 - 1} \times EBG \quad [4]$$

where: $Net\ Requirement_{growth}$ is the net mineral requirement for body weight gain; EBW is empty body weight; β_0 is the net requirement for the maintenance of macrominerals; β_1 is the regression slope; ε_i residual effect.

Dietary requirements for each macromineral were calculated via the sum of both net maintenance and net growth requirements divided by the retention coefficient, as described in equation 5.

$$Dietary\ requirements_M = \frac{Net\ requirement_{Maintenance} + Net\ requirement_{Growth}}{Retention\ Coefficient} \quad [5]$$

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

Statistical Procedures

The models of all macrominerals for maintenance requirements were tested by PROC MIXED Procedure of SAS software (SAS Institute Inc., 2013) version 9.4; SAS Institute Inc., Cary, NC, USA) to evaluate differences between pregnant and non-pregnant cows both for β_0 and β_1 estimated parameters. Parameters of macromineral retention for weight gain were estimated by NLIN Procedures of SAS Software (SAS Institute Inc., 2013) version 9.4; SAS Institute Inc., Cary, NC, USA), and differences between parameters estimated both for pregnant and non-pregnant cows were tested by PROC NLMIXED procedure of SAS Software (SAS Institute Inc., 2013) version 9.4; SAS Institute Inc., Cary, NC, USA). The level of $P \leq 0.05$ was used for all tests as the critical probability level for the occurrence of type I error.

RESULTS AND DISCUSSION

Conversion of EBW into SBW and ADG and EBG

Dataset related to BW and ADG is described in Table 1. Pregnancy showed effect on body mass conversion (P < 0,001). Thus, regressions to convert EBW into SBW and EBG into ADG were different for pregnant and non-pregnant. The models for non-pregnant cows were described as:

$$EBW_{NP} = 0.998_{\pm 0.0594} \times SBW \qquad [6]$$

($r^2 = 0.9657$; RMSE = 69.95)

$$EBG_{NP} = 0.9306_{\pm 0.0483} \times ADG \quad [7]$$

($r^2 = 0.9737$; RMSE = 0.1016)

where EBW and BW are expressed in kilograms and kilograms per day, respectively. The intercept was not significative for the models 6 and 7 ($P = 0.0608$ and $P = 0.0892$ respectively). Therefore, models were set without the intercept. For pregnant cows, the models to convert EBW into SBW and EBG into ADG were:

$$EBW_{preg} = 0.924_{\pm 0.0288} \times SBW \quad [8]$$

($r^2 = 0.9625$; RMSE = 31.68)

$$EBG_{preg} = 0.9849_{\pm 0.0387} \times ADG \quad [9]$$

($r^2 = 0.9418$; RMSE = 0.1167)

where EBW and BW are expressed in kilograms and kilograms per day, respectively. The intercept was not significative for the models 8 and 9 ($P = 0.0822$; $P = 0.1653$), thus the models were arranged without intercept.

Prediction of body mass based on EBW and EBG are more satisfactory to predict nutrient requirements (Valadares Filho et al., 2016). As the predictions in this study was built related to the body mass, systematic error is increased when the model includes gastrointestinal content (Owens et al., 1995; Chizzotti et al., 2008). Thus, nutrient requirement models estimated by comparative slaughter exclude gastrointestinal content as part of body composition.

Homeorheric changes in consequence of pregnancy condition is described in several species. In growing animals, studies claim that pregnancy stimulates the deposition in maternal tissues (Salmon-Legagneur, 1965; Heap and Lodge, 1967; Robinson, 1986). Sguizzato et al., (2020a) showed difference in maternal tissues throughout gestation in mature cows, where the authors found lower weight of carcass and non-carcass in the last third of gestation than in the first stage of gestation. Thus, distinct models to describe body mass of non-pregnant and pregnant cows agree with information in the literature.

Intake, excretion, and retention were similar in pregnant and non-pregnant groups for all macrominerals (Table 3). It was evident in the results related to net requirement for maintenance of all macrominerals (Table 4), where the parameters did not present differences between pregnant and non-pregnant groups ($P > 0.05$). Therefore, the net requirements for maintenance of both groups were considered the same value.

Net Requirements of Macrominerals for Maintenance

The approaches developed in this study are the first predictions developed for dairy cows in tropical climate. Additionally, the first models considering macrominerals before 190 d of pregnancy. Net requirement for maintenance for all macrominerals was estimated based on the mineral balance, which the estimated intercept (β_0) in the linear regression from the relationship between macromineral intake and retention represents the value for net requirement for maintenance (Figure 1). For maintenance, there are not effects in consequence of pregnancy for any mineral (Table 4).

The net requirement for maintenance of Ca, P, Mg, K, Na, and S were 13.48, 8.35, 4.44, 45.89, 10.08, and 7.82 mg/kg of EBW, respectively (Table 4; Figure 1). Additionally, the predicted slope of the same regression (β_1) implies the true retention coefficient for each mineral (Table 3).

Mineral balance of all macrominerals showed some considerable excretion for all macrominerals in urine (Table 3). In this study, calcium had the lowest excretion via urine when compared with other minerals. However, calcium concentration in the urine corresponds around 9.50% of the total excretion of this mineral. On the other hand, potassium concentration in the urine composition was 81.61% of the total excretion. Based on it, the retention coefficient is suitable to estimate the dietetic requirement for each macromineral. (Costa e Silva et al., 2015) described the same pattern of excretion for macrominerals, where the authors argue that the retention coefficient is more reliable to predict retention by mineral balance methodology.

NRC (2001) recommends the utilization of absorption coefficient to estimate the availability of minerals for tissues, where it quantifies only the minerals in the feces as mineral excretion. For example, NRC (2001) considered 100% of the potassium is absorbed by the animal (Table 5). However, the most portion of excreted potassium is in the urine (Ward, 1966). Therefore, estimations of true available macrominerals to the metabolism should be estimated based on retention coefficient for macrominerals.

Calcium is the most abundant mineral in the body and the most abundant component in bone structure (Underwood and Suttle, 1999). Further, serum calcium concentration in homeostasis condition is between 9.0 and 10.0 mg/dL (Goff, 2018). Hence, the net requirement of calcium for maintenance is essential to keep normal level in the bloodstream. In this study, net Ca for maintenance was 13.5 mg/kg of SBW for non-pregnant cows and 12.5 mg/kg of SBW for pregnant cows. Both values for non-pregnant and pregnant cows are lower than the recommendation of

(NRC, 2001; 5.4 mg/kg of BW) and INRA (2018; 15.0 mg/kg BW per day), but greater than the recommendation from Valadares Filho et al. (2016; 11.7 mg/kg BW). In this study, all the animals are Holstein vs. Gyr crossbred, mature cows, and rearing under tropical climate. Thus, effects based on climate and genetics might be the main reason to vary the values of calcium requirements for maintenance.

Retention coefficient for calcium in this study was 65% (Table 3). NRC (2001) and AFRC (1991) considered absorption coefficient equals 70% and 68%, respectively. Considering retention coefficient, Valadares Filho et al. (2016)recommends 56.8% for calcium. Calcium excretion via urine does not vary as much as in feces (Goff and Horst, 1993). Therefore, close values of recommended absorption coefficient and retention coefficient in this study for calcium could be explained by the low variability in urine composition.

P is the second most abundant mineral in the body, where main tissue to store it is the bones (Suttle, 2010). The net requirement of P for maintenance in this study was 8.3 mg/kg SBW for non-pregnant cows, and 7.7 mg/kg SBW for pregnant cows. These values were lower than requirement systems. Valadares Filho et al. (2016) recommend 13.5 mg/kg BW of phosphorus, and ARC (1980) and NRC (2001) recommend 12.0 mg/kg BW and 16.0 mg/kg BW, respectively. Cows in this study had reached the mature body size, what implies that the variation in bone structure is low (Shahin and Berg, 1985). Consequently, the needs of P for metabolism are reduced. Probably, it was the main reason for reduced net P for maintenance in this study.

P coefficient of retention was set in this study in 74.2% (Table 3). Valadares Filho et al. (2016) found retention coefficient of P equals 67.8%. When we look at the systems considering absorption coefficients, ARC (1980), AFRC (1991), and NRC (2001) predicted 60%, 58%, and 80% for P, respectively. As described for Ca, feces are the main route of P excretion in ruminants.

Additionally, the main reason for a variety in P requirements is the phosphorus recycling, where it could change depending on the phosphorus concentration in the diet, under or overestimating phosphorus value (Suttle, 2010) Furthermore, the main source of P in the diet was dicalcium phosphate, which shows a high absorption in the gastrointestinal tract. These reasons might explain the differences among all coefficients describe in the literature and in this study (Table 6).

Magnesium requirements are important to avoid hypomagnesaemia, especially in pregnant cows. Additionally, tropical pastures are poor in Mg bio availability, which increases the risk of hypomagnesaemia occurrence. Net requirements of Mg for maintenance are 4.05 mg/kg SBW for non-pregnant and 3.75 mg/kg SBW for pregnant cows (Table 3). These predictions are greater than NRC (2001) recommendation (3 mg/kg BW) and lower than Valadares Filho et al. (2016; 5.9 mg/kg BW). From BR-CORTE (2010), for zebu cattle the recommendation was 3.3 mg/kg BW. In this study, the results for Mg are close to the reference value presented by requirement systems.

Retention coefficient for Mg was set in 40% in this study. True retained magnesium estimated by Valadares Filho et al. (2016) was 35.5%, and from NRC (2001) was 17%. Predicted coefficient of retained magnesium is greater than traditional systems, even NRC (2001) excluded urine excretion. The possible reason to find greater values of retention coefficient is the climate condition. Castro et al. (2019) reported the same evidence comparing Holstein and Holstein vs. Gyr calves. In consequence of warm climate, changes in magnesium balance occur (Sanchez et al., 1994), which could be an adaptation of cattle rearing in tropical conditions. Further information is needed to obtain cleared evidence on it.

Potassium is abundant in ruminant diets because forages have high K concentration in their composition, and interactions among K and other minerals are an issue. For example, potassium is one of the major minerals that should be reduced when cows need to be fed with negative DCAD

(Goff, 2018). Thus, the excess of K is the main concern rather than for other minerals where the deficiency is the problem (Suttle, 2010).

In this study, net requirements of K for maintenance were 45.8 mg/kg SBW and 42.4 mg/kg SBW for non- pregnant and pregnant cows, respectively. Those values are close to the prediction from NRC (2001), which they recommend 38.0 mg/kg BW. The differences between this study and NRC (2001) could be explained by the different climates and breed. NRC (2001) was developed based information from dairy herds in temperate climate regions, instead of warmer regions where this study was performed. Swearing and urine are important rout of K excretion in the body. Higher temperatures increase the losses via sweat (Ward, 1966; Underwood and Suttle, 1999; Suttle, 2010). Additionally, *Bos indicus* cattle releases more potassium in sweat than *Bos taurus* (Johnson, 1970). As the animals was *Bos indicus* vs *Bos taurus* crossbred, It could explain the greater net potassium for maintenance in this study.

The retention coefficient for K proposed by this study was 56.4%. It was quite greater than the estimation used by Valadares Filho et al. (2016) (48.4%). NRC (2001) considered only K excretion by feces, and the major excretion of potassium in this study is by urine, what meets by Costa e Silva et al., (2015). Consequently, considering the absorption coefficient for K is not the best coefficient to predict dietary requirement of K because an overestimation for retained potassium will be counted.

Sodium is an important cation on osmotic pressure, and water intake and flux. In this study the recommendation of sodium for net requirements for maintenance of non-pregnant and pregnant cows were 10.1 mg/kg SBW and 9.3 mg/kg SBW, respectively. Both values are greater than the recommendation from (ARC, 1980) and Valadares Filho et al., (2016), where they recommended 6.8 and 6.3 mg/kg BW, respectively. On the other hand, sodium for maintenance in this study are lower than the recommendation from NRC (2001; 15.0 mg/kg BW). The lower sodium requirements in this study for dairy cattle is probably based on the Na:K balance. The high potassium intake may increase the sodium excretion via urine. Hu and Kung (2009) reported a linear increase of Na concentration in the urine when the Na:K ration increases. Probably, it could be an effect caused by this balance on Na excretion.

The retention coefficient for sodium in this study was 56.4% (table 3). It is greater than the recommendation by Valadares Filho et al. (2016). However, it is much lower than the (NRC, 2001) recommendation of 90%. As potassium, the main route of excretion of sodium is by the urine. It shows that the absorption coefficient overestimates the retention capacity of cows. It is clear on table 2, where we found more than one third of sodium excretion is via urine.

Sulfur is part of several amino acids and hormones in the metabolism. Further, it is the major component to get a negative DCAD in diets formulated to pre-calving cows. In this study, the prediction for net requirements of S for maintenance were 7.80 mg/kg SBW for non-pregnant cows and 7.23 mg/kg SBW for pregnant cows.This value was lower than the recommendation provided by Valadares Filho et al. (2016), where they found 10.4 mg/kg BW. Few studies are published evaluating sulfur requirements in the literature. NRC (2001) recommends for sulfur a fixed value of 2.0 g/kg DM. Approach developed based on comparative slaughter, Costa e Silva et al. (2015) found 9.4 mg/kg BW of S for maintenance of young Nellore cattle. It is quite greater than our prediction, but a lower prediction for mature cows makes sense because of the lower nutrient deposition. The retention coefficient for S was 85.0%, and it is greater than reported by Costa e Silva et al. (2015) and Valadares Filho et al. (2016), where they recommend 77.1% and 67.3%, respectively.

Net Requirement of Macrominerals for Weight Gain

Net requirement for BWG was obtained for non-pregnant and pregnant cows independently, then both regression models were compared for significant differences. Also, all the models were described considering the ADG equals to 1 kg.

For Ca, the model for gain of non-pregnant cows were: $Net\ Ca_{gain} = 0.2818 \times EBW^{0.6202}$; and prediction of pregnant cows were $Net\ Ca_{gain} = 3.1319 \times EBW^{0.1484}$. Based on the retention model, both β_0 and β_1 were different (β_0 – P = 0.0374; β_1 - P = 0.0006). Thus, the net requirement for gain should consider the pregnancy as a critical condition to calculate the Ca demanded by cows.

Net requirement of P for gain of non-pregnant cows were predicted as $Net\ P_{gain} = 0.7669 \times EBW^{0.3843}$; and for pregnant cows $Net\ P_{gain} = 2.4456 \times EBW^{0.1435}$. Both regression parameters of retention profile showed differences of P retention by non- pregnant and pregnant cows (β_0 - P = 0.0376; β_1 - P = 0.0136).

Sulfur retention for gain showed differences for non-pregnant and pregnant cows (β_0 - P = 0.0334; β_1 - P = 0.0062). Thereby, sulfur prediction for gain of non-pregnant cows was $Net\ S_{gain} = 0.1708 \times EBW^{0.5185}$, and S requirements for gain of pregnant cows was $Net\ S_{gain} = 1.2422 \times EBW^{0.1333}$.

Magnesium, potassium, and sodium retention for gain did not show statistical differences between non-pregnant and pregnant cows (magnesium: β_0 - P = 0.2675; β_1 - P = 0.0819. For gain, magnesium of non-pregnant and pregnant cows was $Net\ Mg_{gain} = 0.0508 \times EBW^{0.4163}$ and

$Net\ Mg_{gain} = 0.617 \times EBW^{-0.0700}$ respectively. Potassium for gain of non-pregnant cows was $Net\ K_{gain} = 0.2178 \times EBW^{0.3514}$ and for pregnant cows was $Net\ K_{gain} = 0.7269 \times EBW^{0.1191}$. For sodium, the prediction for gain of non-pregnant cows was $Net\ Na_{gain} = 0.0201 \times EBW^{0.7219}$, and the model of pregnant cows was $Net\ Na_{gain} = 0{,}1597 \times EBW^{0.3185}$

For all macrominerals for gain, the models related to body mass and mineral retention were adjusted by allometric equations, what met with Valadares Filho et al. (2016) showed the best adjustment for nutrients. Exception for magnesium for pregnant cows, all other macrominerals presented a positive slope in the regressions. It means that the mineral content increases when the body mass increases. It is suitable for the animals used in this study because they already had reached the mature weight (average body weight = 550 kg). Thus, the animals would rise body mass and macromineral retention proportionally. Another point is the lower values of slope for pregnant than for non-pregnant cows, it was expected in consequence of pregnancy. For fetus development, maternal tissue decreases the deposition to increase the flux of nutrient for fetal growth. Consequently, maternal tissues in pregnant cows show lower values of macromineral retention than non-pregnant cows. Negative slope value of magnesium for pregnant cows was close to zero, it means that the Mg deposition in maternal tissue does not vary widely by pregnancy. As a result of low Mg demand for metabolism and fetal growth. Therefore, the mobilization of magnesium from maternal tissue is almost unaltered.

All the macrominerals were set to predict the dietary mineral requirements, what consists by the sum of the net requirements for maintenance and gain divided by the true retention coefficient (Table 7). Because of the differences in retention pattern of macrominerals. The dietary requirements for non-pregnant and pregnant cows were calculated separately.

In summary, considering two cows, pregnant and non-pregnant, with BW = 550 kg and same ADG 1.0 kg. For pregnant cow, the dietary requirements for calcium, phosphorus, magnesium, sodium potassium, and sulfur would be 22.5, 12.7, 6.1, 14.7, 44.0, and 8.0 g/d, respectively. For non-pregnant cow would be 31.5, 17.1, 7.1, 17.1, 48.0, and 10.0 g/d. Comparing these two cows, the requirements of non-pregnant cows are greater because in this study we compared requirements for maintenance and gain without the gestational components for pregnant cows.

Comparing with NRC (2001) recommendations, those cows would be fed with 21.9 g/d of calcium, 17.4 g/d of phosphorus, 10.8 g/d of magnesium, 10.6 g/d of sodium, 49.8 g/d of potassium, 17.3 g/d of sulfur. For total macromineral requirements, the recommendations were close, only for sulfur the recommendation in this study was much lower. It happens because NRC (2001) considered a fixed model based on responsive-dose trial to recommend this mineral.

CONCLUSIONS

Body mass of pregnant and non-pregnant cows has different pattern of body composition. Consequently, dietary macromineral requirements should be calculated for pregnant and non-pregnant cows separately. Retention coefficient is more suitable to adjust mineral concentration in the diet because of the true excretion must consider urine composition for major of macrominerals. Requirement approaches from this study are the first in the literature evaluating pregnancy influence on macromineral requirement for maintenance and body weight gain, what would provide accuracy predictions to optimize nutrient utilization by pregnant and non-pregnant dairy cows.

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Table 1: Ingredients of diet supplied to both pregnant and open cow groups

Ingredients	**Diet**
Proportion (g/kg DM)	
Corn silage	930.0
Cotton meal	50.0
Limestone	5.0
Salt	5.0
Urea	9.0
Ammonium sulfate	1.0
Mineral mix*[1]	0.2
Chemical composition	
Dry matter (g/kg)	376.0
Organic matter (g/kg DM)	929.0
Crude protein (g/kg DM)	111.0
Neutral detergent fiber (g/kg DM)	497.
Ether extract (g/kg DM)	37.0
Non-fiber carbohydrates (g/kg DM)	284.0
Calcium (g/kg DM)	3.9
Phosphorous (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; phosphorus - 0.7 g/kg; magnesium – 2.1 g/kg; potassium – 0.89 g/kg; sodium – 0.3 g/kg; sulfur – 63.5 g/kg; cobalt - 348 mg/kg; chromium – 2.6 mg/kg; copper – 3.3 mg/kg; iron – 2.1 mg/kg; manganese – 4.7 mg/kg; zinc – 7.8 mg/kg; selenium – 318.0 mg/kg.

Table 2: Descriptive statistics to estimate macromineral requirements of pregnant Holstein x Gyr crossbred cows

			SBW[1] (kg)		EBW[2] (kg)		ADG[3]	EBG[4]
Condition	Nº	Item	Initial	Final	Initial	Final	(kg/d)	(kg/d)
Non-pregnant cows	12	Average	458.0	544.3	382.1	474.1	0.61	0.58
		Minimum	408.0	471.0	332.2	394.3	0.10	0.03
		Maximum	492.0	676.0	416.1	579.4	1.76	1.93
		SD[5]	27.15	68.81	27.11	69.85	0.710	0.509
Pregnant cows	42	Average	478.5	577.7	402.6	508.1	0.71	0.68
		Minimum	408.0	460.0	332.2	388.6	-0.01	0.13
		Maximum	587.0	756.0	510.9	670.4	1.60	1.55
		SD	43.42	83.71	43.36	80.06	0.451	0.447

[1] SBW = Shrunk body weight;
[2] EBW = Empty body weight;
[3] ADG = Average daily gain;
[4] EBG = Empty body for gain;
[5] SD = Standard deviation;

Table 3: Means of input, output, and retention to estimate macromineral balance to predict requirements

	Non-pregnant cows				Pregnant cows			
Mineral	Intake	Feces	Urine	Retention	Intake	Feces	Urine	Retention
		grams/day				grams/day		
Calcium	46.3	20.7	2.2	23.4	48.1	20.3	2.5	25.4
Phosphorus	19.4	7.5	1.3	10.7	20.2	7.5	1.5	11.3
Magnesium	14.1	6.4	3.9	3.7	14.8	6.5	3.8	4.4
Potassium	51.3	8.7	38.4	4.3	53.7	9.3	35.8	8.5
Sodium	18.3	9.9	5.3	2.9	18.9	9.3	6.0	3.7
Sulfur	6.9	2.5	1.9	2.4	7.1	2.5	2.0	2.6

Table 4: Estimation of parameters (β_0 and β_1) by mineral retention to predict net requirements of macrominerals for maintenance

Mineral	β_0	SEM[1]	P - value	β_1	SEM	P - value	RMSE[2]	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] SEM = standard error of mean

[2] RMSE = root mean square error

Table 5: Estimation of parameters (β_0 and β_1) by mineral retention in carcass and non-carcass to predict net requirements for weight gain

Mineral	Non-pregnant cows				Pregnant cows				NP[2] vs Preg.[3] cows (P-value)	
	β_0	SEM[1]	β_1	SEM	β_0	SEM	β_1	SEM	β_0	β_1
Calcium	0.1739	0.1171	1.6202	0.1091	2.7272	1.1939	1.1484	0.0697	0.0374	0.0006
Phosphorus	0.5540	0.2706	1.3843	0.0793	2.1387	0.6945	1.1435	0.0517	0.0376	0.0136
Magnesium	0.0359	0.0542	1.4163	0.2452	0.6634	0.5575	0.9300	0.1341	0.2675	0.0819
Potassium	0.1612	0.1699	1.3514	0.1723	0.6495	0.3830	1.1191	0.0943	0.2489	0.2305
Sodium	0.0117	0.1011	1.7219	1.3978	0.1211	0.4491	1.3185	0.5901	0.8264	0.9491
Sulfur	0.1125	0.0830	1.5185	0.1194	1.0961	0.4437	1.1333	0.0645	0.0334	0.0062

[1] SEM = standard error of mean

[2] NP = non-pregnant cows

[3] Preg. = pregnant cows

Table 6: Specific estimation of absorption/retention coefficients for macrominerals by different studies and nutrient requirement systems

Study/System[1]	Calcium	Phosphorus	Magnesium	Potassium	Sodium	Sulfur
ARC (1980)	0.68	0.60	0.17	1.00	0.91	-
AFRC (1991)	0.68	0.58	-	-	-	-
NRC (1996)	0.50	0.68	0.17	-	0.91	-
NRC (2001) Forages	0.30	0.80	-	-	0.81	-
NRC (2001) Concentrate	0.60	-	-	-	1	-
CSIRO (2007)	-	0.70	-	-	-	-
Gionbelli (2010)*	0.55	0.56	0.16	0.19	0.04	-
Costa e Silva (2015)*	0.72	0.82	0.98	0.70	0.58	0.67
BR-CORTE(2016)*	0.57	0.68	0.36	0.37	0.43	0.77
This Study*	**0.65**	**0.74**	**0.42**	**0.56**	**0.43**	**0.85**

[1] Nutrient requirement system

*Retention coefficient

Table 7: Dietary requirements for pregnant and non-pregnant cows for each macromineral considering net requirement for maintenance and different weight gain

BW[1]	ADG[2]	Non-pregnant cows						Pregnant cows					
		Ca	P	Mg	Na	K	S	Ca	P	Mg	Na	K	S
kg	kg/d	g/d						g/d					
500	0.2	14.1	7.7	5.3	12.6	41.2	5.5	11.9	6.8	4.8	11.4	38.1	4.9
	0.5	19.8	10.9	5.8	13.7	42.2	6.9	15.4	9.1	5.1	12.2	38.9	5.9
550	0.2	15.4	8.4	5.8	13.8	45.3	6.0	12.9	7.1	5.3	12.5	41.9	5.3
	0.5	21.4	11.6	6.3	15.0	46.3	7.5	16.5	9.2	5.6	13.3	42.7	6.3
600	0.2	16.6	9.0	6.3	15.0	49.4	6.5	13.9	7.7	5.7	13.6	45.6	5.8
	0.5	23.0	12.4	6.8	16.4	50.4	8.1	17.5	9.8	6.0	14.5	46.5	6.8
650	0.2	17.9	9.6	6.8	16.3	53.5	7.0	14.9	8.2	6.2	14.8	49.4	6.2
	0.5	24.6	13.1	7.4	17.7	54.5	8.7	18.6	10.3	6.5	15.6	50.2	7.2
700	0.2	19.1	10.3	7.4	17.5	57.6	7.5	15.9	8.7	6.7	15.9	53.2	6.6
	0.5	26.1	13.9	7.9	19.0	58.6	9.2	19.6	10.9	7.0	16.7	54.0	7.7

[1] BW = body weight;

[2] ADG = average daily gain

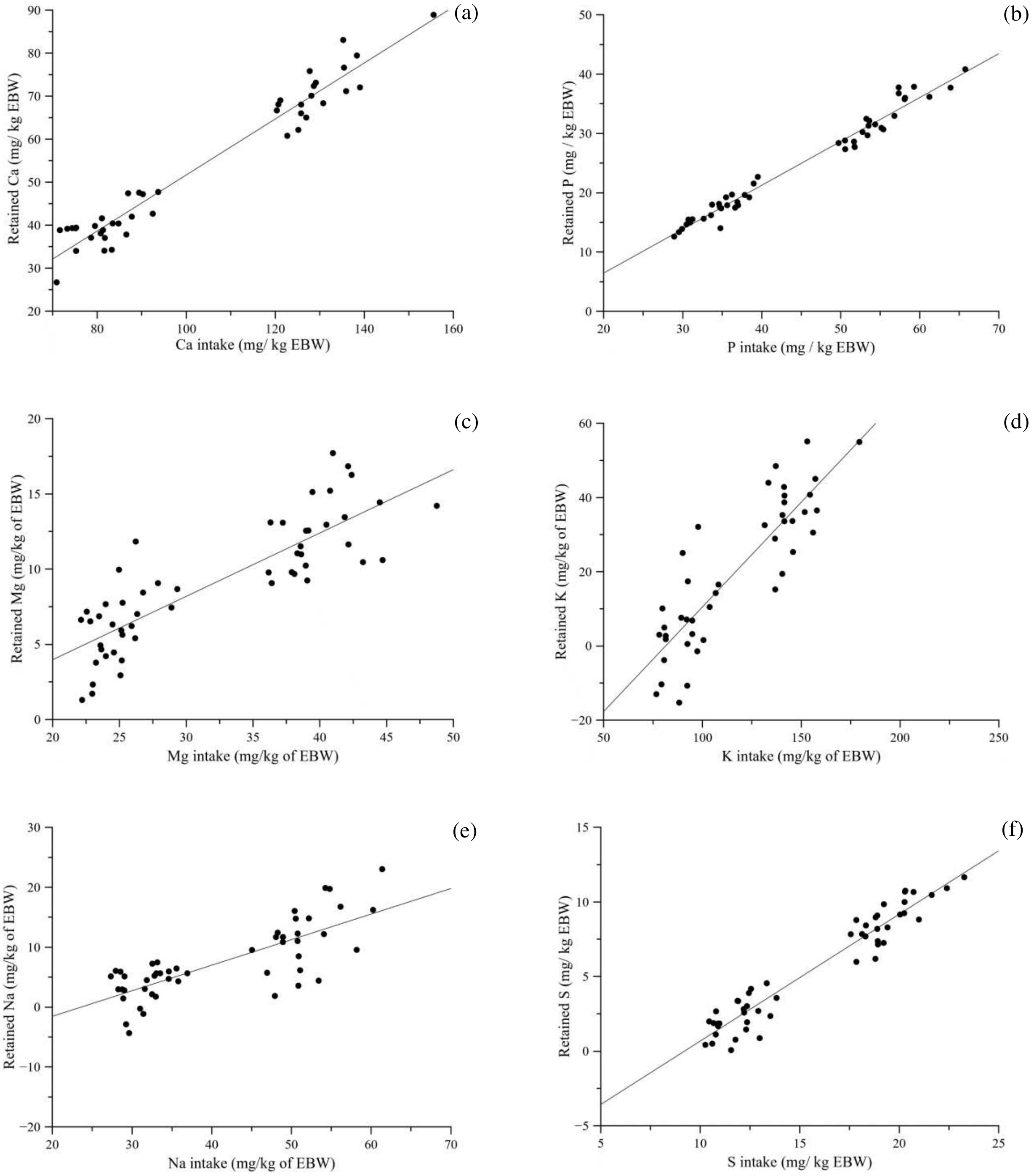

Figure 1: Linear model based on intake and excretion to predict net requirement for maintenance of calcium (a), phosphorus (b), magnesium (c), potassium (d), sodium (e) and sulfur (f).

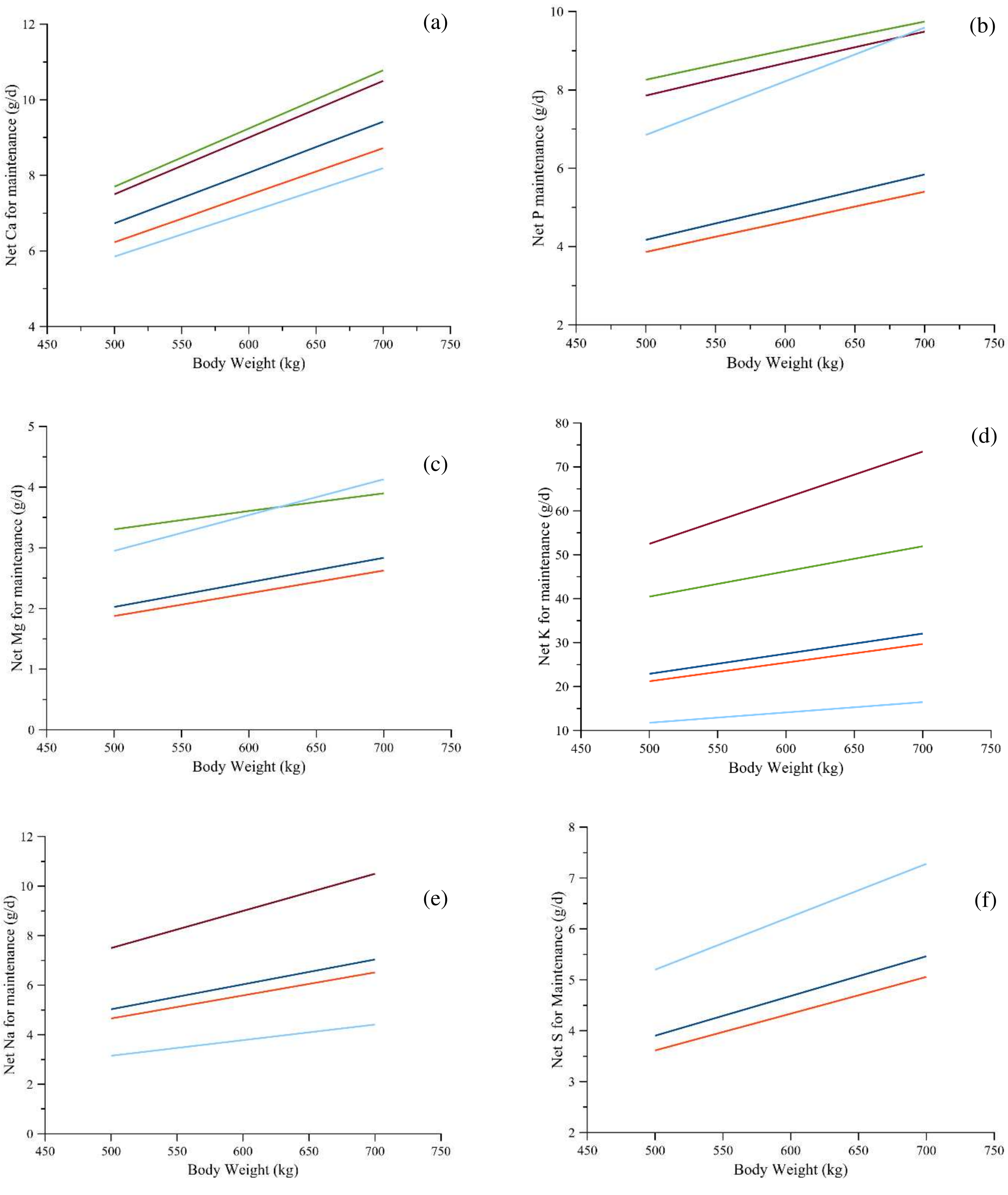

Figure 2: Comparison of predictions of non-pregnant (dark blue line) and pregnant cows (red line) macrominerals among traditional requirement systems: NRC (2001) (green line), BR-CORTE (2016) (light blue line), and INRA (2018) (dark red line) of calcium (a), phosphorus (b), magnesium (c), potassium (d), sodium (e) and sulfur (f).

CHAPTER 2: Macromineral requirements for pregnancy of Holstein × Gyr cows

Interpretative summary: By Camisa-Nova et al. Studies related to nutrient requirements of pregnant cows are scarce. However, many studies involving fetal programing have considered the importance of balance diets for cows throughout pregnancy. Thus, this is the first study that developed approaches to estimate the macromineral demand throughout pregnancy of dairy cows. New approaches presented more precise models to meet the pregnant cow demand for macrominerals because the pattern of models are similar with fetus development in the uterus. Therefore, the present study revealed excellent information about the macromineral requirements for pregnant dairy cattle.

Running Head: Macromineral requirements for pregnancy

Macromineral requirements for pregnancy of Holstein × Gyr cows

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ABSTRACT

This present study aimed to set approaches to predict the macromineral requirements throughout pregnancy of Holstein × Gyr cows. Fourty-two pregnant and twelve non-pregnant cows enrolled in this trial. Cows were grouped by feed regimen, where one group was kept in low body weight gain and the second group was fed *ad libitum*. Both pregnant and non-pregnant cows were submitted to comparative slaughter throughout pregnancy. The gestational components of non-pregnant cows were used as a correction for a maternal contribuition on gestational components. Based on macromineral concentration in gestational components, macromineral predictions of net requirements for pregnancy was calculated. Additionally, the initial point of net requirements for pregnancy was set for all macrominerals. All the predictions of pregnancy for macromineral requirements presented an exponential pattern. Thus, the macromineral demand throughout the pregnancy increases, and it occurs before the last third of gestation. Therefore, predictions in the present study consider the requirements in early and mid-gestation. Initial points of net requirements for pregnancy were: 121, 85, 43, 27 d of pregnancy for Ca, P, Mg, and S, respectively. Sodium and potassium did not present adjustment to estimate initial point of requirements for pregnancy. In conclusion, the predictions proposed in this study were estimatives to meet the macromineral requirement throughout entire pregnancy, disregarding the fixed values for requirements of pregnant cows for some macromineral and the concept of the initial point of requirements for pregnancy from 190 d.

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

INTRODUCTION

Dairy products will increase the demand through the years because of greater capital income and nutritional characteristics of these products to suppley human demand for some nutrients (Britt et al., (2018). In parallel, livestock in tropical regions have been increasing in importance in milk production in the global market. As well known, particular challenges on milk production in tropical areas are evident, and several technologies have been developed to support the higher productivity. *Bos taurus* × *Bos indicus* crossbred animals have been an important strategy to meet better adaptation by animals for climate conditions associated to higher milk production (Ramírez-Rivera et al., 2019). In Brazil, 80% of total milk production comes from Girolando cattle (Canaza-Cayo et al., 2015). This estimative shows the importante of crossbred cows for tropical livestock.

However, studies related to these animal breeds are scarcy, and more information are needed to enhance production and efficiency of dairy farms under this condition. Salah et al. (2014) reported differences on energy and protein requirements of ruminants rearing in tropical regions, indicating that specific approaches should be developed to predict nutrient requirements for ruminants under warmer temperatures.

Although minerals are presented in less proportion in the body composition than protein and fat, they play a variety of functions in the organism. Their functions are separated into different types: structural, physiological, catalytic, regulatory, and immunological response (Valadares Filho et al., 2016; Wilson et al., 2016). Moreover, mineral requirements change based on species, level of production, and physiological stage because mineral deposition varies in different tissues, and changes in absorption and retention are closely related to animal`s demand for each mineral, where mineral transporters in the wall of the intestine changes in number to increase or decrease the uptake by erythrocytes (Suttle, 2010).

Several studies about fetal programming have mentioned the effects of disbalance of maternal nutrition on fetal development, physiological and endocrinological dysfunctions, and changes on nutrient deposition in fetuses (Long et al., 2009; Du et al., 2010; Duarte et al., 2013) as well as in maternal tissues, such as placenta and mammary gland (Rotta et al., 2015b; c). Consequently, understanding the requirements of dairy cows throughout gestation is crucial to feed precisely cows on this physiological stage.

NRC (2001) recommend nutrient requirements for pregnanct cows from 190 d of gestation based on one study conducted by House and Bell (1993) where they evaluated only the last third of pregnancy. The last third of gestation is the period where the exponential growth of fetuses is evident. However, new information about maternal nutrition has risen to clarify the nutrient demand of pregnant cows earlier the last third of pregnancy. Sguizzato et al., (2020) and Provazi (unpublished) have worked on energy and protein requirements through pregnancy, respectively. Sguizzato et al., (2020) described the initial point of the energy demand of pregnant Holstein × Gyr cows before the 190 d. Based on these studies, new approaches to nutrient demand by pregnant cows should be proposed.

Studies on macromineral requirements are lacking to provide reliable predictions for these nutrients. Traditional nutrient requirement systems around the world only consider the final stage of gestation as a significant increase of nutrient demand for gestational maintenance (AFRC, 1991; NRC, 2001; NASEM, 2016; INRA et al., 2018), in some cases, nutrient requirements systems do not provide recommendation about macrominerals for pregnant cows (Valadares Filho et al., 2016) .

Based on previous studies on nutrient requirements for pregnancy, the hypothesis in this study is that the macromineral requirements of gestation for dairy cows start before the last third of pregnancy. Further, the efficiency of utilization of minerals could change throughout pregnancy period. This study aimed to measure the macromineral retention on -tissues involved to pregnancy, and develop estimatives for macromineral requirements to supply macromineral demand for pregnancy in Holstein × Gyr cows.

MATERIAL AND METHODS

The trial was performed in stalls at Universidade Federal de Viçosa, MG, Brazil. All the procedures and sampling in this study were evaluated and approved by the animal care and handling committee of the host university (CEUAP/UFV – 47/2012).

Fourty-two pregnant and twelve non-pregnant Holstein × Gyr cows were enrolled in this experiment. All the cows were allocated into individual pens (30 m²) with individual feed bunker and automatic water system. Feed regimen followed the same protocol developed by Camisa Nova et al. (2021). In summary, cows were divided into two distinct groups: cows fed with a restricted feed management to obtain low ADG through pregnancy (0.1 kg), and the group of cows where the fed*ad libitum* to obtain an ADGabout 1 kg. Both groups were fed twice a day with the same diets formulated with corn silage and concentrate at a ratio of 93:7 on a DM basis.The difference for the groups was the amount of feed provided for them.

Comparative Slaughter Method and Sampling

The slaughters were set based on the pregnancy time. The animals belong to the pregnant group were submitted to comparative slaughter throughout four different days of gestation: 140, 200, 240, and 270. In parallel, the open cows were slaughtered at the same days that pregnant cows to be used as the reference of maternal composition of gestational components to estimate the balance of true composition of gestational components throughout pregnancy. Valadares Filho et al. (2016) recommend to calculate gestational components separately of maternal body composition, and this methodology was applied in this trial. Consequently, the non-pregnant cows were slaughtered at 200, 240, and 270 d. The slaughter of open cows at day 140 of pregnancy was not considered because we as initial stage of fetus development, what implied in low difference between groups at this day of pregnancy.

Also, the slaughter procedures were performed as described by Camisa Nova et al. (2021). Then, gestational components (fetus, placenta fetal fluids and mammary gland) were obtained, weighed, and sampled. Each gestational component was weighted and sampled individually. The fetus, placenta, uterus, mammary gland were submitted to sample preparation by dissection and grinding for posterior macromineral composition analyses.

Calculation of the Macromineral Requirements for Gestation

Available mineral for pregnancy in the metabolism was calculated based on the rest of all retained macromineral in the body minus the dietary macromineral requirements for maintenance and body weight gain (equation 1). Data about maintenance and gain was taken from predictions developed by Camisa Nova et al. (2021).

$$Available\ mineral_{preg} = Mineral\ input - \frac{Net\ req._{maint.} + Net\ req._{gain}}{Retention\ coefficient} \quad [1]$$

The retention of each macromineral for pregnancy was calculated based on the sum of macromineral retained in all gestational component (uterus + placenta + fetal fluid + fetus + mammary gland). Then, the true retained mineral for pregnancy were calculated based on the differences of gestational component composition between pregnant and non-pregnant cows throughout the pregnancy. Posteriorly, the true retention throughout pregnancy was estimated by an exponential model described by equation 2.

$$RM_{pregnancy} = \beta_0 \times e^{\beta_1 \times DP} \quad [2]$$

where: $RM_{pregnancy}$ is the retained mineral for pregnancy; DP is the days of pregnancy; β_0 is the intercept; β_1 is the regression slope; ε_i residual effect.

Subsequently, the net requirement for pregnancy for all macrominerals were developed based on the first derivative equation of exponential model of retained mineral for pregnancy. The first derivative of equation 2 is described in equation 3.

$$Net\ Requirement_{pregnancy} = \beta_0 \times \beta_1 \times e^{\beta_1 \times DP} \quad [3]$$

where: $Net\ Requirement_{pregnancy}$ is the net mineral requirement for pregnancy (g/d); DP is the days of pregnancy; β_0 is the intercept; β_1 is the regression slope; ε_i residual effect.

Based on the true retention of macrominerals and available mineral in the metabolism for pregnancy, the efficiency of utilization for all macrominerals were calculated based on the interactive method as described by the equation 4:

$$\Delta = MI - \left(\frac{NetMM_{maint.} + NetMM_{gain}}{Retention\ Coefficient} + \frac{Net\ MM_{preg}}{k_{preg}}\right) \quad [4]$$

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

Additionally, a new approach to estimate the retention efficiency for macrominerals was proposed to set the efficiency throughout pregnancy. The new model proposed assumes a nonfixed efficiency of macromineral utilization throughout pregnancy, which is described as equation 5:

$$\Delta = MC - \left(\frac{NetMM_{maint.} + NetMM_{gain}}{Retention\ Coefficient} + \frac{Net\ MM_{preg}}{\Delta k_{preg}}\right)$$

where: MC is the Consumption of any macromineral by cows (g/d); $NetMM_{maint}$ is the net requirement of any macromineral for maintenance (g/d); $NetMM_{gain}$ is the net requirement of any macromineral for gain (g/d); $NetMM_{preg}$ is the net requirement of any macromineral for pregnancy (g/d); Δk_{preg} corresponds to the variation of retention efficiency for any available macromineral for pregnancy.

After the obtained variables, the dietary requirements for each mineral were calculated by the sum of the net requirement for maintenance and growth divided by the true retention coefficient. Then, the requirement for pregnancy was calculated based on the utilization efficiency of the mineral content throughout the gestational period.

Estimative of initial point of net requirements for macrominerals of pregnant cows was set based on the methods proposed by Sguizzato et al., (2020). This new methodology is based on the lower confidence limit of mineral retention. In other words, the initial point for net requirements for pregnancy is predicted at the estimation from the proposed model for any nutrient and the standard error of the estimative is equal zero. From this day, the net requirement for pregnancy is considered significant.

Statistical Procedures

Predictions of parameters of the model related to pregnancy was first tested using NLIN procedure, then the predictions were tested by PROC NLMIXED procedure of SAS Software (SAS Institute Inc., 2013) version 9.4; SAS Institute Inc., Cary, NC, USA). The level of $P \leq 0.05$ was used for all tests as the critical probability level for the occurrence of type I error.

RESULTS AND DISCUSSION

Gestational components and pattern of macromineral retention

For all macrominerals, the available portion of each mineral for pregnancy was calculated based on the balance of mineral intake minus the dietary requirements for maintenance and gain (Table 1). As proposed by Gionbelli et al. (2015) and adopted by (Valadares Filho et al., 2016), uterus, fetal fluids, placenta, fetus, and mammary gland were considered as parts of gestational components. The mammary gland was considered a gestational component because the major mammary gland growth occurs in consequence of pregnancy. In mammal species, the development of mammary gland due to pregnancy could vary from 48 through 94% of total development (Tucker, 1987).

Nutrient requirement systems around the world suggest net mineral requirements for pregnancy from 190 d (ARC, 1980; NRC, 2001; NASEM, 2016; INRA et al., 2018; Tedeschi and Fox, 2018) . However, these systems are based on the mineral retention in the gravid uterus without the mammary gland. In this study, the mammary gland retained a considerable amount of some minerals before the last third of gestation (Table 2). Physiologically, the mineral retention might be a consequence of differentiation of mammary epithelial cells and hormonal responses through gestation (Rillema, 2002). In pregnant animals, cellular differentiation and elongation in the mammary gland start in mid-gestation and increase during the last third of gestation (Akers, 2002; Hurley and Loor, 2011). Thus, the mineral requirements of pregnancy should compute the mammary gland as a gestational component.

All macrominerals revealed an exponential pattern of retention (g) in gestational components throughout days of pregnancy (Table 3). Based on studies evaluating variables involving gestational components, it were expected that exponential models would be suitable to describe them, such as gestational component weight, energy, and mineral accretion throughout days of pregnancy (Ferrell et al., 1982; Reynolds et al., 1990; Sguizzato et al., 2020b).

Fetus and mammary gland were the major gestational components retaining Ca and P. A considerable amount of both minerals was found in mid-gestation (Table 2). Ferrell et al. (1982) found for beef crossbred heifers that daily rates of accretion of Ca and P in the fetus begin during early and mid-pregnancy. It meets with the findings in this study. Also, exponential models were found for Ca and P deposition in the gravid uterus by House and Bell (1993) and Meschy (2007) evaluating mineral retention in the last third of gestation, which show agreement with the findings in this study even considering the different initial point of Ca requirements for pregnancy.

House and Bell (1993) set linear regressions to estimate Mg, K, and Na content into fetus composition and a linear adjustment could be related to the period of gestation studied for these authors, which they evaluated only the last third of gestation. Ferrell et al. (1982) evaluated mineral retention from 100 d of gestation through the parturition for beef cattle. The authors suggest an exponential model to estimate mineral content in the conceptus for all macrominerals, what agrees for Mg, K, and Na predictions from this study.

This study was the first trial to evaluate S content in gestational components.. The mammary gland uptakes a significant accretion of S during mid-gestation. The fetus also presented considerable retention in the last third of gestation (Table 2).

Net macromineral requirements for pregnancy

The net requirement of all macrominerals for pregnancy were set from the first derivative from the mineral retention model (Table 4). Additionally, evidence in the literature shows that initial point of the nutrient requirement for pregnancy is not 190 d (Ferrell et al., 1982; Du et al., 2010). Thus, a new initial point for net requirements of pregnancy for each macromineral were estimated through the method proposed by (Sguizzato et al., 2020a).

Contrasting the models proposed by NRC (2001), INRA (2018), and thie present study from 190 through 262 d of gestation, it is possible to observe that NRC (2001) recommends a greater amount of Ca for pregnancy. Further than 262 d of pregnancy until calving net Ca prediction from this study is greater (Figure 2a). Prediction from NRC (2001) for Ca was developed based on newborn weight equals 46 kg (House and Bell, 1993), and in this study, the average calf weight at parturition was 33 kg, what is an acceptable body weight at calving for Holstein *vs* Gyr crossbred animals (Silva et al., 2017; Azevedo et al., 2020; Sguizzato et al., 2020a). On the other hand, the prediction from this study might increase the Ca demand at the end of gestation because of the colostrum synthesis in the mammary gland. INRA (2018) prediction for Ca was greater than our recommendation in this study. It could be explained based on the breed used to develop the predictions. INRA (2018) does not show adjustments for warm areas. Thus, their dataset is based on temperate climate breed, which is different than the animals used in this study. The estimated initial point of pregnancy requirements is 121 d of gestation (Figure 2a, red arrow).

NRC (2001) and INRA (2018) suggested exponential models to estimate P demand for pregnancy, which meets with the approach proposed in this study. Predictions were quite close in both systems and the present study. As for Ca, the different newborn weight increases the P demand in NRC (2001) and INRA (2018). However, the inclusion of the mammary gland in this study could be the reason to find close predictions for pregnancy from 190 d of gestation.

Mg, Na, and K have fixed value recommendations for pregnancy both by NRC (2001) and INRA (2018). Approaches from this study present exponential pattern as Ca and P. However, the fixed value in NRC (2001) is recommended because of hypomagnesemia occurrence. In this study, cows did not produce milk parallel to the gestation, consequently, the risk of hypomagnesemia was low. Further information on Mg requirements for lactating Holstein × Gyr cows is needed. The initial point for Mg requirements for pregnancy was 43 d (Figure 1c, red arrow). This initial requirement prediction for Mg could be related to the low amount needed in the metabolism. Na and K did not present adjustment for the initial point for pregnancy demand. Further studies are needed to understand the K and Na dynamic throughout pregnancy.

The fixed values recommended for Na and K by NRC (2001) and INRA (2018) were greater than the approaches proposed for Na and K in this study. The fixed value from both minerals by nutrient requirement systems was met just close to 270 d of pregnancy. Thus, a fixed value could be not appropriate to use for pregnancy for Na and K.

Net S requirements for pregnancy were estimated by the factorial method. Both NRC (2001) and INRA (2018) used a fixed model for all demand of sulfur estimated in 2 g/d. The current and previous study performed by Camisa Nova et al. (2021) estimated S requirements for maintenance, weight gain, and pregnancy. Follow the predctions from this study is more suitable because the adjustments for each net requirement could optimize the utilization of S and decrease the excretion via urine and feces. The initial point for S was set from 27 d (Figure 2f, red arrow). The amount of S in the body is quite low. However, studies involved to S amino acids on fetus losses are recently performed (Wiltbank et al., 2014; Toledo et al., 2017), where the authors found lower embryony reabsorption in the beginning of gestation. Thus, S function and increment in the diet in early gestation should be carefully assessed.

As the mineral partitioning for maintenance, weight gain, and pregnancy was calculated, the efficiency of use of each macromineral for pregnancy was estimated. Furthermore, an adjusted efficiency throughout gestation was proposed to predict utilization of any macromineral by cows accurately (Table 4). Studies have reported that the requirements change based on several factors, such as breed, age, physiological status (NRC, 1995). Correspondingly, the efficiency of the use of nutrients might change as well (CSIRO, 2007; Marcondes et al., 2010) (CSIRO, 2007; Marcondes et al., 2010).

Regarding efficiency of use of each macromineral according to days of pregnancy, a fixed and adjusted efficiency throughout pregnancy were calculated (Table 4). The distribution of residual component showed more homogeneous and lower variability, for what means the adjusted efficiency was more suitable than a single efficiency (Figure 3). However, adjusted predictions for efficiency throughout pregnancy did not present reliable results to calculate dietary mineral requirements. It could occur because of mineral sources beyond the diet. For example, Ca may be absorbed from maternal bone instead of just from mineral consumption. Therefore, the authors considered using the method based on the retention coefficient proposed by several nutrient requirement systems.

Dietary requirements of all macrominerals for maintenance, weight gain, and pregnancy were calculated and demonstrated in Table 5. As expected in previous results in this study, fixed values for pregnancy could over or underestimate requirements for pregnant cows. For instance, dietary requirements for Na were lower by recommendation from NRC (2001) that in this study. In contrast, Mg dietary recommendation from NRC (2001) was lower than the predictions for Mg in this study. Therefore, changes in requirements throughout gestation should be considered.

The Ca:P ratio varies throughout pregnancy for predictions in this study. In mid and late pregnancy Ca:P ratio was 1.80:1 and 2.03:1, respectively. These Ca:P ratio agrees with recommendation from ARC (1980), where is recommended Ca:P ratio between 1:1 and 2:1. Further, (NRC, 2000) reported that Ca:P ratio from 1:1 to 7:1 does not show differences in performance.

On dietary requirements for S was estimated about 0.2% of DMI. Although it was equal to the recommendation from NRC (2001), the partition of S into maintenance, weight gain, and pregnancy was calculated. Thus, the proposed requirements for S are more suitable to formulate diets for pregnant dairy cows. Additionally, the adjustment of exponential model provides possibility to increase S in the diet gradually.

CONCLUSIONS

The present study revealed several new considerations on macromineral requirements for pregnant cows. Inclusion of all macrominerals should be carefully evaluated for pregnant dairy cows. Although~~,~~ the last third of gestation is the main stage of nutrient retention in the gravid uterus and mammary gland, considerations on macromineral demand before the last third should be done to avoid lack of nutrients for fetus and pregnant cows. Utilization of fixed demand of mineral for pregnancyshould not be considered according the present study. All macrominerals presented exponential increase of macromineral requirements for pregnancy. Thus, adjustment of macromineral supply throughout pregnancy should ponder to formulate rations for pregnant cows.

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Table 1. Summary of dataset used to predict net requirements for maintenance and gain, and retention coefficient for each macromineral adapted from Camisa Nova et al. (2021)

Item	Unit[1]	Calcium	Phosphorus	Magnesium	Potassium	Sodium	Sulfur
Intake	g/d						
Non-pregnant cows		46.3	19.4	14.1	51.3	18.3	6.84
Pregnant cows		48.1	20.2	14.8	53.7	19.0	7.12
Feces	g/d						
Non-pregnant cows		20.7	7.48	6.42	8.65	9.97	2.53
Pregnant cows		20.3	7.45	6.53	9.34	9.29	2.50
Urine	g/d						
Non-pregnant cows		2.18	1.28	3.95	38.40	5.38	1.94
Pregnant cows		2.53	1.46	3.79	35.80	6.02	2.03
Retention	g/d						
Non-pregnant cows		23.4	10.7	3.70	4.27	2.97	2.38
Pregnant cows		25.4	25.4	4.44	8.51	3.67	2.59
Net requirement$_{\textbf{maint.}}$	mg/kg EBW[1]	13.5	8.35	4.06	45.90	10.1	7.82
Retention coefficient		0.65	0.74	0.42	0.56	0.43	0.85
Net Requirement$_{\textbf{gain}}$[3]	g/kg EBG[2]						
Non-pregnant cows							
β_0		0.28	0.77	0.05	0.17	0.02	0.22
β_1		0.62	0.38	0.42	0.52	0.72	0.35
Pregnant cows							
β_0		3.13	2.45	0.62	0.73	0.16	1.24
β_1		0.15	0.14	-0.07	0.12	0.32	0.13

[1] EBW = empty body weight.

[2] EBG = empty body for gain.

[3] $Net\ requirement_{gain} = \beta_0 \times EBW^{\beta_1} \times EBG$

Table 2. Descriptive statistics of macromineral content in each gestational component.

	Days of pregnancy							
Mineral retention	140		200		240		270	
	Mean	SD[1]	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	10.44
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	11.71
Sodium (g)								
Mammary gland	22.4	10.93	27.2	8.787	29.9	10.6	37.0	15.03
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	30.15
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	21.71
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

[1] SD = standard deviation.

Table 3. Macromineral retention regression, net requirements of pregnancy, and efficiency of utilization through pregnancy

Item	Retention for pregnancy[1] (grams)	Standard Error of Mean		RMSE[2]	R[2]
		β_0	β_1		
Calcium	$0.5026\ e^{0.02489 \times DP}$	0.3230	0.0025	8.88	0.94
Phosphorus	$1.0081\ e^{0.02049 \times DP}$	0.4802	0.0018	5.00	0.88
Magnesium	$0.1173\ e^{0.01748 \times DP}$	0.0470	0.0016	0.26	0.86
Sodium	$5.1177\ e^{0.0115 \times DP}$	1.2827	0.0010	2.15	0.75
Potassium	$3.6890\ e^{0.0119 \times DP}$	0.8774	0.0009	1.57	0.88
Sulfur	$1.7758\ e^{0.0148 \times DP}$	0.8214	0.0018	2.69	0.86

[1] $Mineral\ retention_{preg} = \beta_0\ e^{\beta_1 \times DP}$; DP = days of pregnancy.

[2] RSME = root square mean error;

Table 4. Net requirement of macrominerals for pregnancy, and efficiencies of mineral utilization for pregnancy

Macromineral	Net requirement$_{preg}$[1] (grams/d)	Efficiency of mineral retention by gestational components[2]	
		k	$Adj\ k$
Calcium	$0.0125\ e^{0.02489 \times DP}$	0.139	$(5.00 \times 10^{-12}) \times DP^{4.4165}$
Phosphorus	$0.0207\ e^{0.02049 \times DP}$	0.201	$(2.29 \times 10^{-7}) \times DP^{2.5039}$
Magnesium	$0.0021\ e^{0.01748 \times DP}$	0.012	$(2.00 \times 10^{-7}) \times DP^{2.0225}$
Sodium	$0.0588\ e^{0.0115 \times DP}$	0.147	$(9.21 \times 10^{-7}) \times DP^{2.2135}$
Potassium	$0.0439\ e^{0.0119 \times DP}$	0.080	$(2.07 \times 10^{-7}) \times DP^{2.3813}$
Sulfur	$0.0263\ e^{0.0148 \times DP}$	0.389	$(1.59 \times 10^{-6}) \times DP^{2.2850}$

[1] First derivative equation from mineral retention equation; DP = Days of pregnancy.

[2] k = constant efficiency of macromineral retention throughout pregnancy; $Adj\ k$ = Adjusted efficiency of macromineral retention by pregnancy time.

Table 5. Dietary macromineral requirements for maintenance, gain, and pregnancy recommendation from current study and NRC (2001)

BW[1]	ADG[2]	DP[3]	Current study (g/d)						NRC (2001) (g/d)					
			Ca	P	Mg	Na	K	S	Ca	P	Mg	Na	K	S
550	0.2	150	16.2	8.98	5.89	14.6	45.8	15.7	-	-	-	-	-	-
		190	17.6	9.74	5.96	15.0	46.1	15.9	17.3	15.2	10.9	11.4	50.0	17.3
		230	21.3	11.5	6.10	15.7	46.5	16.3	21.7	17.8	10.9	11.4	50.0	17.3
		270	31.3	15.4	6.40	16.9	47.3	17.1	27.5	20.1	10.9	11.4	50.0	17.3
	0.5	150	17.3	9.82	5.64	14.1	43.1	16.8	-	-	-	-	-	-
		190	18.7	10.6	5.71	14.6	43.4	17.0	20.3	16.7	11.0	11.9	50.5	17.3
		230	22.4	12.3	5.86	15.3	43.9	17.4	24.6	19.5	11.0	11.9	50.5	17.3
		270	32.4	16.3	6.15	16.4	44.6	18.2	30.4	21.8	11.0	11.9	50.5	17.3

[1] BW = body weight (kg).

[2] ADG = average daily gain (kg).

[3] DP = days of pregnancy.

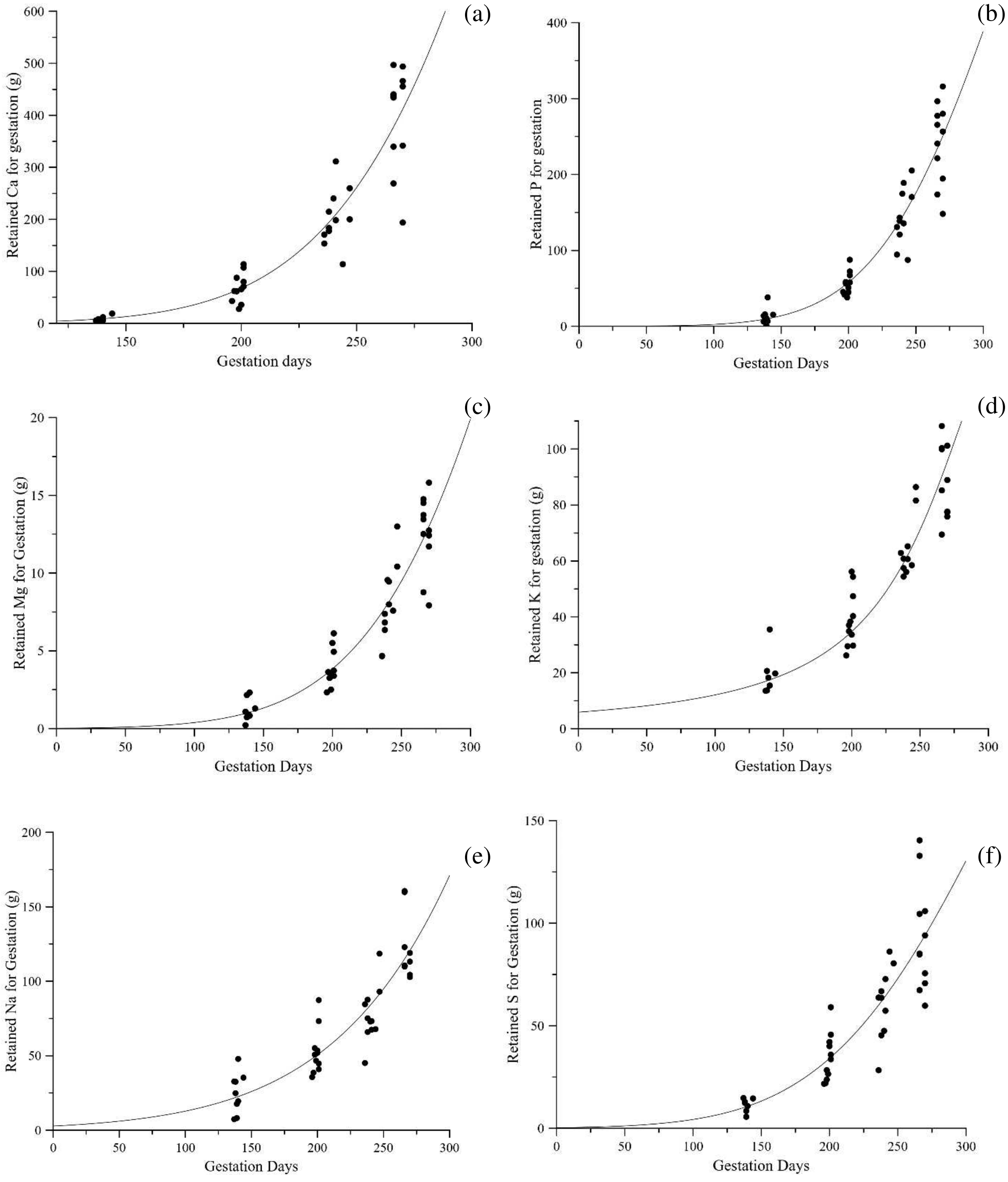

Figure 1: Retention of each macromineral in gestational components throughout the pregnancy (a – calcium; b – phosphorus; c – magnesium; d – potassium; e – sodium; f – sulfur).

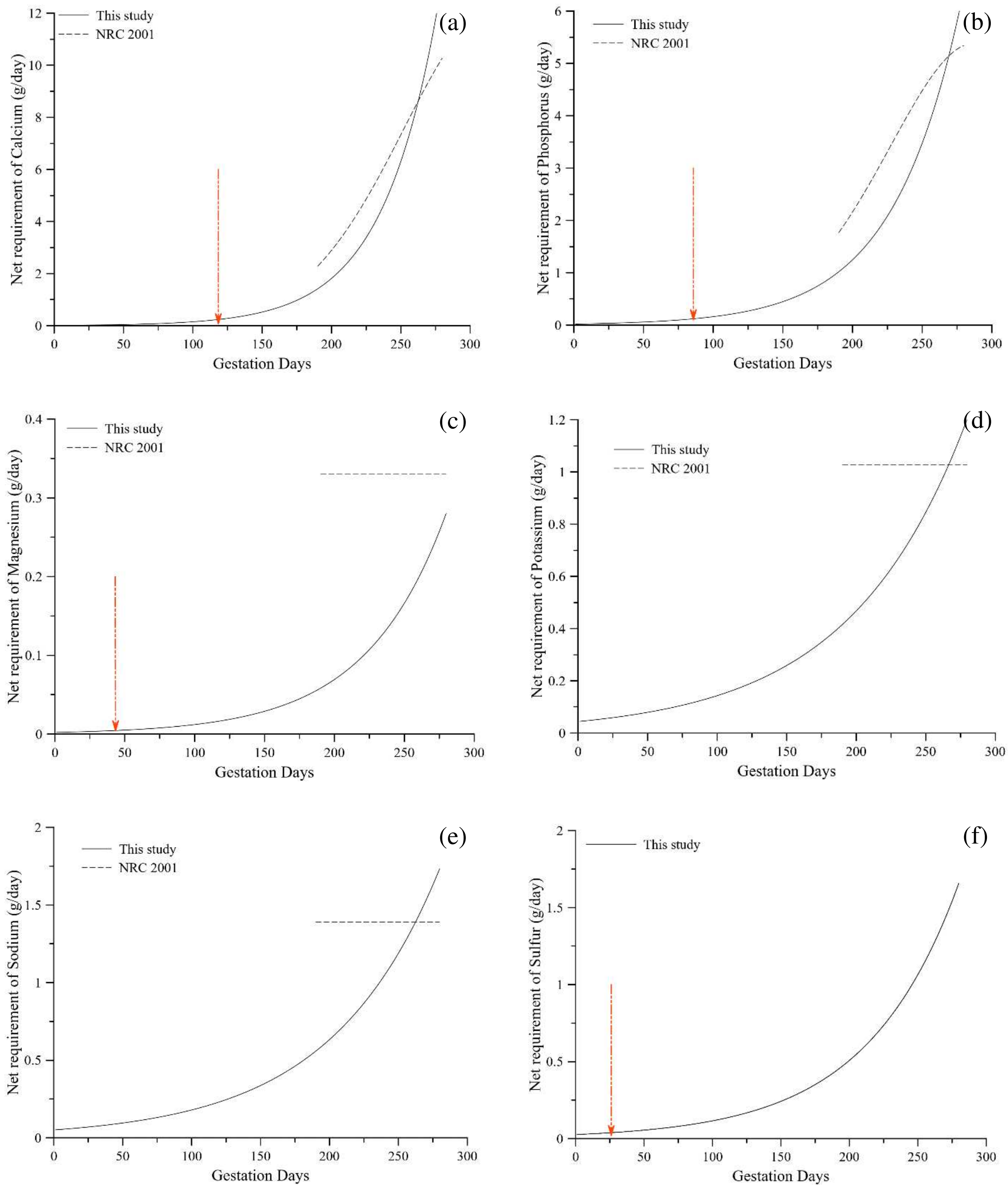

Figure 2: Plots of new approaches to predict macromineral requirements (a – calcium; b – phosphorus; c – magnesium; d – potassium; e – sodium; f – sulfur) throughout pregnancy, and comparison with NRC (2001) recommendations. Initial point of each macromineral requirements by pregnant cows (red arrows; a – calcium = 121 days; b – phosphorus = 85 days; c – magnesium = 43 days; d – potassium = Not predicted; e – sodium = not predicted; f – sulfur = 27 days).

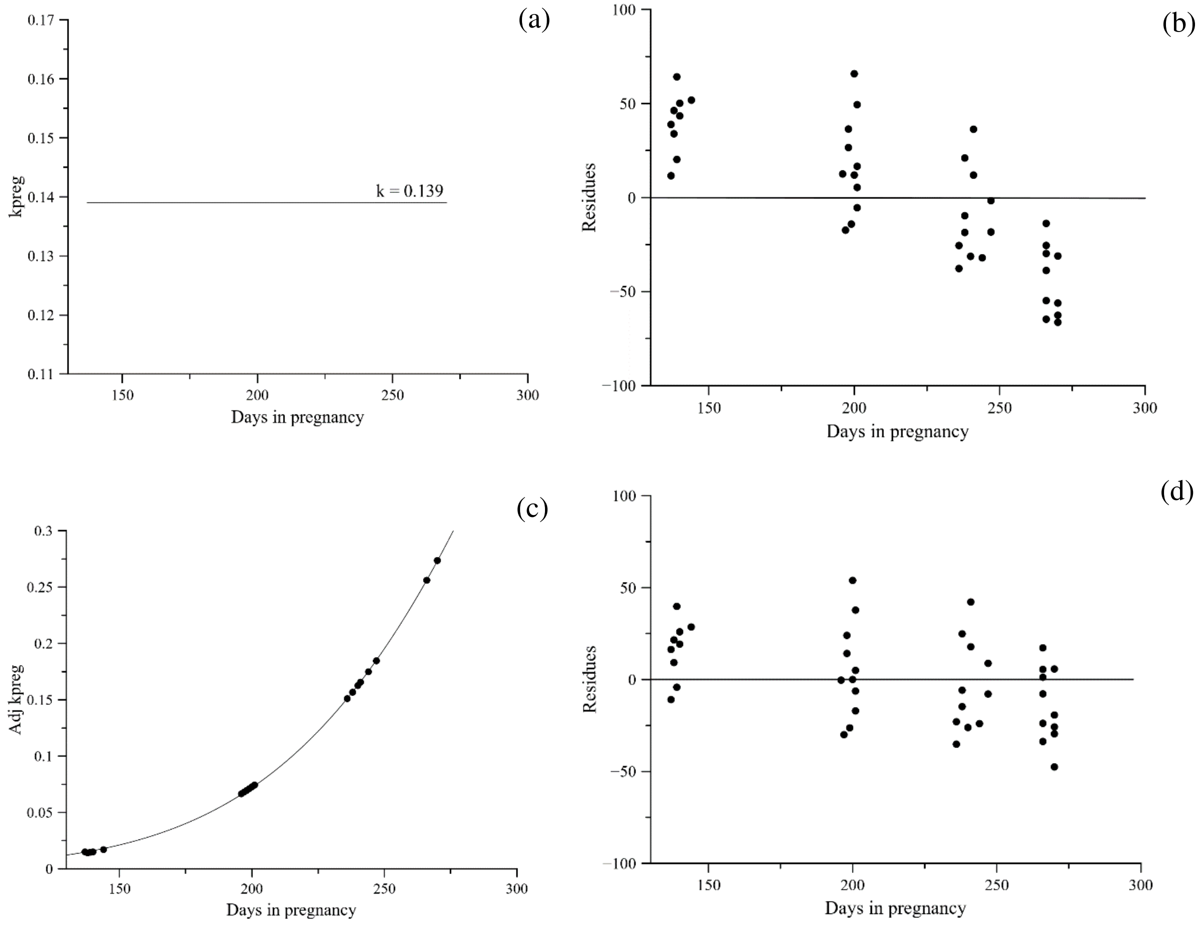

Figure 3: Comparison of constant efficiency (k_{preg}), adjusted efficiency by days of pregnancy ($Adj\ k_{preg}$), and the variability of the residues for calcium.