http://dx.doi.org/10.1590/1678-4162-9240

***Arq. Bras. Med. Vet. Zootec.*, v.69, n.4, p.1013-1020, 2017**

Mineral profile of crossbred F_1 Holstein x Gyr dairy cows during the transition period in summer and winter

[*Perfil mineral de vacas F_1 Holandês x Gir durante o período de transição no verão e inverno*]

T.F. *Moreira*[1], E.J. *Facury Filho*[1], A.L.B.S.A. *Costa*[2], R.M. *Meneses*[1], F.P. *Casagrande*[3], F.O.P. *Leme*[1], J.A.Z. *Uribe*[1], A.U. *Carvalho*[1]

[1]Aluno de pós-graduação – Escola de Veterinária – Universidade Federal de Minas Gerais – Belo Horizonte, MG
[2]Escola de Veterinária – Universidade Federal de Minas Gerais – UFMG – Belo Horizonte, MG
[3]Universidade Federal da Bahia – UFBA – Salvador, BA

ABSTRACT

The transition period is often a great challenge for dairy cows and mineral imbalances are frequent. With the aim to better understand the mineral profile of F_1 Holstein x Gyr dairy cows and their performances under the different conditions of summer and winter, we collected blood samples to measure calcium, magnesium and phosphorus. Samplings were performed during summer and winter, on 15 and 13 pluriparous F_1 Holstein x Gyr dairy cows, respectively. Blood sampling started 4 weeks prior to the expected calving date until 30 days postpartum. The mean concentrations of all three minerals had a different pattern during the transition period in each season, representing the interaction time x season. Calcium concentration was lower in winter and more animals suffered from subclinical hypocalcemia (100%) then in summer (38.46%). Magnesium concentration was also lower in winter and 46.67% of animals had hypomagnesemia, contributing for the higher hypocalcemia frequency observed in the same season. A high proportion of animals had hyperphosphatemia what can represent an environmental problem and more attention should be given to it. The high frequency of animals with subclinical hypocalcemia is alarming once that can lead to greater consequences.

Keywords: hypocalcemia, hypomagnesemia, milk fever, hyperphosphatemia

RESUMO

O período de transição é uma fase de grandes desafios para vacas leiteiras, e desequilíbrios minerais são frequentes. O objetivo ao desenvolver este trabalho foi de entender melhor o perfil mineral de vacas leiteiras F1 Holandês x Gir e suas performances *sob as diferentes condições de verão e inverno. Para isso, foram mensurados cálcio, magnésio e fósforo sanguíneo. As coletas de sangue foram realizadas durante os períodos de verão e inverno, e utilizaram-se, respectivamente, 15 e 13 vacas leiteiras pluríparas, todas F1 Holandês x Gir. As coletas de sangue começaram quatro semanas antes da data prevista do parto até 30 dias pós-parto. As concentrações médias e o padrão de variação dos três minerais foram distintos em cada estação do ano, representando a interação tempo de coleta x estação do ano. A concentração de cálcio foi menor no inverno, período em que todos os animais apresentaram hipocalcemia subclínica, enquanto no verão 38,46% apresentaram essa condição. A concentração de magnésio foi maior no verão e nenhum animal teve hipomagnesemia, enquanto no inverno 46,67% dos animais apresentaram hipomagnesemia subclínica, contribuindo para a maior frequência de hipocalcemia observada no inverno. Uma alta proporção de animais teve hiperfosfatemia, o que pode representar um problema ambiental. A alta frequência de animais com hipocalcemia subclínica é alarmante principalmente devido às consequências geradas por essa condição.*

Palavras-chave: hipocalcemia, hipomagnesemia, febre do leite, hiperfosfatemia

Recebido em 14 de junho de 2016
Aceito em 29 de setembro de 2016
E-mail: tiagofacuryvet@gmail.com

INTRODUCTION

Crossing specialized *Bos taurus* breeds with *Bos indicus* has been practiced for more than 70 years in the tropical areas. The objective is, for example, to combine the higher productivity of Holstein animals with the higher resistance to tick infestation and heat stress from the Zebu, generating a more profitable animal adapted to tropical conditions (Madalena, 1998; Matos, 2001).

Nowadays, Brazil is the fifth largest country in cow's milk production in the world and has the largest commercial bovine herd (Food…, 2013), which is mainly, composed by crossbreed Zebu/Holstein cows with different breed compositions (Madalena, 1998; Belchior *et al.*, 2015).

Dairy production in Brazil is mainly based on grazing systems, but, in most parts of the country, supplementation with other source of roughage and concentrate is often necessary during the dry period. This variation in feed supply due to climate implies in two distinct managements and have a great influence on the performance of animals (Moreira *et al.*, 2015).

F1 *Bos taurus* x *Bos indicus* dairy cows are becoming more popular because of their increased production in the last few years, due to genetic selection, combined with high heterosis and longevity (Madalena, 1998; Madalena and Junqueira, 2004). However, the higher production levels currently achieved by the crossbreed cows can lead to an increase in the occurrence of metabolic alterations (Fleischer *et al.*, 2001).

In this scenario, mineral imbalances play an important role. This is especially important during the transition period, the most challenging period in the lactation cycle of the dairy cow and when most health problems occur (Chapinal *et al.*, 2011). Hypocalcemia is one of the most common and important disorders at this time and may affect more than 70% of the herd in a subclinical form (Souza Júnior *et al.* 2011; Moreira *et al.*, 2015), leading to less milk production, impaired reproduction, mastitis, retained placenta and abomasal displacement (Chapinal *et al.*, 2011; Oetzel, 2013; Sepúlveda-Varas *et al.*, 2015).

The diagnosis and prevention of mineral imbalances is very important once it has effects in animal health and dairy farm economics. In order to assess mineral profile, serum analyzes are the most appropriated form. For this reason, the objective of this work was to evaluate the mineral profile of F_1 *Bos taurus* x *Bos indicus* dairy cows during the transition period to compare their performances between summer and winter and to determine the frequency of animals suffering from mineral imbalance in each time.

MATERIAL AND METHODS

This study was approved by the Comitê de Ética em Experimentação Animal (CETEA; Ethics Committee on Animal Experimentation) of the Universidade Federal de Minas Gerais (UFMG) under the protocol number 82/2011.

The study was conducted at a dairy farm in Martinho Campos, Minas Gerais state, Brazil (Latitude 19° 19' S; Longitude 45° 14' W; 674 m above sea level). The herd had 120 lactating cows, producing 2,000 liters of milk/ day. Cows were milked twice daily (morning and evening).

The research consisted of two experimental periods, from January to April 2011 (summer) and from May to August 2011 (winter). The climate of the region is defined according to the Koppen classification as Aw, a tropical savanna climate with hot and rainy summers and dry and mild winters. The average temperatures were 25.5°C in the summer and 20.6°C in the winter. The maximum and minimum temperatures were respectively 36°C and 15.2°C in the summer and 34.3°C and 7.9ºC in the winter. The total precipitation was 707.5mm in the summer and 20.2mm in the winter.

Climatic data (maximum, minimum, and average temperatures; relative humidity and rainfall) were recorded each month of experimental period (Fig. 1).

The cows were kept in a semi-intensive system in which they remained in grazing paddocks all year round. The dominant grass species were *Panicum maximum*, *Cynodon* sp. and *Brachiaria brizanta.* Cows were fed a mixed ration in the trough after each milking, composed of concentrate and corn silage during summer, and concentrate, corn silage and sugar cane during winter. The concentrate had 17% of crude protein and was constituted by ground corn, soybean and mineral during summer and sugar cane, ground corn, soybean, citrus pulp, cottonseed and mineral during winter. In both seasons the forage:concentrate ratio was of 75:25. Water and mineral salt were provided ad libitum.

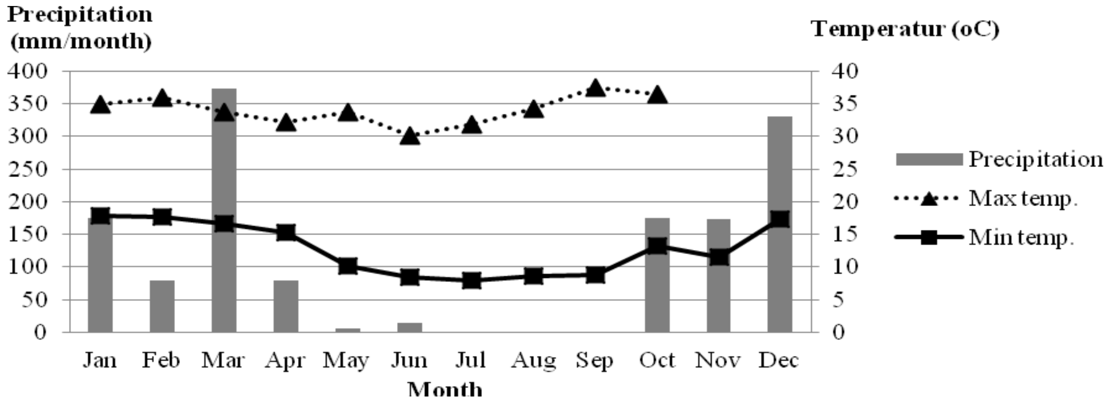

Figure 1. Maximum and minimum temperature (^oC) and precipitation (mm/month) during the year 2011 in Martinho Campos / MG. Data from the National Institute of Meteorology (Inmet) Pompéu.

We collected blood from 15 cows during summer and 13 cows during winter. All cows were crossbred ½ Holstein ½ Gyr, pluriparous between second and fifth lactation and with an average daily production of 24L of milk in both summer and winter.

During prepartum, blood was sampled weekly, starting on the 4^{th} week prior to the expected calving date. We took samples on the calving day and on the 2^{nd}, 5^{th}, 10^{th}, 15^{th}, 21^{th} and 30^{th} days postpartum by coccygeal venipuncture into 6 mL vacutainer tubes with gel separator and clot activator. All samples were carried out before feeding the animals. Samples were placed on ice immediately after collection and serum was separated by centrifugation. Serum analyses were made by spectrophotometry in an automatic device with the use of commercial kits for calcium, phosphorus and magnesium.

Using the mineral concentrations, we calculated the frequency of subclinical hypocalcemia (Ca <8,5mg/dL; Goff, 2004), hypomagnesemia (Mg<1,8mg/dL; Goff, 2008), and hyperphosphatemia (P>8,0mg/dL; Goff, 2000) in each moment and for the whole season.

The design was a split plot arrangement, in which the seasons consisted of plots and the times of blood collection were the subplots. All data was tested for normality. Data was analyzed by time, season and time x season interaction. Times of blood sampling were compared by the Scott-Knott test and differences between seasons was compared by SNK test (Sampaio, 2002). The total proportion of animals in summer and winter that had hypocalcemia, hypomagnesemia and hyperphosphatemia was compared by two-sample proportion test using the software STATA 12.1 (Stata Corp., College Station, Texas, USA). Animals that had at least one measurement under the cut point were considered positive. Probabilities of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Sampling time and season presented an interaction for all the three minerals evaluated. This means that the serum concentration of these minerals displayed a different pattern in each season during the transition period. This also means that all minerals were influenced by the transition period and had different average concentrations between seasons. The displayed patterns of the minerals are showed in Fig. 2.

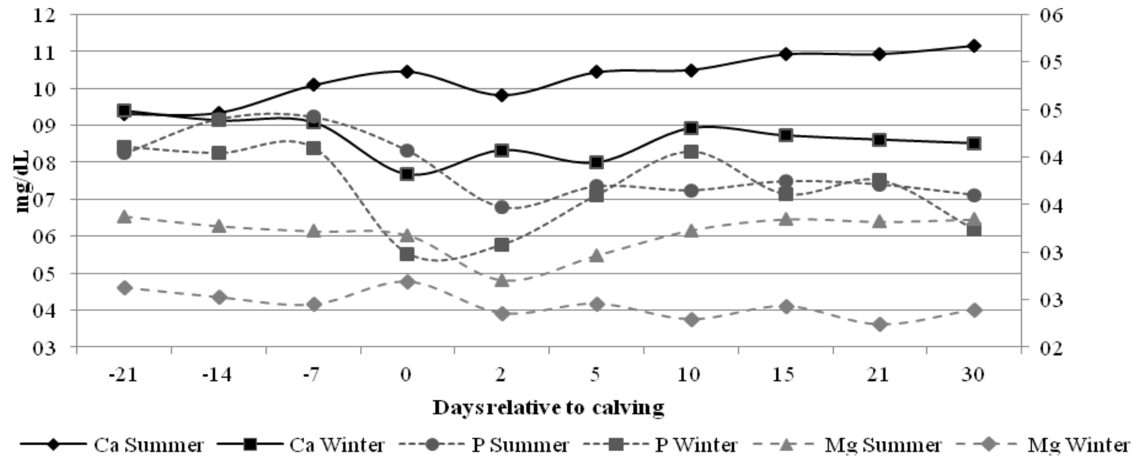

Figure 2. Concentrations of serum calcium (left axis; g/dL), magnesium (right axis; mg/dL) and phosphorus (left axis; g/dL) F_1 Holstein x Gyr dairy cows during the transition period in summer and winter in semi-intensive system.

Regarding the differences between seasons serum calcium, concentration was higher after calving in summer when compared to winter, while magnesium concentration was always higher in summer. Phosphorus was lower during postpartum on summer than winter (Table 1).

Table 1. Mean concentrations of calcium (mg/dL), magnesium (mg/dL) and phosphorus in F_1 Holstein x Gyr dairy cows in the fourth (-4 wk.), third (-3 wk.), second (-2 wk.) and first (-1 wk.) weeks prepartum, on calving (Calv.) and in the second, fifth, 10^{th}, 15^{th}, 21^{th} and 30^{th} days postpartum in semi-intensive system in summer and winter

		Days relative to calving										
	Season	-3 wk	-2 wk	-1 wk	Calv.	2^{nd} d	5^{th}d	10^{th}d	15^{th}d	21^{th}d	30^{th}d	Mean
Caucium	Summer	9.32Ab	9.35Ab	10.09Ab	10.45Aa	9.82Ab	10.44Aa	10.50Aa	10.92Aa	10.92Aa	11.15Aa	10.20
	S.D.	1.21	1.95	2.52	1.53	1.29	0.99	1.25	1.12	0.98	1.06	1.43
	Winter	9.40Aa	9.13Aa	9.09Aa	7.67Bb	8.33Bb	7.99Bb	8.93Ba	8.73Ba	8.61Ba	8.51Ba	8.65
	S.D.	0.79	0.67	0.55	1.36	1.51	1.23	1.24	0.93	0.72	0.84	1.00
	Mean	9.36	9.24	9.59	9.06	9.08	9.22	9.71	9.82	9.77	9.83	9.43
Magnesium	Summer	3.38Aa	3.28Aa	3.22Aa	3.18Aa	2.71Ab	2.97Ab	3.23Aa	3.35Aa	3.32Aa	3.35Aa	3.18
	S.D.	0.61	0.68	0.47	0.25	0.47	0.29	0.18	0.23	0.41	0.27	0.40
	Winter	2.63Ba	2.53Ba	2.45Ba	2.69Ba	2.36Ba	2.46Ba	2.30Ba	2.44Ba	2.24Ba	2.40Ba	2.46
	S.D.	0.29	0.25	0.28	0.66	0.52	0.60	0.70	0.37	0.38	0.53	0.45
	Mean	3.00	2.90	2.84	2.94	2.53	2.71	2.77	2.89	2.78	2.87	2.82
Phosphorus	Summer	8.24Aa	9.18Aa	9.24Aa	8.32Aa	6.78Ab	7.35Ab	7.24Ab	7.49Ab	7.40Ab	7.12Ab	7.92
	S.D.	1.96	3.12	2.71	2.28	2.13	1.50	1.63	1.18	1.44	2.35	1.99
	Winter	8.41Aa	8.24Aa	8.40Aa	5.53Bb	5.76Ab	7.10Aa	8.28Aa	7.13Aa	7.51Aa	6.18Ab	7.37
	S.D.	1.55	1.38	1.33	1.21	1.82	1.55	3.69	1.59	2.04	1.65	1.80
	Mean	8.33	8.71	8.82	6.93	6.27	7.22	7.76	7.31	7.46	6.65	7.65

Means followed by different capital letters in columns differ by the Scott-Knott test (P <0.05). Means followed by different lowercase letters in lines differ by the SNK test (P <0.05). SD = Standard deviation.

The mean serum concentrations of the analyzed minerals were between the physiological values at most part of the time. Average calcium concentration had been under physiological value (8.5mg/dL) just during winter at the calving day and in the second and fifth day postpartum. The average serum magnesium concentrations was always between the physiological values and phosphorus was above reference values during the prepartum in both seasons.

Analyzing just the mean concentration does not demonstrate the real picture of mineral status of the herd. As the serum concentrations of the evaluated minerals are related to disease and health impairment, it is more important to measure the proportion of animals below and above the biologic threshold, so we can have a more precise diagnosis of the farm situation (Oetzel, 2013; Moreira *et al.*, 2015).

Analyzing thus, 100% of animals in the study have had subclinical hypocalcemia in winter, a higher proportion ($P< 0.001$) then the one found during summer, when 38.46% of cows had subclinical hypocalcemia. Regardless of the high prevalence of subclinical hypocalcemia, there was no case of clinical hypocalcemia in both seasons.

The incidence of hypomagnesemia was also higher in animals that calved during winter (46.67%) then animals that calved in summer, when no animals had serum magnesium concentration under physiological limit ($P< 0.01$).

Hyperphosphatemia showed no difference ($P > 0.05$) between summer and winter, being both very high, with 100% and 86.67% of cows presenting hyperphosphatemia in winter and summer, respectively. The proportion of animals with hypocalcemia, hypomagnesemia and hyperphosphatemia at each sampling time can be seen in Fig. 3.

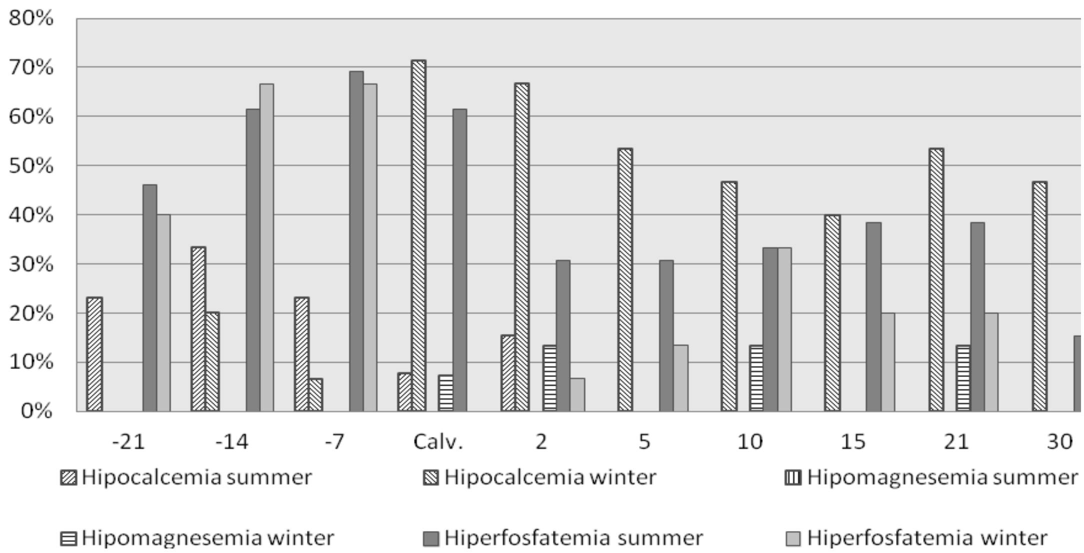

Figure 3. Frequency of hypocalcemia, hypomagnesemia and hyperphosphatemia in each sampling time during the transition period of F_1 Holstein x Gyr dairy cows in summer and winter in semi-intensive system.

These results are extremely disturbing, especially the high incidence of hypocalcemia and hyperphosphatemia. Hypocalcemia can have a great impact on health and consequently in the production and profitability of the animal (Goff, 2008). Cows that had serum calcium concentrations lower than 8.5 mg/dL have a high probability for immunosuppression, once the Ca^{++} is essential for trigging immune cells signals (Kimura *et al.*, 2006; Goff, 2008, 2014). Because neutrophil activity is downregulated in hypocalcemic cows, they are more likely to suffer from infectious diseases like retained placenta, clinical mastitis, metritis (Goff, 2014; Martinez *et al.*, 2014; Sepúlveda-Varas *et al.*, 2015). Furthermore, hypocalcemic cows are more prone to develop metabolic diseases such as clinical hypocalcemia and abomasal displacement (Martinez *et al.*, 2014; Sepúlveda-Varas *et al.*, 2015).

Currently there is the understanding that metabolic processes have an intricate pathway through the homeostatic process and a failure of one of these processes will inevitably impact on the efficiency of others. Mineral metabolisms are no different and there are complex homeostatic links between them (Jacobson *et al.*, 1972; Lean and DeGaris, 2010). Because of that, it is important to analyze all together to get a real picture of the situation and have an accurate diagnose. Considering this, the higher Mg concentration and the lower frequency of hypomagnesemia in summer can be one of the causes of the higher calcium concentrations and lower frequency of hypocalcemia found during summer. Magnesium plays an essential role in calcium homeostasis and their deficiency can affect Ca metabolism by reducing parathyroid hormone (PTH) secretion and by reducing tissue sensitivity to PTH. Because of this close correlation, the increased dietary concentrations of Mg can drastically reduce milk fever risk (Lean *et al.*, 2006; Goff, 2008).

Another interaction that probably contributed to the high incidence of hypocalcemia was the great number of animals that had hyperphosphatemia. Phosphorus can disturb calcium metabolism by reducing the concentration of 1,25-dihydroxycholecalciferol, the active form of vitamin D, and consequently reducing intestinal absorption and bone resorption (Jacobson *et al.*, 1972 ; Goff, 2008, 2014).

The serum Mg concentration is very dependent of the Mg received from the diet once there are almost no physiological controls of Mg homeostasis (Dalley 1992; Goff, 2008). Despite the fact that PTH has a great effect in Mg concentration, by decreasing renal excretion and stimulating bone resorption, serum magnesium concentration alone is not enough to trigger PTH release (Jacobson *et al.*, 1972; Dalley 1992). Control of magnesium serum concentration just occurs when its concentrations drops below 1.3 mg/dL, which was very rare in this experiment. Because of that, we can suppose that the differences in Mg concentrations between seasons is mainly caused by the different diet contents of the mineral.

The incidence of hyperphosphatemia is a result of an excessive phosphorus supplementation, once their serum concentration is mainly dependent of the content and availability in the diet, as magnesium concentration. That is because phosphorus homeostasis is barely regulated, only being controlled when in excess by increased salivary secretion and fecal excretion. The higher incidence of hyperphosphatemia can have an impact on the health of the animal as described, but there is another important consideration about the ecological issue that hyperphosphatemia can have. The excess of phosphorus supplementation is excreted in feces, increasing the output of this mineral to the environment. Phosphorus might have a potent effect especially on surface water, causing contamination and eutrophication with great consequences to the ecosystem balance, as decrease in diversity and habitat destruction. Because of that, phosphorus supplementation in livestock animals is a growing issue and the precise supplementation of this minerals is a goal to achieve (Rodríguez *et al.*, 2015).

The breed composition can influence the prevalence of hypocalcemia, as is well documented in that Jersey cows are more prone to develop this condition then Holstein cows (Goff, 2014). Unfortunately, there are no studies about the likelihood of zebu cattle developing hypocalcemia and, regardless of the high incidence of subclinical hypocalcemia in the present study, is not possible to say that the F_1 Holstein x Gyr cows are more or less likely to suffer from hypocalcemia than Holstein cows. In fact, the authors believe that the breed did not contribute significantly to the results, as cows with more proportion of Holstein genes in almost identical condition had similar frequency of hypocalmecima and average serum calcium concentration (Moreira *et al.*, 2015). Is is noteworthy that crossbreed F_1 Holstein x Gyr are nowadays able to have average daily productions superior to 20L/day, similar to Holstein cows managed under the same condition (Madalena 1998; Moreira *et al.*, 2015). This can have an impact in the mineral imbalances once high producing cows have a greater chance for developing hypocalcemia (Fleischer *et al.*, 2001).

The high number of cows suffering from subclinical hypocalcemia in many Brazilian studies in different regions and conditions is disturbing. In many of these studies, the frequency of subclinical hypocalcemia was above 60% and the lowest prevalence was of

33% (Paula *et al.*, 2011; Souza Júnior *et al.*, 2011; Greghi *et al.*, 2014; Moreira *et al.*, 2015; Silva Filho *et al.*, 2015). There is a remarkable difference when we compare these results with a Chilean study with grazing cattle conducted from 2003 to 2011, using a cut point of 8.0mg/dL for hypocalcemia, that find a much lower frequency of only 3.4% of animals below the cut point (Wagemann *et al.*, 2014). One other Brazilian study performed by Alvarenga *et al.* (2015) showed better results with 16.7% of animals with subclinical hypocalcemia. These results demonstrate that is possible to decrease the incidence of hypocalcemia and that further researches are necessary to understand factors that are causing this high incidence in so many studies in Brazil.

CONCLUSIONS

The transition period was challenging for the F_1 Holstein x Gyr cows that could not keep the serum concentrations of most minerals within physiological limits. Season has a great impact on mineral metabolism and winter surprisingly represented the most challenging season with lower concentrations of calcium and magnesium, and greater frequency of hypocalcemia and hypomagnesemia.

The differences in nutrition and food supply according to the season were directly responsible for the differences in magnesium and phosphorus concentrations and, because of the strong association between minerals metabolism, lower magnesium concentration contributed for the high hypocalcemia incidence in the winter, as the higher phosphorus concentration may play a role in hypocalcemia in both seasons.

The large proportion of animals experiencing subclinical hypocalcemia is of great concern, once this condition is considered a ‘gateway’ disease that leads to many consequences to animal health. The constant reporting of high frequency subclinical hypocalcemia in many Brazilian studies is alarming and more attention should be given to this subject.

Hyperphosphatemia have a health impact for the cows, but aside from that, the excess of this mineral might cause several important ecological issues, what makes a more accurate P supplementation needed to diminish P excretion to the environment.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the cooperation of the owners, the herdsman, and the staff from the farm. We also acknowledge the Universidade Federal de Minas Gerais and Brazilian Research Council (CNPq) for supporting this work.

REFERENCES

ALVARENGA, E.A.; MOREIRA, G.H.F.A.; FACURY FILHO, E.J. *et al.* Avaliação do perfil metabólico de vacas da raça Holandesa durante o período de transição. *Pesqui. Vet. Bras.*, v.35, p.281-290, 2015.

BELCHIOR, A.P.C.; LOPES, L.B.; GONÇALVES, V.S.P.; LEITE, R.C. Prevalence and risk factors for bovine tuberculosis in Minas Gerais state, Brazil. *Trop. Anim. Health Prod.,* v.48, p.373-378, 2015.

CHAPINAL, N.; CARSON, M.; DUFFIELD, T.F. *et al.* The association of serum metabolites with clinical disease during the transition period. *J. Dairy Sci.*, v.94, p.4897-4903, 2011.

DALLEY, D.E. *Studies of magnesium metabolism in ruminants.* 1992. 149f. These (PhD) - Lincoln University, Christchurch, Christchurch, NZL.

FLEISCHER, P.; METZNER, M.; BEYERBACH, M. *et al.* The relationship between milk yield and the incidence of some diseases in dairy cows. *J. Dairy Sci.*, v.84, p.2025-2035, 2001.

FOOD and agriculture organization of the United Nations "production/ livestock primary/ milk, whole fresh cow" and “live animals/ cattle”. 2013. Available in: <http://faostat3.fao.org/browse/Q/QL/E>. Accessed in 24 May 2016.

GOFF, J.P. Calcium and magnesium disorders. *Vet. Clin. N. Am. Food Anim. Pract.*, v.30, p.359-381, 2014.

GOFF, J.P. Macromineral disorders of the transition cow. *Vet. Clin. N. Am. Food Anim. Pract.*, v.20, p.471-494, 2004.

GOFF, J.P. Pathophysiology of calcium and phosphorus disorders. *Vet. Clin. N. Am. Food Anim. Pract.*, v.16, p.319-337, 2000.

GOFF, J.P. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. *Vet. J.*, v.176, p.50-57, 2008.

GREGHI, G.F.; NETTO, A.S.; SCHALCH, U.M. *et al.* Suplemento mineral aniônico para vacas no periparto : parâmetros sanguíneos, urinários e incidência de patologias de importância na bovinocultura leiteira. *Pesqui. Vet. Bras.*, v.34, p.337-342, 2014.

JACOBSON, D.R.; HEMKEN, R.W.; BUTTON, F.S.; HATTON, R.H. Mineral nutrition, calcium, phosphorus, magnesium, and potassium interrelationships. *J. Dairy Sci.*, v.55, p.935-944, 1972.

KIMURA, K.; REINHARDT, T.; GOFF, J.P. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. *J. Dairy Sci.*, v.89, p.2588-2595, 2006.

LEAN, I.J.; DEGARIS, P.J.; MCNEIL, D.M.; BLOCK, E. Hypocalcemia in dairy cows: meta-analysis and dietary cation anion difference theory revisited. *J. Dairy Sci.*, v.89, p.669-684, 2006.

LEAN, I.; DEGARIS, P. *Transition cow management veterinarians and farm advisers*. Melbourne: Dairy Australia, 2010. 52p.

MADALENA, F.E. Hybrid F1 Bos taurus Bos indicus dairy cattle production in the state of Minas Gerais, Brazil. In: WORLD CONGRESS ON GENETICS APPLIED TO LIVESTOCK PRODUCTION, 6., 1998. Armidale. *Proceedings…*, Armidale: [s.n.], 1998. p.199-202.

MADALENA, F.E.; JUNQUEIRA, F.S. The value of sexed bovine semen. *J. Anim. Breed. Genet.*, v.121, p.253-259, 2004.

MARTINEZ, N.; SINEDINO, L.D.P.; BISINOTTO, R.S. *et al.* Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. *J. Dairy Sci.*, v.97, p.874-887, 2014.

MATOS, L.L. Do pasto ao leite com tecnologia. In: SIMPÓSIO DE NUTRIÇÃO E PRODUÇÃO DE GADO DE LEITE, 2., 2001, Belo Horizonte. *Anais*... Belo Horizonte: UFMG, 2001. p.50-65.

MOREIRA, T.F.; ZAMBRANO, J.A.U.; PAULA, V.M. *et al.* Perfil mineral de vacas mestiças Girolanda no período de transição em sistema semi-intensivo em duas estações do ano. *Pesqui. Vet. Bras.*, v.35, p.249-257, 2015.

OETZEL G.R. Oral calcium supplementation in peripartum dairy cows. *Vet. Clin. N. Am. Food Anim. Pract.*, v.29, p.447-455, 2013.

PAULA V.M., FREITAS M.D., MOREIRA T.F. *et al.* Perfil mineral e bioquímico de vacas leiteiras no período de transição em um sistema semi-intensivo em Minas Gerais. In: CONGRESSO BRASILEIRO DE BUIATRIA, 9., 2011, Goiânia. *Anais…* Goiânia: FMVZ,2011. p.650-654. (Resumo).

RODRÍGUEZ, G.B.; FLORES, M.D.M.; SAYED, A.F.; ÁLVAREZ, N.I.O. Response to phytase addition on production variables, and excretion of phosphorus in faeces of finishing lambs. *Life Sci. J.*, v.12, p.33-36, 2015.

SAMPAIO, I.B.M. *Estatística aplicada a experimentação animal*. 2.ed. Belo Horizonte: FEPMVZ, 2002. 265p.

SEPÚLVEDA-VARAS, P.; WEARY, D.M.; NORO, M.; VON KEYSERLINGK, M.AG. Transition diseases in grazing dairy cows are related to serum cholesterol and other analytes. *PloS one*, v.10, p.1-13, 2015.

SILVA FILHO, A.P.; AFONSO, J.A.B.; CARVALHO, C.C.D. *et al.* Hormonal e mineral de vacas no momento do parto normal e com distocia. *Vet. Zootec.*, v.22, p.418-428, 2015.

SOUZA JÚNIOR J.A.; MOREIRA T.F.; LASMAR P.V.F. *et al.* Efeito da adição de monensina ou propilenoglicol na dieta de vacas leiteiras no periparto sobre concentrações séricas de minerais. In: CONGRESSO BRASILEIRO DE BUIATRIA, 9., 2011, Goiás. *Anais…* Goiânia: FMVZ,2011. p.636-639. (Resumo).

WAGEMANN, C.; WITTWER, F.; CHIHUAILAF, R.; NORO, M. Estudio retrospectivo de la prevalencia de desbalances minerales en grupos de vacas lecheras en el sur de Chile. *Arch. Med. Vet.*, v.46, p363-373, 2014.