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Characterization of major mineral contents in milk of four cattle breeds

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ABSTRACT

The average mineral content of cow milk is 8-9 g/L and the elements occur in different chemical forms. Despite minerals represent a small portion of cow milk, they are fundamental for human health and cheese-making process. Moreover, the variation of the concentration of some elements is an indicator of udder health. The aim of this thesis was to investigate sources of variation of the major mineral contents (Ca, K, Mg, Na and P) in milk of four cattle breeds (Holstein-Friesian, Brown Swiss, Simmental and Alpine Grey). A total of 954 cows were sampled once in 62 single-breed herds located in Bolzano province (Italy) between April and November 2014. Milk samples were analysed for milk chemical composition and coagulation properties. Major minerals were determined on 268 individual milks using the Inductively Coupled Plasma Optical Emission Spectrometry preceded by nitroperchloric mineralization. Pearson correlations between major mineral contents of milk, chemical composition and coagulation traits were estimated. Sources of variation of milk minerals were investigated using a mixed linear model that included the fixed effects of breed, stage of lactation and parity, and the random factors of herd nested within breed and residual. There were moderate to strong correlations between minerals, with the strongest estimates between Ca, P and Mg (0.77 to 0.81). These three minerals were moderately correlated with milk traditional traits (fat, protein and casein), with values comprised between 0.38 and 0.41, and Ca was favourably correlated with rennet coagulation time, curd-firming time and curd firmness. Breed effect was not important in explaining the variation of major minerals in milk, and the only significant difference was assessed between Ca content in milk of Brown Swiss (1,278 mg/kg) and Simmental (1,417 mg/kg) cows. Days in milk affected significantly all mineral contents. Overall, Ca, Mg, Na and P exhibited the lowest and the greatest concentration at the beginning and end of lactation, respectively, whereas the overall trend for K resembled that of milk yield across lactation. Parity influenced significantly only Na and P. In particular, Na concentration was lower in milk from first- than later-parity cows, whereas P showed an opposite trend. Results of the present thesis indicate that mineral contents of milk did not differ across breeds. However, further research is needed to support these findings, but using a greater number of samples. For this purpose, it would be interesting to compare the results obtained by reference method considered in this work, with those obtained by innovative, faster and cheaper technologies such as mid-infrared spectroscopy (MIRS).

Key words: cow breed, ICP-OES, milk mineral

RIASSUNTO

Caratterizzazione del contenuto di macroelementi minerali nel latte di quattro razze bovine

Il contenuto medio di minerali nel latte vaccino è 8-9 g/L ed essi sono presenti in diverse forme chimiche. Nonostante i minerali rappresentino una frazione ridotta del latte vaccino, sono essenziali per la salute umana e per il processo di caseificazione. Inoltre, la variazione di concentrazione di alcuni elementi è un indicatore dello stato sanitario della mammella. Il presente lavoro di tesi si è proposto di caratterizzare il contenuto dei principali minerali nel latte di quattro razze bovine (Frisona, Bruna, Pezzata Rossa e Grigio Alpina). Per tale scopo, sono state campionate 954 vacche in 62 aziende monorazza in provincia di Bolzano (Italia), da aprile a novembre 2014. I campioni di latte sono stati analizzati per determinare la composizione chimica e le proprietà coagulative. I principali minerali sono stati quantificati in 268 campioni di latte con il metodo “Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)”, previa mineralizzazione nitroperclorica. Sono state stimate le correlazioni di Pearson tra i minerali, i caratteri qualitativi ed i caratteri tecnologici del latte. Le fonti di variazione del contenuto minerale del latte sono state studiate utilizzando un modello lineare misto che includeva gli effetti fissi della razza, dello stadio di lattazione e dell’ordine di parto, e gli effetti casuali dell’allevamento entro razza e del residuo. Si sono riscontrate correlazioni medio-forti e significative tra i minerali, in particolare tra Ca, P e Mg (da 0,77 a 0,81). Questi tre minerali sono risultati moderatamente correlati con grasso, proteina e caseina (da 0,38 e 0,41), e il Ca ha mostrato correlazioni favorevoli con il tempo di coagulazione, il tempo di rassodamento e la consistenza del coagulo. L’effetto razza non è risultato significativo nello spiegare la variabilità dei minerali nel latte, e l’unica differenza significativa si è riscontrata tra il contenuto di Ca nel latte di Bruna (1.278 mg/kg) e nel latte di Pezzata Rossa (1.417 mg/kg). Lo stadio di lattazione ha influenzato significativamente il contenuto minerale nel latte. In generale, Ca, Mg, Na e P hanno evidenziato concentrazioni più basse ad inizio lattazione e più alte a fine lattazione. Per quanto concerne il K, l’andamento lungo la lattazione è risultato simile a quello della produzione di latte. L’ordine di parto ha influenzato significativamente soltanto Na e P. La concentrazione di Na è risultata più bassa nel latte di vacche primipare rispetto alle pluripare, mentre il P ha mostrato un andamento opposto. I risultati ottenuti in questa tesi suggeriscono che il contenuto dei principali minerali nel latte vaccino non risenta di un effetto razza. Tuttavia, sono necessarie ulteriori ricerche per approfondire questo aspetto, ma su una base dati più ampia. In tal senso, sarebbe interessante comparare i risultati ottenuti con il metodo di riferimento (ICP-OES), con quelli ottenibili impiegando tecnologie innovative, veloci ed economiche, come la spettroscopia nel medio infrarosso (MIRS).

Parole chiave: razza bovina, ICP-OES, minerali del latte

1. INTRODUCTION

1.1 THE MINERAL ELEMENTS

Minerals are essential elements swallowed through the diet. They are classified into three groups: macrominerals, microminerals and trace elements. The macrominerals are sodium (Na), calcium (Ca), potassium (K), phosphorus (P), magnesium (Mg) and chloride (Cl); the microminerals are iron (Fe), zinc (Zn), copper (Cu), fluorine (F), iodine (I), selenium (Se), manganese (Mn), chrome (Cr) and cobalt (Co); trace elements group several minerals not essential for the organism such as bromine (Br), nickel (Ni), lithium (Li), silicon (Si) and tin (Sn). Minerals represent 4% of total human body mass and they are found in tissues, liquids, cells and organs. Minerals have structural, biochemical, catalytic and nutritional functions, thus they are fundamental for human health (Zamberlin et al., 2012).

Cow milk and dairy products are valuable sources of essential minerals (on average 10 to 20% of daily dietary intake) (Zamberlin et al., 2012). Minerals represent a small portion of milk (about 8-9 g/L) and they occur in different chemical forms: inorganic ions and salts or as parts of proteins, nucleic acids, fats and carbohydrates (Gaucheron, 2005; Gao et al., 2009; Summer et al., 2009). The demand of dairy products and, among them, of fortified products, is increasing in the global market. The consumers requires healthy food and thus they pay serious attention to the nutritional composition. Some studies argued that minerals have better functional effects when combined with other compounds such as vitamins, proteins and fatty acids (Soyeurt et al., 2009). Minerals are studied not only to evaluate their effects on human health, but also for their technological properties, in particular for cheese production. Indeed, more than 70% of Italian milk is transformed into cheese and Ca, P and Mg contents influence milk coagulation properties (MCP) (Stefanon et al., 2002; Malacarne et al., 2014; Toffanin et al., 2015a). Other elements like Na, Cl and K are involved in diagnosis of specific diseases such as mastitis in dairy cows (Hamann and Krömker, 1997; Summer et al., 2009).

1.2 MAJOR MINERALS IN COW MILK

The major minerals in cow milk are Ca, P, Mg, Na and K. The average concentration, standard deviation and range of these elements calculated from the literature (Gaucheron et al., 1996; Murcia et al., 1999; Gaucheron, 2005; Bartowska et al., 2006; Cashman, 2006; Summer et al., 2009; Van Hulzen et al., 2009; Zamberlin et al., 2012; Pereira, 2014) are reported in Table 1.

Table 1 – Major minerals in cow milk.

Mineral	Mean, mg/L	SD[1], mg/L	Range, mg/L
Calcium	1,194	39.2	1,120-1,235
Phosphorus	1,112	406	825-1,995
Magnesium	117	8.54	100-125
Sodium	531	83.7	446-669
Potassium	1,550	136	1,360-1,769

[1] SD = standard deviation.

1.2.1 Calcium

Calcium is *“the mineral of milk”* (Cashman, 2006) and the mean value is 1,194 mg/L (Table 1). The recommended dietary Ca intake is 1,200 mg/day (Table 2).

Table 2 – Recommended daily intake of calcium, phosphorus and magnesium (Zamberlin et al., 2012).

Category	Age (years)	Mineral		
		Calcium (mg)	Magnesium (mg)	Phosphorus (mg)
Infants	0.0-0.5	200	30	100
	0.5-1.0	260	75	275
Children	1-3	700	80	460
	4-8	1,000	130	500
Males	9-13	1,300	240	1,250
	14-18	1,300	410	1,250
	19-30	1,000	400	700
	31-50	1,000	420	700
	51-70	1,200	420	700
	>70	1,200	420	700
Females	9-13	1,300	240	1,250
	14-18	1,300	360	1,250
	19-30	1,000	310	700
	31-50	1,000	320	700
	51-70	1,200	320	700
	>70	1,200	320	700
Pregnancy	14-18	1,300	400	1,250
	19-30	1,000	350	700
	31-50	1,000	360	700
Lactation	14-18	1,300	360	1,250
	19-30	1,000	310	700
	31-50	1,000	320	700

Approximately 21 to 45% of milk Ca is absorbed in the human intestine and the rate of absorption depends on vitamin D, lactose and the age of the individual. The bioavailability of Ca from milk is greater than that of Ca from vegetables, probably because Ca is bound to caseins in milk (Zamberlin et al., 2012). A daily Ca intake lower than the recommended one could have serious negative effects for human health, like the onset of osteoporosis, very common in menopausal women, hypertension, and colon and breast cancer (Haug et al., 2007; Soyeurt et al., 2009). Calcium is responsible for regular cardiac rhythm maintenance, blood pressure and clotting regulation, hormone secretion, muscle contraction, enzyme activation and body fat and weight. Moreover, it is important for teeth, skin and hair health (Cashman, 2006; Caroli et al., 2011).

In milk, Ca is an important component of casein micelles and it contributes to the stability of the micelles themselves. Caseins are the most representative fraction of proteins in cow milk and they play an important role in the cheese-making process. Calcium concentration depends on casein amount in milk (Carroll et al., 2006). Indeed, two-third of this mineral can be found as Ca phosphate in the colloidal phase, bound to casein micelles or as Ca ions, linked to phosphoserine caseins residues. The remaining part can be found in the soluble phase, bound to citric acid, and bound to α-lactalbumin. Calcium is completely solubilized in milk at pH 3.5 (Gaucheron, 2005; Zamberlin et al., 2012; Pereira, 2014).

1.2.2 Phosphorus

Bovine milk is an important source of P, with an average concentration of 1,112 mg/L (Table 1). The recommended dietary P intake is about 700 mg/day, but it is about 1,250 mg for adolescents and pregnant women (Table 2). The absorption of P in the intestine is promoted when its amount increases in the diet and also by low level of this mineral in blood. Saliva is an important source of P (Knowlton and Herbein, 2002). This mineral is essential for bone, skin, hair and teeth health, energy metabolism, fatty acids transport, phospholipid synthesis, aminoacids metabolism and protein synthesis. Phosphorus is also a component of nucleic acids and consequently it is involved in cellular metabolism and in buffer and enzyme systems. An excessive intake of P can reduce the reabsorption of Ca, with negative effects on skeleton health (Cashman, 2006).

Phosphorus in milk is involved in the stabilization of caseins and is present in two forms: organic and inorganic. Organic P is bound to molecules such as proteins, organic acids, phospholipids and nucleotides, mainly in colloidal phase, and inorganic ionic P is mainly present in soluble fraction. Phosphorus constituting Ca fosfate in casein micelles is also considered inorganic (Gaucheron et al., 1996; Pereira, 2014). This forms depends on pH value of the mean; in fact when milk pH drops at 5.2 (the normal milk pH is 6.7), inorganic P is completely solubilized and consequently Ca fosfate molecules are completely destroyed. The modification is irreversible (Gaucheron, 2005). Moreover, this mineral is involved in the milk acidity regulation and represents, together with caseins, 4/5 of the titratable acidity (TA) (Stefanon et al., 2002). Phosphorus is moderately correlated with TA and pH. Indeed, Toffanin et al. (2015a) reported a coefficient of correlation of 0.54 between P and TA, and Stefanon et al. (2002) found a negative correlation between P and pH (-0.61).

1.2.3 Magnesium

Magnesium is an ubiquitous food mineral. Milk is a good source of Mg with an average content of 117 mg/L (Table 1). The recommended dietary intake of Mg is about 310-320 mg per day for females and 400-420 mg per day for males (Table 2). The absorption of Mg in the small intestine is promoted by lactose, but decreases when dietary P concentration increases (Knowlton and Herbein, 2002; Zamberlin et al., 2012). Magnesium is important for human health because it regulates many physiological processes, such as bone growth, blood pressure, protein and nucleic acids metabolism, neuromuscular trasmission and muscle contraction. Besides, Mg acts as co-factor of many enzymes and has an important role in reducing asthma. Magnesium deficiency can increase the risk of osteoporosis, mainly in menopausal women, the risk of atherosclerosis and lead to oxidative stress. The reduction of Mg level in blood may occur after kidney disease and use of some diuretic substances; this phenomenon is very common in old people because they have less appetite and consequently follow an unbalanced diet (Cashman, 2006; Haug et al., 2007; Zamberlin et al., 2012). In milk, 65% of Mg is found in the soluble phase, as Mg citrate, Mg phosphate and free ions. The remaining part is in colloidal phase and is bound to casein micelles (Zamberlin et al., 2012).

1.2.4 Sodium

Sodium is a monovalent cation mainly located in extracellular fluids. If compared to other major minerals, its concentration in bovine milk is relatively low, with an average of 531 mg/L (Table 1). Anyway, dairy products (e.g. cheese) are good sources of Na because salt is often added during their production. The recommendend dietary Na intake is about 1.5 g per day (Table 3).

Sodium is basic for human health because it is involved in the maintenance of acid-base balance, the cellular membrane potential, and the regulation of osmotic pressure, but an excessive intake of this mineral causes an increase of urinary Ca excretion (calciuria) and it may be responsible of serious problems in high-blood pressure sufferers. Sodium in milk is found mainly as free ions, but it can be also bound to chloride (NaCl) (Cashman, 2006; Zamberlin et al., 2012).

Table 3 – Recommended daily intake of potassium and sodium (Zamberlin et al., 2012).

Category	Age (years)	Mineral element	
		Potassium (g)	Sodium (g)
Infants	0.0-0.5	0.4	0.12
	0.5-1.0	0.7	0.37
Children	1-3	3.0	1.0
	4-8	3.8	1.2
Males	9-13	4.5	1.5
	14-18	4.7	1.5
	19-30	4.7	1.5
	31-50	4.7	1.5
	51-70	4.7	1.3
	>70	4.7	1.2
Females	9-13	4.5	1.5
	14-18	4.7	1.5
	19-30	4.7	1.5
	31-50	4.7	1.5
	51-70	4.7	1.3
	>70	4.7	1.2
Pregnancy	14-18	4.7	1.5
	19-30	4.7	1.5
	31-50	4.7	1.5
Lactation	14-18	5.1	1.5
	19-30	5.1	1.5
	31-50	5.1	1.5

1.2.5 Potassium

Potassium is one of the most important intracellular cations, but in a lower concentration is present also in the extracellular fluids. Potassium is found in cow milk, mainly in aqueous phase, with an average concentration of 1,550 mg/L (Table 1). The recommended dietary K intake is about 4.7 g/day (Table 3). This mineral has many functions for human health, like trasmission of nerve impulses, muscle contraction, regulation of blood pressure, maintenance of acid-base equilibrium and water-electrolyte balance. Furthermore, some studies have supported that K is involved in skeleton health and in the prevention of Na-induced calciuria promoting reabsorption of Ca in the kidney and reducing urinary Ca excretion (Bartowska et al., 2006; Cashman, 2006; Zamberlin et al., 2012).

1.3 MILK MINERALS ANALYSIS

Many methods are used to analyze milk minerals and some of them are specific for particular elements.

1.3.1 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

The Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is one of the fastest analysis to simultaneously determine several minerals in milk samples, not only at high concentrations. This technique is expensive, but has good sensitivity and accuracy in determining minerals such as Ca, P, Na, Mg, K, Mn, Zn and Fe. The ICP-OES can be conducted starting from milk sample or from milk sample previously treated with mineralization (AOAC). The mineralization step aims to reduce the organic substance to CO_2 and H_2O obtaining only soluble inorganic fraction. The mineralization has some disadvantages, mainly related with costs, time of analysis, sample contamination and analyte loss. For these reasons the direct analysis of milk samples is preferred. Moreover, it has been shown that the results obtained without mineralization do not deviate significantly from those obtained with mineralization. Before analysis, ICP-OES is calibrated with standards at known concentration made by appropriate dilutions in pure water (Soyeurt et al., 2009; Van Hulzen et al., 2009; Toffanin et al., 2015a).

1.3.2 Atomic Absorption Spectroscopy (AAS)

The Atomic Absorption Spectroscopy (AAS) is an analytic technique widely used in research studies which aims to determine inorganic ions in solution. The determination is both qualitative and quantitative. This method is based on the quantification of the energy released by an atom when it passes from an excited state to the ground state (www.wikipedia.org; Carroll et al., 2006; Summer et al., 2009).

1.3.3 Ion Chromatography

The ion chromatography is a technique used for the qualititative and quantitative determination of anions. Before analysis, milk sample is prepared with dry mineralization. The sample is analysed through a chromatographic column and the result is a cromatographic path. The instrument is calibrated by standards at known concentration. There is an excellent linearity between injected quantity of analyte in the column and chromatographic peak area. Each sample takes less than 32 minutes to be tested and multiple elements could be determined in the milk sample. For these reasons this method is very common in the dairy industry (Gaucheron et al., 1996).

1.3.4 Colorimetric method

The colorimetric method is rather conventional and is less used than methods described above; it involves the reduction of agents (mainly amidol) and the result of analysis is a blue color, more or less intense, in relation to the concentration of analyte. Before the test, the organic fraction of milk sample is destroyed. The instrument is calibrated by standards at known concentration (Allen, 1940; Summer et al., 2004).

1.3.5 Electrical Conductivity (EC)

Another specific method is the measure of the electrical conductivity (EC) of milk which is correlated to the concentration of Na, K and Cl. The determination of EC is important to diagnose mastitic milk samples (Norberg, 2005).

1.3.6 Mid-Infrared Spectroscopy (MIRS)

Mid-infrared spectroscopy (MIRS) has been evaluated as a potential tool to collect data at the population level for phenotypic and genetic purposes, and it is becoming one of the major topics in dairy science because it is fast and cheap. In the mid-infrared region, when matter is crossed by electromagnetic radiation, the bonds of the molecules make movements, which involve a more or less marked absorption of the provided energy. On the basis of supplied energy and the amount absorbed by the irradiated sample, and using spectra mathematical pretreatments, it is possible to determine the sample chemical composition and correlated compounds (De Marchi et al., 2014). MilkoScan is the instrument used for the analysis and it needs to be calibrated properly and then validated. To calibrate the instrument it is necessary to compare the results obtained from MIRS (spectra) with the results obtained from the reference method (e.g. ICP-OES). In order to select the best calibration equation, partial least squares analysis is carried out (Toffanin et al., 2015a). Soyeurt et al. (2009) investigated the potential of MIRS to predict the Ca, P, Mg, Na, and K content of cow milk. Results showed the ability of MIRS to predict Ca and P, reasonable accuracies for Mg and Na, and unsatisfactory results for K.

1.4 FACTORS INFLUENCING MINERAL COMPOSITION OF COW MILK

There are many factors that influence minerals concentration in cow milk, such as breed and additive genetic effects, lactation stage, parity, herd, feeding management, and health status of the mammary gland.

1.4.1 Breed and additive genetic effects

Milk mineral content varies among breeds. Mariani et al. (2002) reported that Ca and P contents of milk from Holstein-Friesian cows averaged 112 mg/100 ml and 89.6 mg/100 ml, respectively. These values were approximately 7% lower than mineral contents of milk from Brown Swiss, Reggiana and Modenese breeds (Table 4).

Table 4 – Calcium and phosphorus contents in milk of different breeds (Mariani et al., 2002).

		Holstein-Friesian	**Brown Swiss**	**Reggiana**	**Modenese**
Calcium, mg/100 ml	IM	115.3	122.5	123.4	121.5
	BM	109.5	118.2	117.3	118.7
Phosphorus, mg/100 ml	IM	91.6	96.7	97.5	99.0
	BM	86.8	90.5	91.6	97.6

IM = individual milk during whole lactation.
BM = bulk milk.

The same authors (Mariani et al., 2002) reported that milk of Holstein-Friesian cows had lower concentrations of all colloidal mineral elements, except for colloidal Mg, than Brown Swiss and Reggiana; on the other hand, it contained more colloidal Ca fosfate than milk of Modenese breed. The differences in terms of milk mineral composition between Holstein-Friesian and Brown Swiss were confirmed by Summer et al. (2004) (Figures 1 and 2).

Figure 1 – Calcium concentrations (mg/100 g) in milk of Italian Brown and Italian Friesian breeds (Summer et al., 2004).

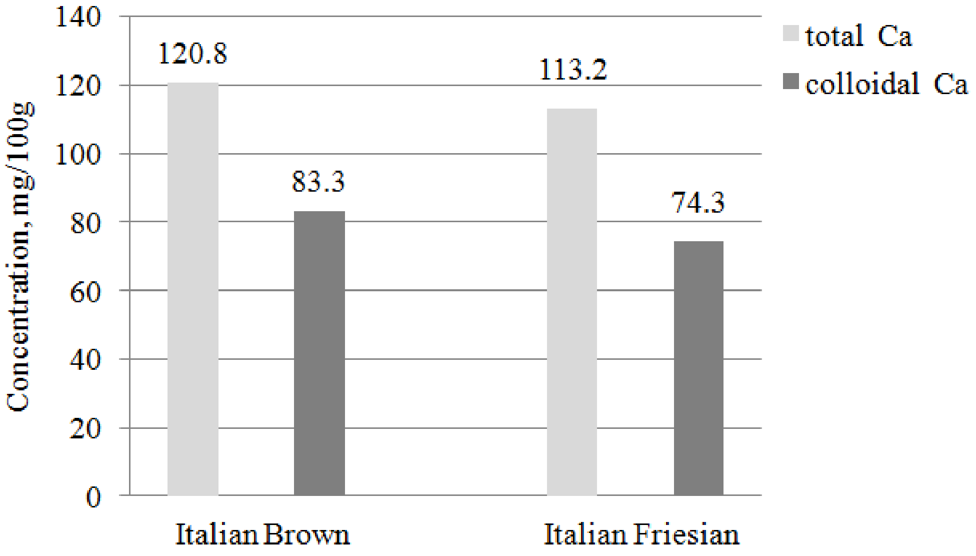
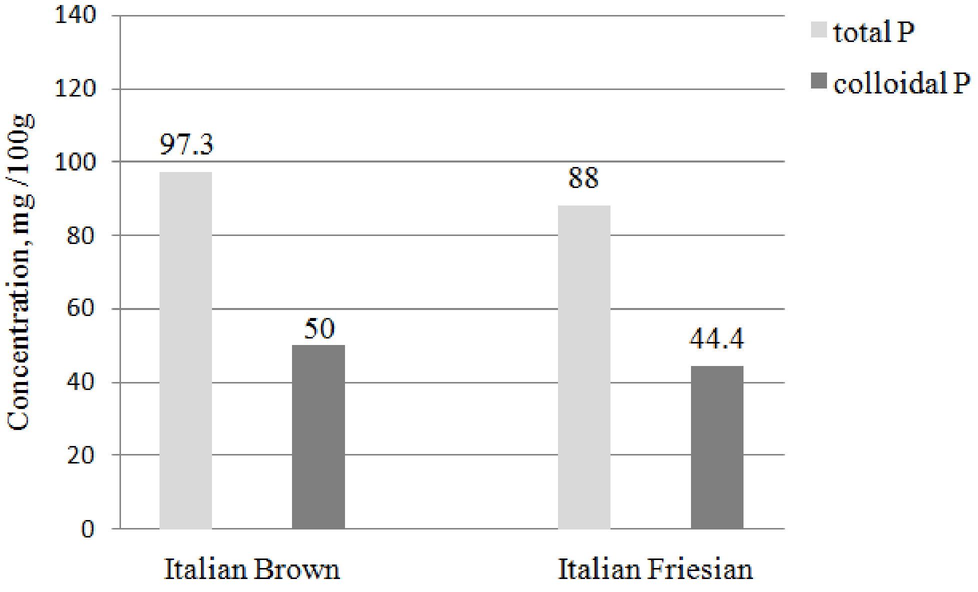


Figure 2 – Phosphorus concentrations (mg/100 g) in milk of Italian Brown and Italian Friesian breeds (Summer et al., 2004).



Calcium and P are significantly related to casein content in milk (Toffanin et al., 2015a). On average, milk of Holstein-Friesian has lower casein content than milk of Brown Swiss cows and this could explain, at least partly, the differences in milk mineral composition between the two breeds (De Marchi et al., 2008).

Mineral elements in bovine milk are quantitative traits. Their phenotypic variability within breed can be partly due to additive genetic effects. There are few studies about the genetics of milk mineral composition because the analytical costs are high. Van Hulzen et al. (2009) analyzed 1,860 milk samples of primiparous Dutch Holstein-Friesian cows to estimate heritability of milk mineral contents (Table 5).

Table 5 – Additive genetic variance (σ^2_A) and heritability (h^2) of milk minerals (Van Hulzen et al., 2009).

Mineral	**σ^2_A**	**h^2 (SE)**[1]
Calcium	6,504.48	0.57 (0.11)
Potassium	7,772.59	0.46 (0.10)
Magnesium	68.62	0.60 (0.11)
Phosphorus	4,861.86	0.62 (0.10)

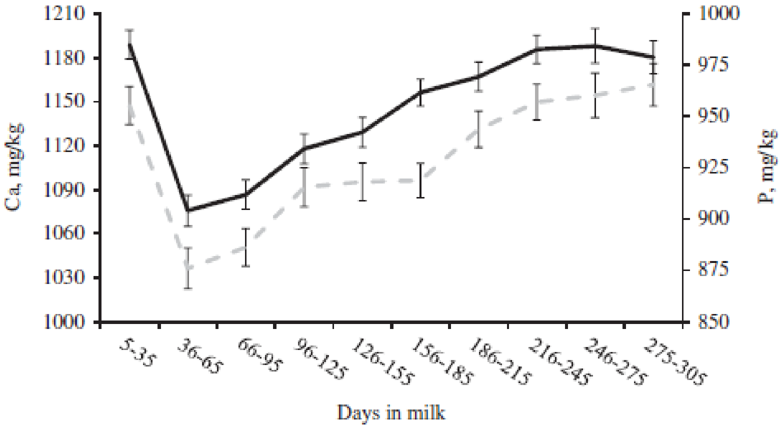
[1] SE = standard error.

Soyeurt et al. (2008) estimated heritabilities of 0.47 for P and 0.42 for Ca, while Toffanin et al. (2015b) reported lower heritabilities of 0.12 for P and 0.10 for Ca. Renner and Kosmack (1974) studied the origin of genetic variance of milk minerals and reported that the genetic variation of Ca concentration could be related to high heritability of caseins. Heck et al. (2008) reported that part of genetic variance in content of P seems to be due to the link between P and casein micelles (casein phosphoserine residues), whereas Mg is less bound to caseins, thus its genetic variation could have another origin (Van Hulzen et al., 2009). Therefore, Ca, Mg and P are traits that can be selected in animals in order to get milk with added value (Van Hulzen et al., 2009). Moreover, because Ca and P are genetically correlated with milk protein, the selection of milk protein entails an indirect selection of these minerals (Toffanin et al., 2015b). Further studies are needed to better understand of genetic aspects of milk minerals, as well as to understand if the selection for K and Na is feasible.

1.4.2 Lactation stage

Minerals concentration change across lactation. Calcium, P and Mg contents are high at the beginning of lactation, decrease rapidly until 6 to 8 weeks and increase thereafter (Figure 3) (Gaucheron, 2005; Toffanin et al., 2015b). However, some studies found different results (Haug et al., 2007; Van Hulzen et al., 2009; Zamberlin et al., 2012).

Figure 3 – Milk calcium (continuos line) and phosphorus (dashed line) concentrations across lactation (Toffanin et al., 2015b).



Potassium content is low in colostrum and it increases gradually in milk (Zamberlin et al., 2012). However, some studies found a different trend (Gaucheron, 2005; Van Hulzen et al., 2009). Sodium concentration is high in colostrum and at the end of lactation (Kume et al., 1998; Gaucheron, 2005; Zamberlin et al., 2012). This trend can be explained by changes in nutritional composition of the diet or by a dilution effect across lactation (Toffanin et al., 2015b).

1.4.3 Parity

Calcium content in plasma of periparturient cows decreases as parity increases (Table 7), which leads to higher probability of contracting milk fever in old than young cows. Magnesium and K content exhibited an opposite trend to that of Ca. Finally, plasma Na and P level were not influenced by parity (Table 6) (Kume et al., 1998).

Table 6 – Mineral concentrations in plasma of periparturient cows (Kume et al., 1998).

	Parity			SE
	1	**2**	**≥ 3**	
Number of cows	10	6	8	
Age (months)	27.1	44.7	66.5	1.7
Plasma[1]				
Ca (mg/dl)	10.25	10.20	9.99	0.04
P (mg/dl)	5.21	4.82	4.87	0.10
Mg (mg/dl)	2.05	2.37	2.29	0.02
Na (mg/dl)	325	329	327	1
K (mg/dl)	16.4	17.2	17.7	0.1

[1] Ca =calcium; P = phosphorus; Mg = magnesium; Na = sodium; K = potassium.

A reduction of minerals, mainly Ca and P, has been detected across parities (Table 7) (Toffanin et al., 2015b). The utilization of these minerals in the udder is lower in old than young cows. Moreover, older animals produce a great milk quantity, thus there is a milk dilution. These two phenomena can explain the lower mineral concentrations in milk of later parity cows (Toffanin et al., 2015b).

Table 7 – Calcium and phosphorus concentrations in cow milk across parities (Toffanin et al., 2015b).

Mineral	Parity				s.e.
	1	**2**	**3**	**4 and later**	
Calcium (mg/kg)	1,185	1,185	1,162	1,152	6
Phosphorus (mg/kg)	965	942	929	931	5

1.4.4 Herd

The phenotypic variability of milk minerals can be due to herd effect. Herd effect means environmental factors generated by, for example, management, feeding and stabulation. Van Hulzen et al. (2009) estimated the herd variance for the concentration of milk minerals and calculated the ratio between additive genetic and herd variance (Table 8).

Table 8 – Herd variance (σ^2_{herd}) and ratio between additive genetic variance and herd variance (Ratio) (Van Hulzen et al., 2009).

Mineral	σ^2_{herd}	Ratio
Ca	1,792.91	3.63
K	5,301.44	1.47
Mg	16.95	4.05
P	1,420.67	3.42

The ratio quantifies the importance of variation in genetic and herd effects. Results show that the genetic was more significant than the environmental effect (Van Hulzen et al., 2009).

1.4.5 Feeding management

Feeding management is another important factor that can influence milk minerals concentration. The mineral percentage in the diet used to fed cows is relatively low and, in addition, the phytate presence often prevents the mineral intestinal absorption. Consequently, blocks of mineral salts are found into the dairy farms to integrate dietary mineral deficiencies. Generally, milk produced by cows which are reared in mountain and fed mainly with pasture fodder contains more minerals (in particular Ca, Mg, Na and K) than milk produced by animals which are fed silages in plain (McDowell, 1996; Bartowska et al., 2006). The variability of Mg amount in milk, which is originated from the dietary Mg intake, is small and not significant (Haug et al., 2007; Zamberlin et al., 2012). Phosphorus is used inefficiently by lactating cows. Phosphorus dietary intake is very correlated to P excretion; lactating cows excrete 50 to 80% of P intake, therefore dietary P supply is basic for milk secretion. The absorption of P through the diet is promoted by a low blood P concentration. The balance of P is regulated also by saliva that contains large quantity of this mineral. Dietary Ca intake does not depend on feeding P concentration, but a dietary P supplement can decrease quadratically milk Ca secretion (Knowlton and Herbein, 2002). Potassium concentration in milk depends on its intake trough the diet, whereas Na amount in milk is not influenced by its dietary intake (Zamberlin et al., 2012). However, an increase of dietary salt (sodium chloride) causes major urinary Ca excretion (Cashman, 2006). Furthermore, it has been shown that an increase of dietary fat levels, leads to an increase of Mg and Ca concentrations in milk (Carroll et al., 2006).

1.4.6 Health status of the mammary gland

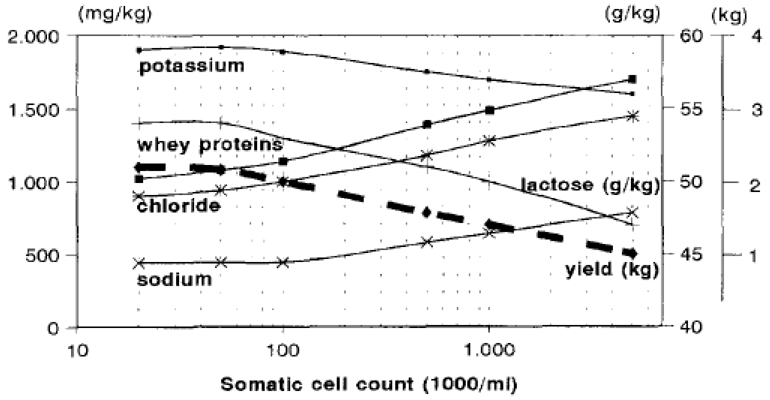
The udder health status of dairy cows has an impact on milk quality. The most frequent, serious and costly disease of the mammary gland is mastitis. Errors during milking, poor hygiene or lesions in the udder are the causes of the onset of mastitis. The subclinical mastitis, the most dangerous, is caused by *Streptococcus* or *Staphylococcus* and has a chronic course; the clinical mastitis is generally in acute form, but it can degenerate in a chronic form if not treated. It is caused by *Streptococcus*, *Staphylococcus or Enterobacter* (Bittante et al., 1993). During mastitis, tight junctions and the active transport system are destroyed and consequently Na and Cl leak into the lumen and increase in milk, while K, Ca and lactose decrease (Norberg, 2005). Mastitic milk is characterized by high pH and high somatic cell count (SCC), and thus it is not suitable for producing cheese. Milk mineral composition is significantly affected by somatic cells; the relationships of $\log_{10}$(SCC) with Na (0.87) and Cl (0.85) are strong and positive, and they are strong and negative with K (-0.64) and Ca (-0.87) (Ogola et al., 2007). Summer et al. (2009) did not find significant differences in Ca content between milk with high and low SCC. However, they detected a significant reduction of total P when moving from low to high SCC (Table 9).

Table 9 – Mineral contents in milk with different somatic cell levels (Summer et al., 2009).

Trait		**Somatic cell count (cells/ml)**		**SE**	**P value**
		<400,000	**>400,000**		
n. samples		13	13		
pH		6.67	6.71	0.02	0.05
Lactose	g/100 g	4.69	4.45	0.04	0.01
Casein	g/100 g	2.52	2.44	0.02	ns
Total Ca	mg/100 g	121.01	117.74	2.39	ns
Colloidal Ca	%	75.35	74.46	0.24	ns
Total P	mg/100 g	89.71	87.76	0.86	0.05
Colloidal P	%	53.37	57.72	1.61	ns
Total Mg	mg/100 g	11.45	11.62	0.26	ns
Colloidal Mg	%	37.23	37.43	1.61	ns
Colloidal	g/100 g	3.65	3.62	0.78	ns
Ca/casein	g/100 g	1.90	1.86	0.06	ns
Colloidal P/casein					
	mg/100 g	44.69	58.50	2.52	0.01
Na	mg/100 g	146.49	137.27	1.66	0.01
K		0.30	0.42	0.02	0.01
Na/K					

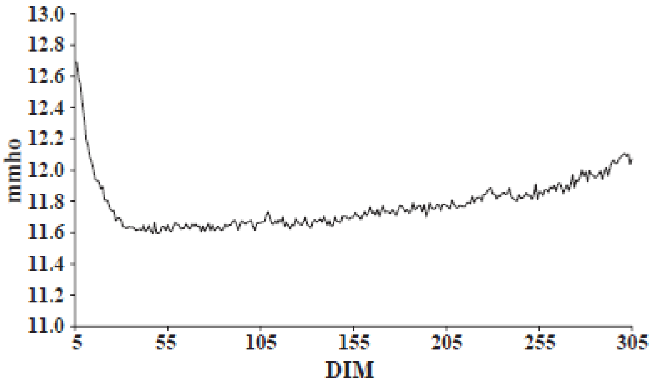
In the presence of intramammary infection, Na and Cl contents of milk increase, leading to an increase of the electrical conductivity (EC). On the contrary, K content decreases at high levels of SCC (Figure 4 and Table 9) (Hamann and Krömker, 1997; Summer et al., 2009).

Figure 4 – Relationship between SCC, minerals of milk and milk yield (Hamann and Krömker, 1997).



Usually, mastitis occurs during the first weeks of lactation. The trend of milk EC across days in milk is reported in Figure 5 (Norberg, 2005).

Figure 5 – Relation between milk electrical conductivity and days in milk (Norberg, 2005).



1.5 MILK MINERAL COMPOSITION AND COAGULATION PROPERTIES

The capacity of milk to coagulate is essential for both quantity and quality of cheese, and it is typically measured using three milk coagulation properties (MCP): rennet coagulation time (RCT), curd-firming time (k_{20}) and curd firmness (a_{30}). Milk coagulation ability depends mainly on milk chemical composition and genetic and seasonal factors (Macheboeuf et al., 1993). Magnesium, Ca, P and the Ca/P ratio show favourable correlations with MCP. Calcium has an aggregating function of paracasein micelles essential for the clot formation. An adequate increase in Ca and P leads to shorter RCT and firmer curd (Mariani et al., 2002; Van Hulzen et al., 2009; Toffanin et al., 2015a). Milk with high casein-P, colloidal-Ca and colloidal-Mg levels is optimal for cheese-making process. Another important factor that influence MCP is acidity. Milk with too low values of pH is usually characterized by high P content and low Ca content; on the other hand, milk with high values of pH is deficient in soluble P. These kinds of milk take longer to coagulate and k_{20} and a_{30} are not optimal (Storry et al., 1983; Stefanon et al., 2002; Malacarne et al., 2014).

1.6 AIM OF THE STUDY

Milk minerals play a central role for human health because they are involved in the regulation of metabolic processes and acid-base balance, and in the prevention of several diseases. They are essential also for casein micelles structure and stability, and thus for cheese-making process. The aim of this study was to assess the sources of variation of the major minerals of milk from dairy (Holstein-Friesian and Brown Swiss) and dual-purpose (Simmental and Alpine Grey) cattle breeds.

2. MATERIALS AND METHODS

2.1 MILK SAMPLE COLLECTION

A total of 954 cows from 2 dairy (Holstein-Friesian, n = 237, and Brown Swiss, n = 237) and 2 dual-purpose (Simmental, n = 240, and Alpine Grey, n = 240) breeds were sampled once in 62 single-breed herds located across the Bolzano province (northeast Italy) from April to November 2014. For each cow, two 50-mL samples were collected and immediately added with preservative. One sample was transferred at 4°C to the laboratory of the South Tirol Dairy Association (Bolzano, Italy) and analysed for milk chemical composition, using a MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark), and for somatic cell count (SCC), which was assessed using a Fossomatic (Foss Electric A/S, Hillerød, Denmark). Values of SCC were transformed to somatic cell score (SCS) using the formula $SCS = 3 + \log_2(SCC/100{,}000)$. The other sample was transported to the Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE) of the University of Padova, and divided into two subsamples: one subsample was stored at -20°C and one was delivered to the laboratory of the Breeders Association of Veneto region (Padova, Italy) for MCP analysis.

2.2 MILK MINERALIZATION

Milk minerals content was determined on 268 individual milks, with approximately the same number of samples for each breed. A nitroperchloric mineralization by MILESTONE START D microwave (1,200 watt, Milestone Srl Sorisole, Bergamo, Italy) was used. The microwave contained a SK-10 rotor at high pressure (64 bar) and control systems with temperature probe and software. Milk samples stored at -20°C were thawed in water at 35°C and homogenized before sampling. A total of 2.5 g of sample was introduced in Teflon vessels at high pressure. Then, 2 mL of H_2O_2 30% and 7 mL of HNO_3 67% were added. The vessel was hermetically sealed and placed in the microwave. The process of mineralization involved three steps. In the first step the sample was heated to 200°C in 15 min; in the second step the sample was kept constantly at 200°C for 18 min; finally, the sample was cooled down to 35°C. The mineralized sample was added with demineralized water to reach a final volume of 25 ml.

2.3 INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY (ICP-OES)

The minerals determination was conducted with ICP-OES, using a SPECTRO ARCOS (SPECTRO Analytical Instruments GmbH, Kleve, Germany). The ICP-OES was employed to determine calcium (Ca) at 315.887 nm, potassium (K) at 766.491 nm, magnesium (Mg) at 280.270 nm, phosphorous (P) at 177.495 nm and sodium (Na) at 589.592 nm. Instrument operating parameters were optimized for aqueous solutions with undissolved organic material. Calibration standards were matched with 1% ethanol absolute (Prolabo VWR International PBI S.r.l. Milano, Italy). The elements to be determined were added from single element solutions (Inorganic Ventures, Christiansburg, VA, USA). The concentrations range of the calibration solutions were between 0 and 25 mg/L for all elements. The accuracy and precision of method were investigated analyzing the certified reference material BCR® – 063R “Skim milk powder” (Institute for Reference Materials and Measurements (IRMM), Geel, Belgium). These standards of “Skim milk powder” were mineralized according to the protocol described in the previous paragraph. The measured and the certified values were in excellent agreement for all the elements.

2.4 MILK COAGULATION PROPERTIES

Milk coagulation properties (MCP) were determined by Formagraph (Foss Electric A/S, Hillerød, Denmark) in the laboratory of the Breeders Association of Veneto region (Padova, Italy), using Hansen standard rennet with a rennet activity of 0.051 IMCU/mL of milk. Three measures of MCP were recorded: rennet coagulation time (RCT, min), defined as the time from the addition of rennet to milk to the beginning of coagulation, curd-firming time (k_{20}, min), which is the time from the beginning of coagulation to the moment the width of the curve reaches 20 mm, and curd firmness (a_{30}, mm), defined as the width of the curve 30 min after rennet addition (Ikonen et al., 1997; De Marchi et al., 2014).

2.5 DATA EDITING

The original dataset was edited as follows: days in milk were restricted to be between 6 and 540 d; herds with less than 5 records were removed; values of minerals were labeled as missing if they deviated more than 3 standard deviations from the respective mean; RCT, k_{20} and a_{30} were considered missing if values were equal or lower than zero; and values of pH were identified as missing if lower than 6.30. The number of records available for each trait after editing is reported in Table 10.

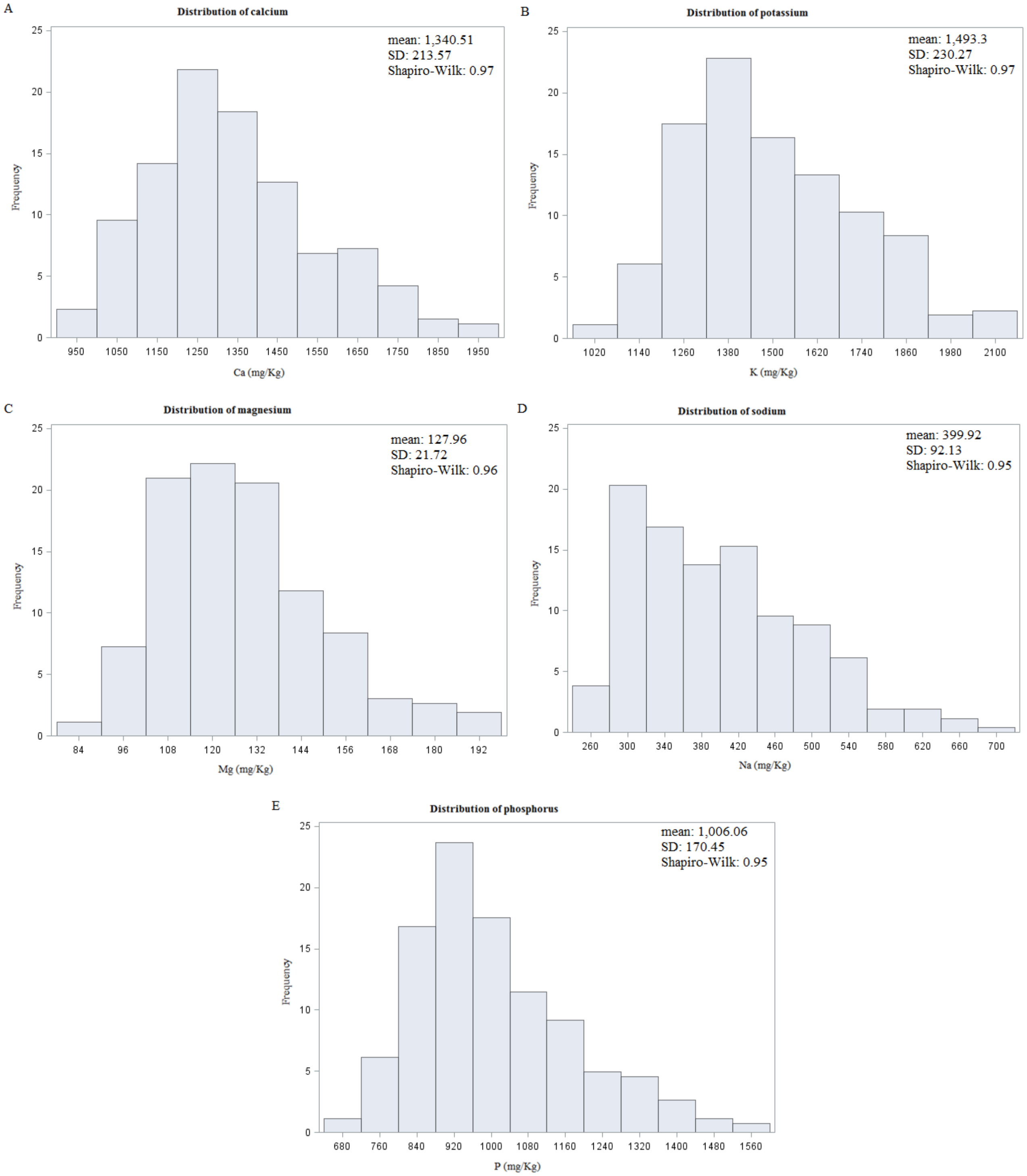
2.6 STATISTICAL ANALYSIS

A preliminary analysis showed that milk minerals were normally distributed (Figure 6). Pearson correlations between major milk mineral contents, quality traits and technological properties were estimated through the CORR procedure of SAS (SAS Institute Inc., Cary, NC, USA). Sources of variation of major milk minerals were investigated using the MIXED procedure of SAS (SAS Inst. Inc.) according to the following linear model:

$$y_{ijklm} = \mu + \text{breed}_i + \text{herd}_j(\text{breed})_i + \text{DIM}_k + \text{parity}_l + \varepsilon_{ijklm},$$

where y_{ijklm} is the dependent variable (Ca, K, Mg, Na or P content); μ is the overall intercept of the model; breed$i$ is the fixed effect of the ith breed (i = Holstein-Friesian, Brown Swiss, Simmental, Alpine Grey); $\text{herd}_j(\text{breed})_i$ is the random effect of the jth herd (j = 1 to 40) nested within the ith breed; DIM_k is the fixed effect of the kth class of stage of lactation of the cow (k = 1 to 6, the first being a class from 6 to 60 d, followed by classes of 60 d each, and the last being a class from 301 to 540 d); parity_l is the fixed effect of the lth parity of the cow (l = first, second, third, and fourth and later parities); and ε_{ijklm} is the random residual $\sim N(0, \sigma^2_\varepsilon)$. In the model, the herd and test-day effects were confounded because cows in each herd were sampled only once, all on the same day. Breed effect was tested using herd within breed as the error term, whereas other fixed effects were tested on the residual. A multiple comparison of means was performed for the fixed effects of breed, DIM and parity ($P<0.05$).

Figure 6 – Distribution of (A) calcium, (B) potassium, (C) magnesium, (D) sodium and (E) phosphorus.



3. *RESULTS AND DISCUSSION*

3.1 DESCRIPTIVE STATISTICS

Descriptive statistics of traditional and technological traits of milk samples collected from four dairy cattle breeds are presented in Table 10.

Table 10 – Descriptive statistics of milk yield, quality traits, technological characteristics and mineral contents.

Trait[1]	N	Mean	SD[2]	Minimum	Maximum
Milk yield and quality					
Milk yield (kg/milking)	873	11.6	4.21	2.00	27.6
Fat (%)	933	4.07	0.72	1.58	9.19
Protein (%)	933	3.60	0.45	2.34	5.5
Casein (%)	933	2.81	0.36	1.71	4.38
Lactose (%)	933	4.77	0.20	3.84	5.48
Milk technological traits					
RCT (min)	813	18.6	4.60	4.30	29.0
k_{20} (min)	602	5.24	1.62	2.00	13.2
a_{30} (mm)	829	27.4	12.7	0.02	57.0
pH	931	6.65	0.07	6.34	6.92
SCC (cells/ml)	933	186,523	343,914	1,046	5,854,000
SCS	933	2.82	1.84	-3.57	8.87
Milk minerals (mg/kg)					
Ca	261	1,341	214	937	1,988
K	263	1,493	230	1,001	2,110
Mg	262	128	21.7	84.1	193
Na	261	400	92.1	255.61	704
P	262	1,006	170	675.74	1,540

[1] RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness; SCC = somatic cell count; SCS = somatic cell score, calculated as SCS = 3 + $\log_2$(SCC/100,000); Ca = calcium content; K = potassium content; Mg = magnesium content; Na = sodium content; P = phosphorus content.

[2] SD = standard deviation.

Milk yield, fat content, protein content, casein content, and lactose content averaged 11.6 ± 4.21 kg/milking, 4.07 ± 0.72 %, 3.60 ± 0.45 %, 2.81 ± 0.36 %, and 4.77 ± 0.20 %, respectively. Average milk chemical composition corresponded to that reported by several studies (De Marchi et al., 2011, 2012; Penasa et al., 2014; Varotto et al., 2014; Toffanin et al., 2015a). The coefficient of variation ranged from 4.2 to 17.7% for milk quality traits and it was 36.2% for milk yield. The much higher variability of milk yield compared to quality characteristics is probably the consequence of the presence of four different breeds in the dataset. Regarding milk technological traits, approximately 13% of milk samples did not coagulate within 30 min from rennet addition and 35% of milk samples did not reach 20 mm of curd firmness. Rennet coagulation time, k_{20} and a_{30} averaged 18.6 ± 4.60 min, 5.24 ± 1.62 min, and 27.4 ± 12.7 mm, respectively. Similar findings were reported by several authors (De Marchi et al., 2012, 2013; Penasa et al., 2014; Varotto et al., 2014; Toffanin et al., 2015a). The mean pH of milk was 6.65

and, as expected, it showed low coefficient of variation (1%), in agreement with findings of De Marchi et al. (2009), Summer et al. (2009) and Varotto et al. (2014). The SCC averaged 186,523 cells/ml and it exhibited a very high standard deviation (343,914 cells/ml). The mean SCS was 2.82 and the coefficient of variation was high (65.2%), suggesting that there is great variability among breeds and among cows within breed. A similar mean value of SCS was reported by Penasa et al. (2010) and De Marchi et al. (2012). Concerning milk mineral composition, Ca, K, Mg, Na, and P contents averaged 1,341 ± 214, 1,493 ± 230, 128 ± 21.7, 400 ± 92.1, and 1,006 ± 170 mg/kg, respectively. These values are in agreement with previous findings (Zamberlin et al., 2012; Pereira, 2014; Toffanin et al., 2015a).

3.2 CORRELATIONS

Pearson correlations between milk minerals were positive and statistically significant ($P<0.001$; Table 11). Calcium was moderately correlated with K (0.55) and Na (0.42), and it was strongly correlated with Mg (0.81) and P (0.77). Toffanin et al. (2015a) estimated a Pearson relationship of 0.68 between Ca and P, which is close to the value of the present thesis. Potassium was moderately correlated with Mg (0.52), P (0.56) and Na (0.35). Magnesium was moderately correlated with Na (0.50) and strongly with P (0.78). Finally, Pearson correlation between P and Na was 0.30.

Table 11 – Pearson correlation coefficients[1] between major milk minerals.

Mineral[2]	**K**	**Mg**	**Na**	**P**
Ca	0.55	0.81	0.42	0.77
K		0.52	0.35	0.56
Mg			0.50	0.78
Na				0.30

[1] All correlations were significantly different from zero ($P<0.001$).

[2] Ca = calcium content; K = potassium content; Mg = magnesium content; Na = sodium content; P = phosphorus content.

Milk minerals were negatively and significantly ($P<0.05$) correlated with milk yield, except for K which was positively correlated with milk yield (0.32; $P<0.001$). Calcium, Mg and P were positively and significantly ($P<0.05$) correlated with fat content (0.26, 0.26 and 0.15, respectively), protein content (0.41, 0.39 and 0.40, respectively) and casein content (0.41, 0.38 and 0.39, respectively). The magnitude of the relationships of Ca, Mg and P with protein and casein were expected because minerals are bound to casein micelles. Similar results were reported by Toffanin et al. (2015a). Potassium showed negative correlations with fat (-0.17; $P<0.01$) and casein (-0.13; $P<0.05$), and Na showed a positive correlation with protein (0.17; $P<0.01$). Finally, a moderate to strong relationship between Na and lactose (-0.58; $P<0.001$) was estimated.

Table 12 – Pearson correlation coefficients between major milk minerals studied and other milk traits.

Trait[1]	**Ca**	**K**	**Mg**	**Na**	**P**
Milk yield and quality					
Milk yield	-0.19**	0.32***	-0.19**	-0.16*	-0.16*
Fat	0.26***	-0.17**	0.26***	0.03	0.15*
Protein	0.41***	-0.11	0.39***	0.17**	0.40***
Casein	0.41***	-0.13*	0.38***	0.11	0.39***
Lactose	0.01	-0.10	-0.16*	-0.58***	0.06
Milk technological traits					
RCT	-0.16*	-0.01	-0.07	0.10	-0.06
k_{20}	-0.22**	0.16*	-0.14	0.04	-0.13
a_{30}	0.24***	-0.06	0.11	-0.14*	0.14*
pH	0.23***	-0.17**	-0.07	-0.14*	0.03
SCS	-0.01	-0.09	0.07	0.32***	-0.02

[1] RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness; Ca = calcium content; K = potassium content; Mg = magnesium content; Na = sodium content; P = phosphorus content.
Statistical significance is given as: *P<0.05; **P<0.01; ***P<0.001.

Calcium was significantly ($P<0.05$) and favourably correlated with RCT (-0.16), k_{20} (-0.22) and a_{30} (0.24), suggesting that an increase of Ca content in milk leads to shorter RCT and firmer curd. Phosphorus content was only related to a_{30} (0.14; $P<0.05$). These results could be indirectly due to the link of Ca and P with casein, as reported by Cassandro et al. (2008). Calcium was significantly correlated with pH (0.23; $P<0.001$), whereas P was not correlated to milk acidity. Toffanin et al. (2015a) reported that P was correlated with TA (0.54) and Stefanon et al. (2002) estimated a strong and negative correlation between P and pH (-0.61). Potassium and Na were negatively correlated with pH (-0.17 and -0.14, respectively; $P<0.05$). Only Na was significantly correlated with SCS (0.32; $P<0.001$), confirming previous findings of Ogola et al. (2007) who reported a strong correlation between Na and $\log_{10}$(SCC) (0.87). Other milk minerals showed no significant correlations with SCS, even if Ogola et al. (2007) estimated correlations of -0.87 and -0.67 between Ca and $\log_{10}$(SCC), and K and $\log_{10}$(SCC), respectively.

3.3 ANALYSIS OF VARIANCE

The structure of data analysed in the present study was based on single-breed herds, which means that herd effect was nested within breed. Even if information on rearing conditions of Holstein-Friesian, Brown Swiss, Simmental and Alpine Grey was not available, it is likely that managerial and feeding strategies were different among herds of different breeds, at least between dairy and dual-purpose farms. Thus, the breed-estimated effect also includes a part of the rearing conditions effect.

Days in milk was significant (P<0.01) in explaining the variability of milk minerals, in agreement with Van Hulzen et al. (2009). Parity was highly significant (P<0.001) only for Na and P. Finally, breed effect did not influence significantly milk mineral contents (Table 13).

Table 13 – F-value and significance of fixed effects included in the analysis of milk macrominerals.

Trait[1]	**Breed**	**Days in milk**	**Parity**	**RSD**[2]
Milk minerals (mg/kg)				
Ca	2.21	6.63***	2.03	178.56
K	0.7	3.41**	2.43	167.06
Mg	0.48	9.4***	0.8	17.21
Na	0.16	6.73***	12.21***	73.07
P	0.93	3.4**	7.63***	128.87

[1] Ca = calcium content; K = potassium content; Mg = magnesium content; Na = sodium content; P = phosphorus content.

[2] RSD = residual standard deviation.

Statistical significance is given as: *P<0.05; **P<0.01; ***P<0.001.

3.4 LEAST SQUARES MEANS

Least squares means of milk minerals across breeds, days in milk and parity are depicted in Figures 7, 8 and 9.

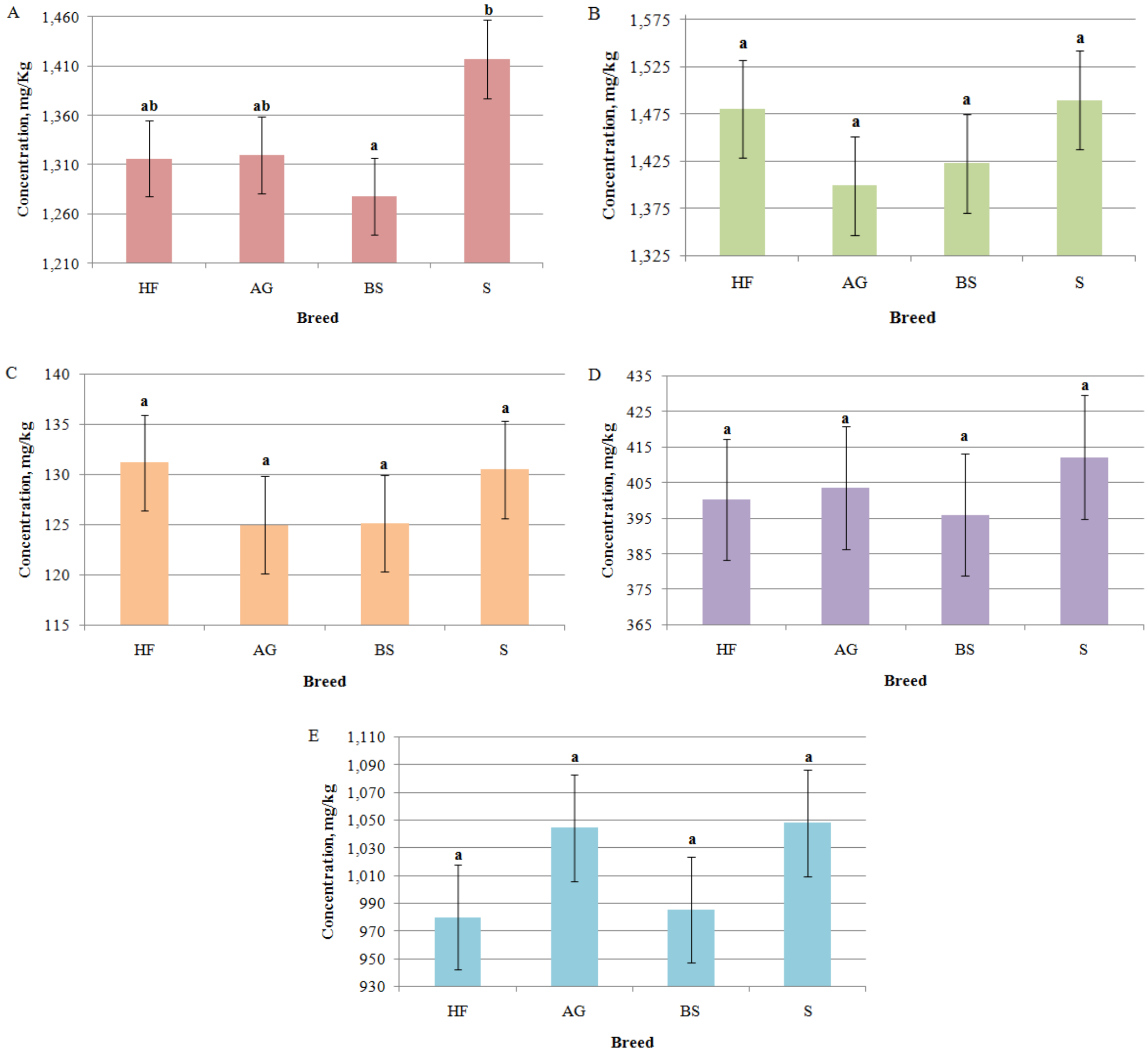
Milk Ca concentration was significantly (P<0.05) different between Brown Swiss and Simmental breeds (Figure 7). Milk from Brown Swiss cows contained the lowest Ca concentration (1,278 mg/kg), whereas milk from Simmental cows showed the greatest Ca content (1,417 mg/kg). Calcium content in Alpine Grey milk (1,319 mg/kg) was similar to Ca content of Holstein-Friesian milk (1,316 mg/kg). Mariani et al. (2002) reported 1,153 mg/kg of Ca in Holstein-Friesian milk and 1,225 mg/kg of Ca in Brown Swiss milk. This latter value is similar to that obtained in the present thesis. Toffanin et al. (2015a) reported an average Ca content of 1,156 mg/kg in milk of Holstein-Friesian cows.

Milk K content did not differ significantly among breeds. On average, Holstein-Friesian and Simmental milk exhibited the highest K content (1,480 and 1,490 mg/kg, respectively). Brown Swiss and Alpine Grey cow milk had 1,423 and 1,399 mg/kg of K, respectively.

A similar situation without significant differences across breeds occurred for Mg, Na and P contents. Milk Mg concentrations were 125 mg/kg for Alpine Grey, 125 mg/kg for Brown Swiss, 130 mg/kg for Simmental and 131 mg/kg for Holstein-Friesian. Simmental milk had the highest Na content (412 mg/kg), followed by Alpine Grey (404 mg/kg), Holstein-Friesian (400 mg/kg) and Brown Swiss (396 mg/kg). Phosphorus content was 980 mg/kg in Holstein-Friesian milk, in agreement with Mariani et al. (2002), who reported 916 mg/kg of P and Toffanin et al. (2015a), who found 934 mg/kg of P. Phosphorus content in milk from Simmental, Alpine Grey and

Brown Swiss cows was 1,045, 1,044 and 985 mg/kg, respectively. The value for Brown Swiss is not far from that obtained by Mariani et al. (2002) in milk of the same breed (967 mg/kg).

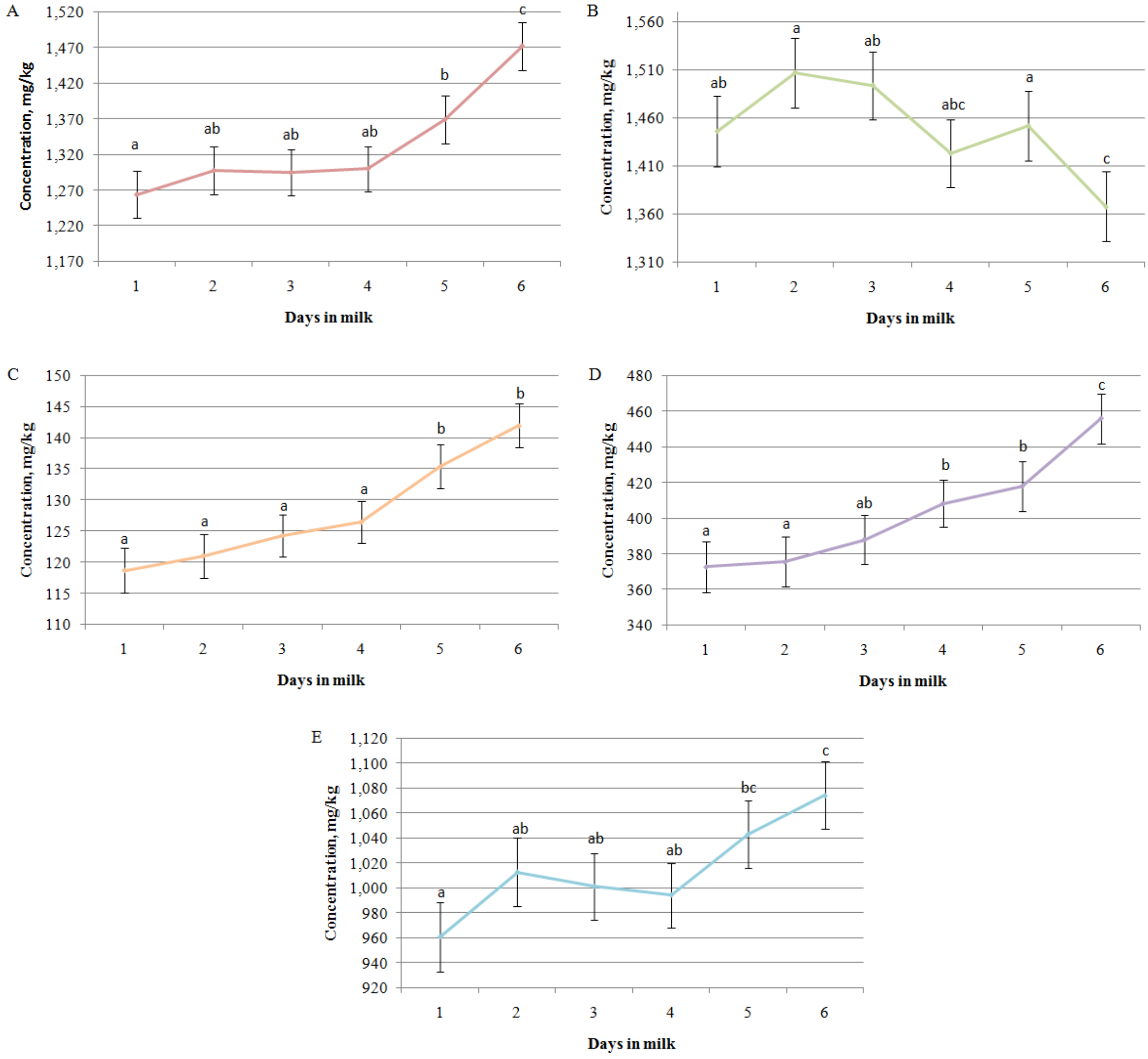
Figure 7 – Least squares means of (A) calcium, (B) potassium, (C) magnesium, (D) sodium and (E) phosphorus across breeds[1]. Least squares means with different letters across breeds mean that they are significantly different (P<0.05).



[1]HF = Holstein-Friesian; AG = Alpine Grey; BS = Brown Swiss; S = Simmental.

Least squares means of milk minerals across days in milk are shown in Figure 8. Calcium content was low in early lactation, increased slightly from 60 to 120 d, remained constant until 240 d and then increased rapidly until the end of lactation. This trend was in agreement with Van Hulzen et al. (2009), whereas Haug et al. (2007) reported that Ca was relatively constant, with some small variations across days in milk. Other studies obtained different trends for Ca across days in milk. Gaucheron (2005), Zamberlin et al. (2012) and Toffanin et al. (2015b) reported Ca concentration that was high at the beginning of lactation, decreased within the first two months after calving and then increased until the end of lactation. Moreover, there were significant differences between all of the first five classes of lactation and the last class; the first class was significantly different even from the fifth class.

Figure 8 - Least squares means of (A) calcium, (B) potassium, (C) magnesium, (D) sodium and (E) phosphorus across days in milk. Least squares means with different letters across parities mean that they are significantly different ($P<0.05$).

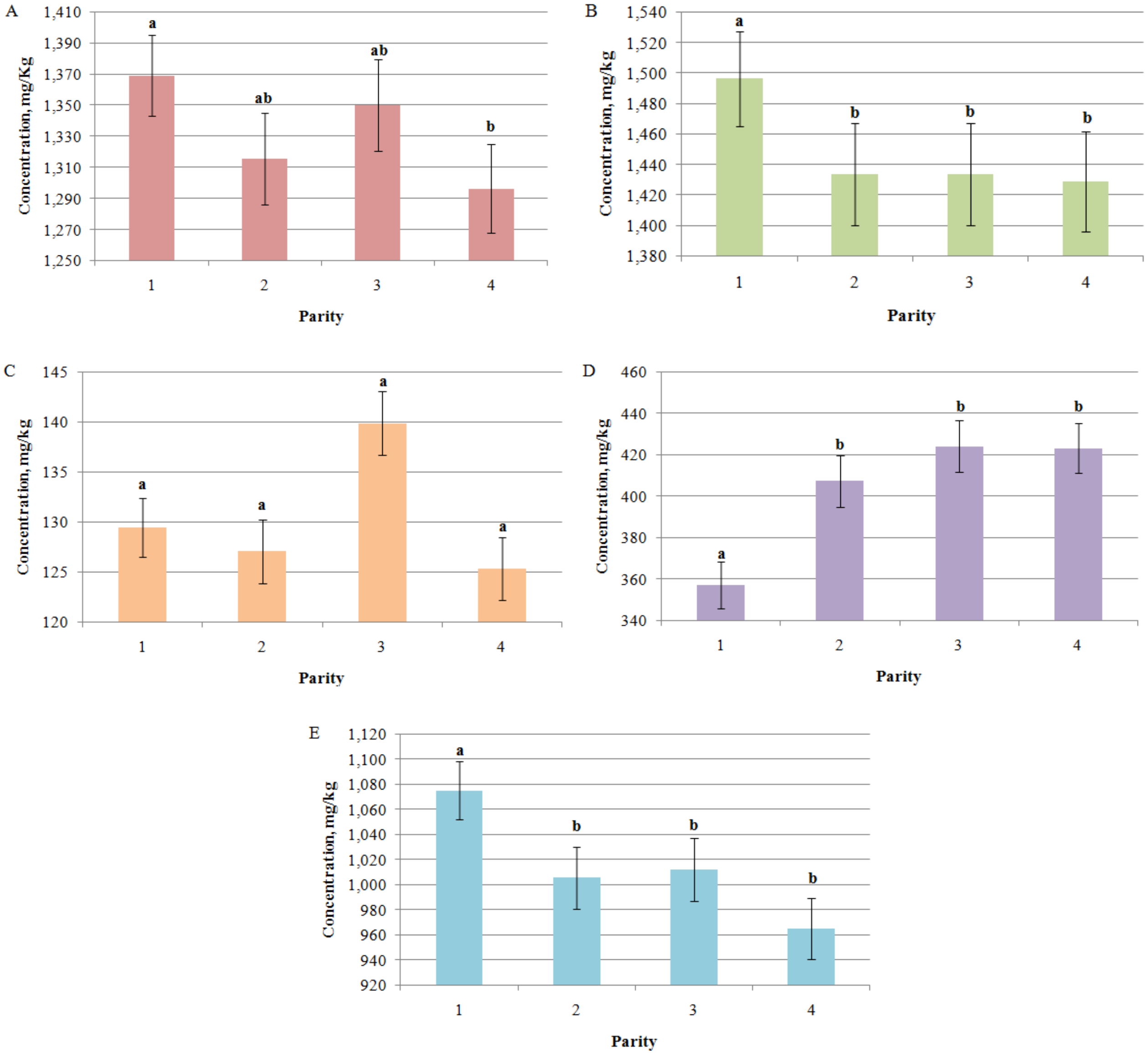


The trend of K corresponded to that found by Gaucheron (2005) and Van Hulzen et al. (2009), but Zamberlin et al. (2012) reported an opposite trend. Furthermore, there were significant differences between the first, second, third and fifth classes of lactation and the last class; the second class was also significantly different from the fourth class.

Magnesium content exhibited increasing values from calving to the end of lactation, in agreement with Van Hulzen et al. (2009). On the other hand, Zamberlin et al. (2012) reported that Mg concentration was high in colostrum and then decreased slightly and remained relatively constant. Gaucheron (2005) found a trend similar to that of Zamberlin et al. (2012), except that Mg increased at the end of lactation. Besides, it was resulted that all of the first four classes of lactation were significantly different from the last two classes. The trend of Na content across days in milk resembled that of Mg. Gaucheron (2005) and Zamberlin et al. (2012) reported higher Na oncentration in early lactation compared to results of the present thesis. Moreover, all of the first five classes of lactation were significantly different from the last class and the first two classes were also significantly different from the fourth and fifth classes. Phosphorus content was low in early lactation, increased slightly from 60 to 120 d, remained quite constant between 120 and 240 d, and then increased until the end of lactation. Gaucheron (2005) reported that P concentration was initially low, then increased and decreased again at the end of lactation, whereas Toffanin et al. (2015b) reported an opposite trend. Van Hulzen et al. (2009) found a different trend in which P level was high in colostrum, and decreased slightly during lactation. Moreover, there were significant differences between all of the first four classes of lactation and the last class; the first class was significantly different even from the fifth class. Differences between the present thesis and previous studies could be due to the low number of cows available for each class of days in milk in the present study.

Least squares means of mineral contents across parities are depicted in Figure 9. Calcium content in milk of primiparous cows was significantly greater than that of fourth parity animals. This result was similar to that reported by Toffanin et al. (2015b). Potassium content was significantly greater in milk of primiparous than multiparous cows. Magnesium concentration did not change significantly across parities. However, the highest content was observed in milk of third parity animals. Sodium concentration was lower in primiparous than multiparous animals. Finally, P content followed a trend similar to that of Ca and K, with a significant greater content in first than later parity cows, as previously obtained by Toffanin et al. (2015b).

Figure 9 - Least squares means of (A) calcium, (B) potassium, (C) magnesium, (D) sodium and (E) phosphorus across parities. Least squares means with different letters across parities mean that they are significantly different (P<0.05).



4. CONCLUSIONS

In this thesis, major mineral contents in milk of four cattle breeds were characterized. There were strong and significant correlations between minerals, in particular between Ca, P and Mg. These three elements were also significantly correlated with milk quality traits. Moreover, among milk minerals, Ca exhibited the highest relationships with coagulation traits. Statistical analysis revealed that breeds did not differ significantly in terms of milk mineral concentration, with the only exception of Ca content in milk from Brown Swiss and Simmental cows. Days in milk affected significantly all mineral concentrations. Overall, Ca, Mg, Na and P contents increased during lactation, whereas the overall trend for K followed that of milk yield. Parity influenced significantly only Na and P. In particular, Na concentration was lower in milk of first- than later-parity cows, whereas P showed an opposite trend. Results of the present work suggest that mineral contents of milk did not differ across breeds. However, further research is needed to confirm these findings, but on a larger dataset. For this purpose, it would be of great interest to compare the results obtained by reference method used in this thesis, with those achievable using innovative, faster technologies such as mid-infrared spectroscopy (MIRS).

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