International Dairy Journal 94 (2019) 65–71

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Zinc supplementation of dairy cows: Effects on chemical composition, nutritional quality and volatile profile of Giuncata cheese

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ARTICLE INFO

Article history:
Received 31 December 2018
Received in revised form
24 February 2019
Accepted 24 February 2019
Available online 18 March 2019

ABSTRACT

The effects of dietary zinc supplementation on chemical composition, nutritional quality and volatile profile of Giuncata cheese were investigated. Dietary zinc addition did not influence milk yield and composition, but induced a marked reduction of somatic cell count. Both in milk and cheese the experimental samples were characterised by a lower concentration of saturated fatty acids and an increase in oleic, vaccenic and rumenic acids. The volatile profile of Giuncata cheese samples was also affected by dietary zinc intake, with an increase in concentration of butanoic acid, hexanoic acid and hexanal. The present results suggest a positive role of zinc in improving animal health and nutraceutical properties of milk and corresponding cheese. Such findings could contribute to the production of cheeses with interesting properties, although further evaluations should be performed to confirm the consumer acceptability of these changes.

1. Introduction

High yielding animals require feeding strategies that guarantee the right contribution of all the necessary microelements, such as zinc, manganese, copper, cobalt, iodine and selenium. For zinc (Zn), its importance in a biological system can be closely related to the different roles performed, which can be categorised into catalytic, co-catalytic, structural and regulatory functions (Mir, Mani, Pal, Malik, & Sharma, 2018). Zn represents the cofactor of several metallo-enzymes (Coleman, 1998) and an essential component of transcription factors that control gene expression, with relevant roles in cell division, development and differentiation (MacDonald, 2000). Zn also plays a relevant role in the stabilisation of RNA, DNA, and ribosome, is involved in insulin production, has antioxidant effects (Kloubert & Rink, 2015) and is involved in the immune system (Bonaventura, Benedetti, Albarede, & Miossec, 2015).

Dietary Zn deficiency in livestock leads to a wide range of disorders like inappetence, growth depression, inefficient feed utilisation, reproductive failure, bone and skeletal deformities, immune system dysfunction, and lower production performance (Suttle, 2010). Zn absorption in the gastrointestinal tract requires, first of all, the microelement release from the complex food matrix through the action of microbial phytases produced by several rumen microbes like *Selenomonas ruminantium*. The free Zn ions are then intercepted by specific low molecular weight carriers credited of a chelating function. Such carriers facilitate the Zn transportation across the specialised barrier of gut epithelial cells; alternatively it is directly absorbed in the ionic form by the highly specific transport proteins in the epithelium. The extra Zn absorbed from the intestine is sequestered within the cells, where it is associated with proteins or remains in free form. Cells regulate the Zn metabolism in such a way that free zinc is present within the nano to picomolar concentration (Mir et al., 2018).

Both veterinary and human medicine have given considerable importance to research aimed at identifying food supplements, to provide a sufficient status of this important microelement in both animals and humans. Inorganic zinc (oxide, sulphate) represents the most common source of zinc supplement, which is supplied to animals through mineral mixtures added to the concentrate diets. Studies on lambs indicate that zinc sulphate and zinc oxide are similar in bioavailability (Kegley & Spears, 1992; Sandoval, Henry, Littell, Cousins, & Ammerman, 1997) and, although the mode of action is unclear, research suggests that the feeding enrichment with specific organic forms of zinc can positively influence the

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https://doi.org/10.1016/j.idairyj.2019.02.014
0958-6946/

animal production responses if compared with those observed in ruminants that are supplemented only with inorganic zinc (Spears, 1996).

The other sources of zinc that may be added to the diet of animals include organic (Zn proteinate, Zn methionine, Zn glycine, Zn propionate), hydroxy and nano zinc oxide (nZnO) that have been recently introduced with the aim to obtain better characteristics in terms of solubility and Zn availability, without causing toxicity (Singh, Maity, & Maity, 2018); however, the predominant form in which Zn is used for the industrial preparation of animal feeds is actually represented by zinc oxide (ZnO).

With specific reference to ruminants, an adequate microelements bioavailability is of fundamental importance also to support the ruminal fermentation mediated by the enzymatic activities of ruminal microbiota. Sonawane and Arora (1976) reported an increased microbial protein synthesis in vitro after incubating rumen fluid with $ZnCl_2$ or $ZnSO_4$; the authors justified this finding as a direct effect of additional Zn in increasing microbial enzymatic activities. Such mechanisms directly contribute to the chemical-nutritional quality of milk and related dairy products. Ruminant products, in fact, are the only foods to contain conjugated linoleic acids (CLAs) and significant amounts of several monounsaturated fatty acids (MUFAs), which represent the only natural source of trans fatty acids (FAs) in the human diet (Doreau, Meynadier, Fievez, & Ferlay, 2016). Several benefits of CLA for human health have been reported, and some important examples concern the modulation of the immune system (Song et al., 2005), the enhancement of bone mineralisation (Platt, Rao, & El-Sohemy, 2007) and their potential activity of slowing the atherosclerosis development (Lock, Corl, Barbaro, Bauman, & Ip, 2004).

Over time few evaluations have been made on the production parameters and the quality of milk obtained from ruminants fed with diets enriched both with organic and inorganic zinc. Salama et al. (2003) showed that milk yield in dairy cows were not significantly affected by Zn-methionine intake in dairy goats. Furthermore, the percentages of protein, lactose, fat, solid nonfat, total solid, and density of milk were not significantly different between treatments. Later, Sobhanirad, Carlson, and Kashani (2010) confirmed this behaviour in dairy cows, without experiencing variations between different zinc sources. Most of the studies have instead focused on the chemical and physical properties of cheeses obtained by using milk fortified with zinc, without testing the microelement effect in the diet of farm animals (Kahraman & Ustunol, 2012).

The aim of the present study was to evaluate the effect of dietary zinc oxide (ZnO) supplementation on Friesian dairy cows, with particular attention on nutritional quality, FA composition, oxidative stability and volatile profile of milk and related Giuncata cheese, a soft dairy product for immediate consumption.

2. Materials and methods

2.1. Experimental design, diets and sampling

Twenty six healthy cows, homogeneous for age (41.5 ± 2.5 months) and lactation days (77 ± 11 days) were used in this study. Animals were randomly divided into two groups of thirteen cows each: a control group (CG) and an experimental group (EG) whose diet was supplemented with ZnO. For the entire trial period, cows were housed in two separate areas of free housing with an access to an identical feeding area. The study was conducted for a period of 42 days, in which all animals received about 22 kg head^{-1} day^{-1} of dry matter of total mixed ration (TMR) whose composition (Table 1) was defined relying the parameters reported on the seventh edition of Nutrient Requirements of Dairy Cattle (NRC, 2001). Samples of TMR were analysed, according to AOAC (1990) methods, for crude protein (CD; method 930.15), ether extract (EE; method 920.39), crude fibre (CF; method 962.09) and ash (method 942.05); detergent procedures reported by Van Soest, Robertson, and Lewis (1991) were used for the determination of neutral detergent fibre (NDF) and acid detergent fibre (ADF).

Table 1
Ingredients and composition of total mixed ration (TMR) administered to each animal of both groups of study.[a]

Component	Level
Ingredients of TMR	
Corn silage (%)	24
First cut alfalfa hay (%)	5
Corn meal (%)	3.5
Soybean meal (%)	3.0
Fine bran (%)	3.1
Barley meal (%)	1.8
$CaCO_3$ (%)	0.2
Vitamins and minerals (%)	0.5
Dry matter (kg head^{-1} per day^{-1})	22.57
Chemical composition of TMR	
Dry matter (%)	56.51
Crude protein (%)	15.35
Ether extract (%)	2.99
Ash (%)	5.26
Neutral detergent fibre (%)	32.63
Acid detergent fibre (%)	20.15
Starch (%)	26.96
Zinc (mg kg^{-1} of dry complete feed)	38 (+59.5)

[a] Crude protein, ether extract, ash, fibre and starch are given on a dry matter basis. The amount of zinc added to the diet of the experimental group is given in parentheses.

The CG received a complete feed formulated taking into account the nutritional needs of cows in mid-lactation, and guaranteeing each animal the daily Zn requirement of about 40 mg for kg of dry complete feed. The EG received the same complete food, formulated according to the same requirements and prepared in the same way, however enriching the daily ration of each cow with additional 60 mg Zn for kg of dry complete feed to obtain a total intake of about 100 mg kg^{-1}.

For the preparation of rations ZnO was used as a powder in which the zinc content was not less than 72%, and the doses management was performed according to the Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. On the 49th day, all cows were milked twice on the same day, once in the morning and once in the evening, and the samples of each animal were mixed for individual analysis, and stored in the dark at 4.5 °C before being analysed; 120 L of bulk milk were collected separately for each group and manipulated in the same way during cheese-making.

For each group, three independent cheese-making procedures were carried out on the same day according to the following manufacturing protocol. Bulk milk was pasteurised at 72 ± 2.5 °C for 3 min, cooled to 38 ± 1.5 °C, and transferred to a container in which container in which there was the addiction of starter bacteria (50 g 100 L^{-1}) followed by acidification; Lyofast YHL 092 E starter cultures (SACCO System, Como, Italy), which consists of specifically selected strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, *L. delbrueckii* ssp. *lactis*, and *Lactobacillus helveticus* were used. Rennet (75% of chymosin and 25% of pepsin; 1:18000 strength; Clerici, Cadorago, Italy) was added (15 g 100 kg^{-1}) to the milk to induce coagulation.

After 30–40 min the texture of the obtained curds was checked and then roughly broken with a skimmer. After a short pause, the curds were further refined by being broken into spheroidal

fragments with a diameter of 17–20 mm. The mass was left to settle at the bottom of the cauldron and heated in whey at a temperature of 40–45 °C. Lastly, the curds were hand-ladled into small baskets that were set on a sloping surface to facilitate the whey drain off. The cheese was then pressed by hand and after 30 min turned once; another 30 min were allowed to pass and it was turned again. At the end of these steps the cheese was considered ready to eat and stored without salting at about 4 °C in plastic tubs.

The final product was characterised by a cylindrical shape with height of about 5 cm and diameter of 15 cm for a weight of about 600 g. From each cheese-making were collected 3 cheese samples of 500–600 g each, which were partly immediately analysed and partly packed under vacuum and frozen at −20 °C until analysis. To evaluate changes in the chemical composition and quality attributes due to storage, sampling and analyses on Giuncata cheese were carried out immediately after the cheese making (T_0) and after 5 days (T_5).

2.2. Chemical analysis of milk and Giuncata cheese

Chemical composition of milk (fat, protein, casein, lactose, and urea) was determined by MilkoScan FT 6000 (Foss Integrator IMT; Foss, Hillerød, Denmark), while somatic cells count (SCC) and total bacterial count (TBC) were performed using, respectively, the Fossomatic TM FC and the BactoScan FC (Foss, Hillerød, Denmark). In cheese, the evaluation of pH, dry matter, total proteins, lipids and ash were performed as previously described by Tofalo et al. (2015). Water soluble nitrogen (WSN) and trichloroacetic acid-soluble nitrogen (TCA-SN) were determined according to Kuchroo and Fox (1982) and Polychroniadou, Michaelidou, and Paschaloudis (1999).

For the determination of total zinc concentration in milk and cheese, samples were firstly mineralised by dry incineration, and then subjected to atomic absorption spectrophotometry using an air/acetylene flame (Nascentes, Arruda, Nogueira, & Nóbrega, 2004). The determination of zinc was performed by referring to a calibration and results were expressed in mg kg^{-1}.

Milk lipid fraction was extracted according to the official method AOAC (2000), while in cheese, the extraction was performed as described by Domagała, Sady, Grega, Pustkowiak, and Florkiewicz (2010) by using a mix of chloroform and methanol (2:1, v/v). Trans-methylation of lipid extracts and separation of fatty acyl methyl esters (FAMEs) was performed following the procedure reported by Castellani et al. (2017). Peak areas were quantified using ChromeCard software, and the relative value of each individual FA was expressed as a percentage of the total FA. The value of each fatty acid was used to calculate the sum of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Taking into account the values associated with each fatty acid, atherogenic and thrombogenic indices (AI and TI, respectively) were calculated in milk and cheese using the formulae proposed by Ulbricht and Southgate (1991); desaturation index (DI) was calculated as proposed by Mele et al. (2007).

2.3. Evaluation of lipid oxidation in cheese by TBARS-test

Lipid peroxidation in Giuncata cheese samples was evaluated by measuring thiobarbituric acid reactive substances (TBARS). The analysis was performed according to the procedure reported by Grotta, Castellani, Palazzo, Haouet, and Martino (2017) with slight modifications. For each sample, an aliquot of 5 g of frozen cheese was mixed, within 2 min of sample withdrawal from the freezer, with 500 μL of 0.1% butylated hydroxytoluene (BHT) in methanol to stop the oxidation mechanisms. The mixture was homogenised with Ultra Turrax T-25 high speed homogeniser (IKA, Staufen, Germany) in 50 mL of an acqueous solution of 7% trichloroacetic acid (TCA), and then subjected to distillation. 2 mL of each distillate were mixed with an equal volume of a 0.02 M thiobarbituric acid (TBA) solution in 90% acetic acid. The solution was kept for 1 h in a thermostated bath at 80 °C, and only after cooling, the absorbance at 534 nm was determined with a spectrophotometer (JENWAY 6305 UV/vis; Jenway, Essex, UK). The amount of oxidated compounds in each sample was calculated by using a calibration curve, and results were expressed in μg of malondialdehyde (MDA) per g of cheese.

2.4. Volatile compounds analysis

Extraction of volatile compounds (VOCs) from cheese samples was performed by solid-phase microextraction (SPME), and GC–MS analysis was performed with a gas chromatograph (Clarus 580; Perkin Elmer, Waltham, MA) coupled with a mass spectrometer (SQ8S; Perkin Elmer). The gas chromatograph was equipped with an Elite-5MS column (length × internal diameter: 30 × 0.25 mm; film thickness: 0.25 μm; Perkin Elmer). Grated cheese (5 g) was mixed with 10 mL of saturated NaCl solution (360 g L^{-1}), and then 10 μL internal standard solution (4-methyl-2-heptanone; 10 mg kg^{-1} in ethanol) were added. The vials were sealed with a polytetrafluoroethylene-silicone septum (Supelco, Bellefonte, PA) and stirred at 60 °C; volatile compounds were extracted from the headspace with a divinylbenzene-carboxen-polydimethylsiloxane SPME fibre (length: 1 cm; film thickness: 50/30 μm; Supelco) with an exposition time of 60 min.

After adsorption, the extracted VOC were thermally desorbed into the gas chromatograph injector splitless mode for 1 min at 250 °C. The oven temperature was held at 50 °C for 1 min, increased at a rate of 3 °C min^{-1} up to 200 °C and held for 1 min, and then increased from 200 °C to 250 °C at 15 °C min^{-1} and held for 15 min. Helium was used as a carrier gas at a flow rate of 1 mL min^{-1}. The mass spectrometer operated in electronic impact ionisation mode at 70 eV, and data were collected in full scan mode, with a scan time of 0.2 s over a mass range of 35–350. Source and interface temperature were held at 250 °C. Volatile compounds were identified by comparison with mass spectra of a library database (NIST Mass Spectral library, Search Program version 2.0, National Institute of Standards and Technology, US Department of Commerce, Gaithersburg, MD, USA) and by comparing the eluting order with Kovats indices. Quantification was carried out by integrating the peak areas of a target ion for each compound. The volatile composition was expressed as relative mean percentage.

2.5. Statistical analysis

Milk yield and chemical composition were analysed according to a mixed model using PROC MIXED of SAS (version 9.0; SAS Institute Inc., Cary, NC, USA) with diet and period (7 days both for milk yield and chemical composition) as fixed factors. The FA composition of individual milk samples collected on the last day of the trial was analysed according to PROC MIXED with diet as a fixed effect. Chemical and FA composition of cheese were analysed by PROC GLM, including diet and time of ripening as fixed effects. Statistical data processing of volatile compounds in cheese was performed by the GLM procedure of SAS of the statistical package SPSS 13.0 (SPSS, Chicago, Ill., USA), using a one-way analysis of variance considering the effect of the feeding strategy as factor of variation. Volatile compounds were performed independently for 1 and 5-day-stored cheeses, the three datasets were processed separately and the ripening effect was not tested. Separation of means was assessed by Student's *t*-test, and differences were considered significant for $P < 0.05$.

3. Results

3.1. Chemical-nutritional composition of milk and Giuncata cheese

Taking into account the milk production over the entire duration of the dietary zinc enrichment, no significant differences were evidenced between the two groups (39.21 ± 1.45 vs 38.53 ± 1.17 kg milk day^{-1} for each cow of CG and ZG respectively), reflecting the fact that such parameter was not affected by diet and diet-period interaction ($P < 0.05$). For what concerns the chemical quality of milk (Table 2), all the parameters analysed did not undergo variations. Similarly, no significant differences were observed as regards the ureic content, TBC and pH, whereas the EG samples showed a lower SSC with respect to the CG ($P < 0.05$). As regards the Zn concentration, in the experimental group were found higher average values, even if not significant.

Regardless of the feeding strategy, no significant differences ($P > 0.05$) in composition of cheeses were evidenced (Table 3). Regarding the storage time, in T_5 samples the dry matter was significantly higher (32.02% versus 37.77% in CG and 31.41% versus 35.98% in EG samples; $P < 0.05$). Protein, lipids and ash were not influenced by storage, as well as the zinc concentration that maintained higher, but not significant values, in the EG with respect to the CG. Proteolysis in cheese is often measured by means of quantification of nitrogen fractions. With regard to the levels of WSN and 12% TCA-SN, no variations were evidenced during storage in both the experimental groups. Regarding the zinc concentration, the feeding strategy and the storage time did not influence this parameter.

3.2. Fatty acids profile and lipid oxidation of Giuncata cheese

The fatty acid composition of milk and corresponding cheese are reported in Table 4. Samples of bulk milk, obtained from EG, evidenced an increase in the content of oleic acid (C18:1 *cis*9; $P < 0.05$), vaccenic acid (C18:1 *trans*11; $P < 0.01$), linoleic acid (C18:2, *n*-6; $P < 0.01$) and rumenic acid (CLA; $P < 0.01$). Similarly, the evaluation of the fatty acids profile in cheese evidenced modifications already evident in milk, with an increase in concentration, in EG, of oleic acid (C18:1 *cis*9; $P < 0.05$), vaccenic acid (C18:1 *trans*11; $P < 0.05$) and rumenic acid (CLA; $P < 0.01$). Based on the FA profile obtained, calculations of atherogenic, thrombogenic and desaturation indices were performed, highlighting a general improvement of nutritional value and health functionality of milk and cheese. Atherogenic and thrombogenic indices lowered in EG ($P < 0.01$ with the exception of TI calculated in cheese samples in which the P value was <0.05), whereas a significant increase in desaturation index was observed only in milk ($P < 0.05$).

An interesting finding of the experimentation is certainly due to the determination of the oxidative state of Giuncata cheese at the end of the cheese-making (T_0) and after 5 days (T_5). The levels of malondialdehyde (MDA) significantly increased in CG samples after 5 days from the cheese-making (0.058 ± 0.007 versus 0.093 ± 0.011 μg MDA g^{-1} for T_0 and T_5 respectively; $P < 0.01$). In the EG samples no significant variations were instead evidenced between T_0 and T_5 samples (0.065 ± 0.008 versus 0.077 ± 0.009 μg MDA g^{-1} for T_0 and T_5 respectively). It is important to strongly consider the fact that both at the beginning and at the end of the storage, no significant differences were evidenced between CG and EG samples ($P > 0.05$).

3.3. Volatile profile of Giuncata cheese

Different chemical families of VOCs were detected in samples of T_0 cheese and T_5 cheese obtained from CG and EG (Table 5). The majority of the identified compounds consists of free fatty acids (FFAs), aldehydes, alcohols, esters and lactones.

Regarding the CG, no variations were highlighted for FFAs, aldehydes, alcohols and aromatic hydrocarbons during ageing, whereas an increase ($P < 0.01$) was observed in the case of esters and lactones. In the case of EG the condition is quite similar, with the only difference that the concentration of lactones did not differ significantly between T_0 and T_5 samples.

Comparing the results between the two groups, the aldehydes are present in higher concentration in EG in the T_0 samples ($P < 0.01$) and this condition unchanged after 5 days of ageing; the only representative of alcohols family, 1-butanol, 3-methyl, is instead present in higher concentrations ($P < 0.01$) in the CG

Table 2
Milk yield and composition of milk obtained from the control group (CG) and the experimental group (EG).[a]

Parameters	Diet		*P*
	CG	EG	
Milk yield (kg)	38.65 ± 1.64	39.73 ± 1.25	n.s.
Composition			
Fat (%)	3.69 ± 0.26	3.43 ± 0.20	n.s.
Protein (%)	3.16 ± 0.17	3.15 ± 0.15	n.s.
Casein (%)	2.31 ± 0.15	2.28 ± 0.13	n.s.
Lactose (%)	4.71 ± 0.24	4.86 ± 0.22	n.s.
Urea (mg 100 mL^{-1})	22.64 ± 1.03	22.99 ± 1.14	n.s.
SCC (×10^3 cells mL^{-1})	386 ± 37	249 ± 23	<0.05
TBC (cfu mL^{-1} × 10^3)	54 ± 10	49 ± 9	n.s.
pH	6.67 ± 0.02	6.66 ± 0.01	n.s.
Zinc (mg kg^{-1})	3.97 ± 0.19	4.11 ± 0.17	n.s.

[a] Abbreviations are: SCC, somatic cell count; TBC, total bacterial count; n.s., not significant.

Table 3
Chemical composition of cheese obtained from the control group (CG) and the experimental group (EG), analysed at the end of cheesemaking (T_0) and after 5 (T_5) days of ripening.[a]

Item	T_0		T_5		*P* (diet × period)
	CG	EG	CG	EG	
Dry matter (%)	32.02^A ± 2.37	31.41^A ± 2.02	36.77^B ± 2.14	35.98^B ± 2.05	<0.05
Fat (%)	52.26 ± 4.13	47.96 ± 3.88	51.51 ± 4.18	49.13 ± 3.84	n.s.
Protein (%)	41.33 ± 3.21	39.39 ± 3.05	39.74 ± 2.94	41.86 ± 3.23	n.s.
Ash (%)	5.75 ± 0.51	6.21 ± 0.56	5.88 ± 0.52	5.69 ± 0.49	n.s.
WSN (%N)	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	n.s.
12% TCA-SN (%N)	0.13 ± 0.01	0.15 ± 0.02	0.14 ± 0.02	0.15 ± 0.01	n.s.
Zinc (mg kg^{-1})	20.69 ± 1.36	21.39 ± 1.56	19.91 ± 1.21	21.14 ± 1.98	n.s.

[a] Data for fat, protein, ash watersoluble nitrogen (WSN) and trichloroacetic acid-soluble nitrogen (TCA-SN) are expressed on a dry matter basis. Values with different superscript lower case and uppercase letters are significantly different by diet and ripening time, respectively; n.s., not significant.

Table 4
Fatty acids of milk and Giuncata cheese obtained from the control group (CG) and the experimental group (EG).[a]

Fatty acid and parameter	Milk			Giuncata cheese		
	CG	EG	*P*	CG	EG	*P*
C4:0	2.23 ± 0.21	2.69 ± 0.25	n.s.	2.31 ± 0.23	2.01 ± 0.19	n.s.
C6:0	2.06 ± 0.20	2.34 ± 0.21	n.s.	1.86 ± 0.18	1.69 ± 0.17	n.s.
C8:0	1.36 ± 0.14	1.68 ± 0.18	n.s.	1.61 ± 0.16	1.39 ± 0.14	n.s.
C10:0	2.06 ± 0.19	2.08 ± 0.19	n.s.	2.46 ± 0.22	2.78 ± 0.25	n.s.
C12:0	3.91 ± 0.37	3.59 ± 0.33	n.s.	3.73 ± 0.33	3.95 ± 0.36	n.s.
C14:0	13.29 ± 1.24	11.97 ± 1.35	n.s.	12.71 ± 1.09	11.89 ± 1.02	n.s.
C14:1	1.42 ± 0.14	1.97 ± 0.19	n.s.	1.71 ± 0.16	2.09 ± 0.19	n.s.
C15:0	1.33 ± 0.14	1.62 ± 0.16	n.s.	1.57 ± 0.15	1.47 ± 0.15	n.s.
C16:0	39.60 ± 3.28	34.07 ± 2.94	n.s.	40.42 ± 3.58	35.35 ± 3.12	n.s.
C16:1	1.85 ± 0.18	2.17 ± 0.21	n.s.	1.77 ± 0.16	1.94 ± 0.18	n.s.
C17:0	0.56 ± 0.06	0.67 ± 0.07	n.s.	0.67 ± 0.07	0.74 ± 0.08	n.s.
C18:0	7.35 ± 0.71	6.51 ± 0.62	n.s.	8.56 ± 0.73	8.19 ± 0.65	n.s.
C18:1 *trans*11	0.47 ± 0.05	0.85 ± 0.08	<0.01	0.37 ± 0.05	0.69 ± 0.08	<0.05
C18:1 *cis*9	19.51 ± 1.51	23.02 ± 1.71	<0.05	16.86 ± 1.49	21.73 ± 1.81	<0.05
C18:1 *cis*11	0.40 ± 0.05	0.53 ± 0.05	n.s.	0.43 ± 0.05	0.57 ± 0.06	n.s.
C18:2	1.73 ± 0.15	2.94 ± 0.27	<0.01	1.98 ± 0.17	2.21 ± 0.19	n.s.
C18:3	0.46 ± 0.05	0.53 ± 0.06	n.s.	0.54 ± 0.06	0.65 ± 0.07	n.s.
Rumenic acid	0.25 ± 0.03	0.56 ± 0.05	<0.01	0.29 ± 0.03	0.48 ± 0.05	<0.01
C20:4	0.16 ± 0.02	0.21 ± 0.03	n.s.	0.15 ± 0.02	0.18 ± 0.02	n.s.
Atherogenic index	3.83 ± 0.34	2.65 ± 0.24	<0.01	3.99 ± 0.35	2.89 ± 0.26	<0.01
Thrombogenic index	4.19 ± 0.36	2.76 ± 0.23	<0.01	4.57 ± 0.41	3.28 ± 0.29	<0.05
Desaturation index	0.10 ± 0.01	0.14 ± 0.01	<0.05	0.12 ± 0.01	0.15 ± 0.02	n.s.

[a] Abbreviation: n.s., not significant.

Table 5
Volatile compounds (VOCs) detected in T_0 and T_5 cheese samples obtained from control group (CG) and experimental group (EG).[a]

VOC	T_0			T_5		
	CG	EG	*P*	CG	EG	*P*
Carboxylic acids						
propanoic acid	n.d.	0.79 ± 0.08	<0.01	1.46 ± 0.11	1.47 ± 0.12	n.s.
butanoic acid	3.59 ± 0.31	3.46 ± 0.29	n.s.	3.90 ± 0.29	6.84 ± 0.55	<0.01
pentanoic acid	1.57 ± 0.13	1.66 ± 0.15	n.s.	1.66 ± 0.12	1.81 ± 0.14	n.s.
hexanoic acid	n.d.	n.d.	n.s.	0.64 ± 0.07	0.94 ± 0.09	<0.05
octanoic acid	15.47 ± 1.14	15.12 ± 1.21	n.s.	9.79 ± 0.77	9.85 ± 0.81	n.s.
nonanoic acid	1.91 ± 0.16	2.08 ± 0.15	n.s.	1.88 ± 0.13	1.93 ± 0.16	n.s.
decanoic acid	17.88 ± 1.12	16.92 ± 1.03	n.s.	16.64 ± 1.23	14.89 ± 1.14	n.s.
Aldehydes						
hexanal	13.65 ± 0.96	18.31 ± 1.46	<0.05	12.77 ± 1.01	16.68 ± 1.33	<0.01
nonanal	5.01 ± 0.39	5.71 ± 0.45	n.s.	5.69 ± 0.48	6.10 ± 0.47	n.s.
Alcohols						
1-butanol, 3-methyl	14.92 ± 1.07	9.10 ± 0.74	<0.01	17.55 ± 1.31	11.99 ± 0.98	<0.01
Esters						
hexanoic acid, ethyl ester	1.38 ± 0.09	1.51 ± 0.13	n.s.	1.45 ± 0.13	1.88 ± 0.16	n.s.
decanoic acid, ethyl ester	n.d.	n.d.	n.s.	1.65 ± 0.14	2.44 ± 0.19	n.s.
Lactones						
δ-nonalactone	1.05 ± 0.08	1.14 ± 0.10	n.s.	1.29 ± 0.13	1.33 ± 0.12	n.s.
δ-decalactone	2.24 ± 0.17	2.92 ± 0.23	n.s.	3.54 ± 0.27	2.04 ± 0.17	<0.05
Aromatic hydrocarbons						
ethylbenzene	4.46 ± 0.32	5.13 ± 0.42	n.s.	4.69 ± 0.39	4.08 ± 0.37	n.s.
p-xylene	2.79 ± 0.20	1.34 ± 0.11	<0.01	1.24 ± 0.12	1.52 ± 0.15	n.s.

[a] The volatile composition was expressed as relative mean percentage ± S.D; n.d., not detectable; n.s., not significant.

samples both at T_0 and T_5. FFAs, esters, lactones and aromatic hydrocarbons did not differ significantly between the two groups both in samples of Giuncata cheese collected at the end of the cheese-making (T_0) and after 5 days (T_5).

4. Discussion

Dietary Zn supplementation did not affect milk yield, in agreement with the results obtained by Pechova, Pavlata, and Lokajova (2006) that explored the effect of Zn supplementation on dairy cows, at the dose of 440 mg per animal per day. The same authors, as in our case, reported also a significant reduction in the SCC value in the experimental group, justifying this phenomenon with an increased Zn supply into the mammary gland with a consequent improvement of the immune function and the reduction of somatic cells release in milk.

The feeding strategy based on Zn supplementation did not affect milk composition in the present study, and this finding is consistent with the previous reports in dairy cows (Cope, Mackenzie, Wilde, & Sinclair, 2009; Wang, Liang, Zhang, Li, & Luo, 2013) and dairy goats (Salama et al., 2003), indicating that milk composition was not sensitive in response to dietary Zn supplementation. A result on which it is necessary to focus the attention concerns the zinc content, which did not change between the control and the experimental group. Despite the apparent contradiction, this finding is in agreement with what was reported by Pechova et al.

(2006), which explained the phenomenon advancing the hypothesis of an impaired incidence of rumen acidosis in the herd before the start of the experiment. According to what was observed in milk, no differences were evidenced in the chemical-nutritional composition of Giuncata cheese samples, both in relation to the feeding strategy and in relation to the storage time. The only differences, as expected, concerned the increase in dry matter at the end of the short storage period.

The results of the present study showed the ability of zinc to influence the FA profile of cow milk and related cheese. As reported by Nudda, McGuire, Battacone, and Pulina (2005), the FA composition found in cheese reflects the composition observed in milk, suggesting that the observed variations in nutritional quality of milk, as a consequence of the experimental feeding strategy, are thereafter maintained in cheese. Dietary zinc positively affected the oleic acid level in milk and cheese. Since the diet administered to the EG does not confer an additional source of oleic acid with respect to the CG diet, this result might be mainly related to the desaturation of stearic acid occurring in the mammary gland by stearoyl coenzyme A desaturase (SCD), a finding also supported by the C14:1/C14:0 ratio, considered an index of Δ^9-desaturation in the mammary gland (Mele et al., 2007).

SCD is an endoplasmic reticulum-bound enzyme that catalyses the Δ^9-desaturation of saturated fatty acyl-CoAs (Miyazaki & Ntambi, 2003); it is encoded by the stearoyl coenzyme A desaturase gene (Smith et al., 2006), whose expression is regulated by Site-2 protease (S2P), a metalloprotease that needs Zn to perform its catalytic function, justifying, at least in part, the highest concentration of oleic acid in milk and cheese obtained from EG with respect to CG.

Dietary zinc supplementation seems to lead also to an increase in milk and related cheese of vaccenic acid and conjugated linoleic acid (CLA). The latter compound is reported to perform an important antioxidant activity that protects bovine mammary epithelial cells from lipoperoxidation and mitigate the level of the reactive oxygen species, leading to an improvement of the mammary gland functionality (Basiricò et al., 2015).

Finally, MUFA and PUFA content increased in milk and cheese obtained after feeding enrichment with zinc, at the expense of SFAs. As a direct consequence of this evidence, atherogenic and thrombogenic indices decreased in the EG. In light of this, it could be argued that dietary zinc supplementation may increase nutritional value and health functionality of milk and related dairy products.

The evaluation of thiobarbituric acid reactive substances was used in this study as a marker for oxidative damage in cheese. Zn has been reported to inhibit free radical lipid peroxidation in biological systems by serving, in several conditions, as free radical scavenger (Fang, Yang, & Wu, 2002). At the end of the cheese-making (T_0), the TBA values of cheese obtained from the control group was significantly lower relative to that in the EG cheese. At the end of storage (T_5), the TBA values slightly increased, as expected, in CG cheese, but decreased in EG cheese suggesting antioxidant protection provided by zinc. Kahraman and Ustunol (2012) reported similar behaviour with zinc-fortified Cheddar cheese, where TBA values decreased over the storage time. The same authors partially justified this behaviour introducing the hypothesis of further reactions of MDA that would therefore be underestimated (Kahraman & Ustunol, 2012).

The effect of dietary zinc on the volatile profile evaluated in cheese appers to be very interesting. Biochemical changes in cheese during ripening may be grouped into primary (lipolysis, proteolysis and metabolism of residual lactose and of lactate and citrate) or secondary (metabolism of fatty acids and of amino acids) events. The most abundant class of volatile compounds was represented by carboxylic acids, composed of acids from C3, propanoic, to C10, decanoic. Butanoic and hexanoic acids, were present at higher concentrations in the EG samples after 5 days of storage ($P < 0.01$ and $P < 0.05$, respectively) from the cheese-making. Butanoic and hexanoic acids are considered to be mainly involved in the determination of cheese flavour, giving origin to cheesy, rancid and sweaty odours. The increased production of such compounds could be probably explained by an increase of lipolysis of the triglycerides by microbial and endogenous milk enzymes, resulting in an augmented release of FFAs (Bertuzzi, McSweeney, Rea, & Kilcawley, 2018).

The general increase of carboxylic acids in ripened cheese could be explained by the extent of starter cell autolysis, a phenomenon supported by the increase in activity of several peptidoglycan hydrolases, commonly named autolysins, that are characterised by a C-terminal domain containing a binding motif for Zn (Huard et al., 2003) that is therefore an important cofactor in the mechanisms that lead to the lysis of the bacterial cells, with the consequent release of peptidases and especially lipases that accelerate the lipolytic event in cheese (Collins, McSweeney, & Wilkinson, 2003). FFAs contribute to the formation of cheese flavour not only directly, but also acting as precursors for the production of volatile flavour compounds through a series of reactions, known collectively as metabolism of fatty acids, which can contribute to the synthesis of methyl ketones, secondary alcohols, aldehydes, lactones and esters.

Aldehydes represent the second group in order of abundance in our study, and seem to be present in higher concentrations in the EG samples in which was evidenced a marked increase of hexanal. This compound is responsible for the “green grasslike” aromas, characterised by green, slightly fruity, lemon, and herbal notes (Bertuzzi et al., 2018). Aldehydes can also derive from secondary reactions that are part of the metabolism of free amino acids; specifically they can derive from the decarboxylation of α-ketoacids produced by the action of aminotransferases, pyridoxal-5′-phosphate (PLP)-dependent enzymes, which use free amino acids as substrate.

Lactic acid bacteria contain intracellular peptidases that are very important for the final stages of proteolysis in cheese during ripening and the ultimate liberation of free amino acids as substrates for catabolic reactions (McSweeney, 2004). Probably the presence of zinc in EG samples favours the activity of metalloenzymes, such as zinc-dependent aminopeptidases that contribute to this process.

After 5 days of storage, the amount of δ-decalactone in EG samples was lower with respect to the CG samples. This compound, in the same way as other lactones, is produced by a one-step transesterification reaction of hydroxylated FFAs which represent the main precursors. Hydroxylated FFAs are released by lipolytic activities or by heating process, furthermore can be also produced from the catabolism of unsaturated fatty acids by the action of microbial lipoxygenases and hydratases (Bertuzzi et al., 2018). Probably in the control group conditions persist that favour these enzymatic mechanisms with respect to that eventually existent in EG samples. A strange behaviour is instead associated with esters, whose synthesis mechanisms and their contribution to the creation of the volatile profile deserve further evaluation.

5. Conclusions

The present results suggest a positive role of zinc in improving the nutritional and nutraceutical properties of milk and corresponding cheese. The experimented feeding strategy may modify the quality of Giuncata cheese obtained from lactating dairy cows. The main finding concerns the increased amount of oleic acid, vaccenic acid and CLA, at the expense of SFA, supporting the hypothesis that consumption of these products could have positive

effects on human health. Besides of this, the volatile profile of dairy products also was affected by dietary zinc intake.

Acknowledgements

Authors are grateful to "Ansape Company" (Raiano (AQ), Italy), for the kind cooperation. This work is part of the project "Innovazione della filiera bovina da latte per produzioni lattiero-casearie ad elevato contenuto salutistico ed ecosostenibile" supported by a grant from Rural Development Plan 2007-2013-MISURA 1.2.4, Regione Abruzzo (Italy), Project manager: Giuseppe Martino.

References

AOAC International. (1990). *Official methods of analysis. Vol. 1* (15th ed.). Arlington, VA: Association of Official Analytical Chemists (AOAC).

AOAC. (2000). *Official methods of analysis* (17th ed.). Washington, DC, USA: Association of Official Analytical Chemists.

Basiricò, L., Morera, P., Dipasquale, D., Tröscher, A., Serra, A., Mele, M., et al. (2015). Conjugated linoleic acid isomers strongly improve the redox status of bovine mammary epithelial cells (BME-UV1). *Journal of Dairy Science, 98*, 7071–7082.

Bertuzzi, A. S., McSweeney, P. L., Rea, M. C., & Kilcawley, K. N. (2018). Detection of volatile compounds of cheese and their contribution to the flavor profile of surface-ripened cheese. *Comprehensive Reviews in Food Science and Food Safety, 17*, 371–390.

Bonaventura, P., Benedetti, G., Albarède, F., & Miossec, P. (2015). Zinc and its role in immunity and inflammation. *Autoimmunity Reviews, 14*, 277–285.

Castellani, F., Vitali, A., Bernardi, N., Marone, E., Palazzo, F., Grotta, L., et al. (2017). Dietary supplementation with dried olive pomace in dairy cows modifies the composition of fatty acids and the aromatic profile in milk and related cheese. *Journal of Dairy Science, 100*, 8658–8669.

Coleman, J. E. (1998). Zinc enzymes. *Current Opinion in Chemical Biology, 2*, 222–234.

Collins, Y. F., McSweeney, P. L., & Wilkinson, M. G. (2003). Lipolysis and free fatty acid catabolism in cheese: A review of current knowledge. *International Dairy Journal, 13*, 841–866.

Cope, C. M., Mackenzie, A. M., Wilde, D., & Sinclair, L. A. (2009). Effects of level and form of dietary zinc on dairy cow performance and health. *Journal of Dairy Science, 92*, 2128–2135.

Domagała, J., Sady, M., Grega, T., Pustkowiak, H., & Florkiewicz, A. (2010). The influence of cheese type and fat extraction method on the content of conjugated linoleic acid. *Journal of Food Composition and Analysis, 23*, 238–243.

Doreau, M., Meynadier, A., Fievez, V., & Ferlay, A. (2016). Ruminal metabolism of fatty acids: Modulation of polyunsaturated, conjugated, and trans fatty acids in meat and milk. In R. R. Watson, & F. De Meester (Eds.), *Handbook of lipids in human function* (pp. 521–542). Amsterdam, The Netherlands: Academic Press.

Fang, Y. Z., Yang, S., & Wu, G. (2002). Free radicals, antioxidants, and nutrition. *Nutrition, 18*, 872–879.

Grotta, L., Castellani, F., Palazzo, F., Haouet, M. N., & Martino, G. (2017). Treatment optimisation and sample preparation for the evaluation of lipid oxidation in various meats through TBARs assays before analysis. *Food Analytical Methods, 10*, 1870–1880.

Huard, C., Miranda, G., Wessner, F., Bolotin, A., Hansen, J., Foster, S. J., et al. (2003). Characterization of AcmB, an *N*-acetylglucosaminidase autolysin from *Lactococcus lactis*. *Microbiology, 149*, 695–705.

Kahraman, O., & Ustunol, Z. (2012). Effect of zinc fortification on Cheddar cheese quality. *Journal of Dairy Science, 95*, 2840–2847.

Kegley, E. B., & Spears, J. W. (1992). Performance and mineral metabolism of lambs as affected by source (oxide, sulfate, or methionine) and level of zinc. *Journal of Animal Science, 70*, 302.

Kloubert, V., & Rink, L. (2015). Zinc as a micronutrient and its preventive role of oxidative damage in cells. *Food and Function, 6*, 3195–3204.

Kuchroo, C. N., & Fox, P. F. (1982). Soluble nitrogen in Cheddar cheese: Comparison of extraction procedures. *Milchwissenschaft, 37*, 331–335.

Lock, A. L., Corl, B. A., Barbano, D. M., Bauman, D. E., & Ip, C. (2004). The anticarcinogenic effect of trans-11 18: 1 is dependent on its conversion to cis-9, trans-11 CLA by Δ9-desaturase in rats. *Journal of Nutrition, 134*, 2698–2704.

MacDonald, R. S. (2000). The role of zinc in growth and cell proliferation. *Journal of Nutrition, 130*, 1500S–1508S.

McSweeney, P. L. (2004). Biochemistry of cheese ripening. *International Journal of Dairy Technology, 57*, 127–144.

Mele, M., Conte, G., Castiglioni, B., Chessa, S., Macciotta, N. P. P., Serra, A., et al. (2007). Stearoyl-coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. *Journal of Dairy Science, 90*, 4458–4465.

Mir, S. H., Mani, V., Pal, R. P., Malik, T. A., & Sharma, H. (2018). *Zinc in ruminants: Metabolism and homeostasis. Proceedings of the national academy of sciences.* India Section B: Biological Sciences. Article 1048.

Miyazaki, M., & Ntambi, J. M. (2003). Role of stearoyl-coenzyme A desaturase in lipid metabolism. *Prostaglandins Leukotrienes and Essential Fatty Acids, 68*, 113–121.

Nascentes, C. C., Arruda, M. A., Nogueira, A. R. A., & Nóbrega, J. A. (2004). Direct determination of Cu and Zn in fruit juices and bovine milk by thermospray flame furnace atomic absorption spectrometry. *Talanta, 64*, 912–917.

NRC. (2001). *Nutrient requirements of dairy cattle: 2001*. Washiongton, DC, USA: National Research Council, National Academies Press.

Nudda, A., McGuire, M. A., Battacone, G., & Pulina, G. (2005). Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and ricotta. *Journal of Dairy Science, 88*, 1311–1319.

Pechova, A., Pavlata, L., & Lokajova, E. (2006). Zinc supplementation and somatic cell count in milk of dairy cows. *Acta Veterinaria Brno, 75*, 355–361.

Platt, I., Rao, L. G., & El-Sohemy, A. (2007). Isomer-specific effects of conjugated linoleic acid on mineralized bone nodule formation from human osteoblast-like cells. *Experimental Biology and Medicine, 232*, 246–252.

Polychroniadou, A., Michaelidou, A., & Paschaloudis, N. (1999). Effect of time, temperature and extraction method on the trichloroacetic acid-soluble nitrogen of cheese. *International Dairy Journal, 9*, 559–568.

Salama, A. A., Caja, G., Albanell, E., Such, X., Casals, R., & Plaixats, J. (2003). Effects of dietary supplements of zinc-methionine on milk production, udder health and zinc metabolism in dairy goats. *Journal of Dairy Research, 70*, 9–17.

Sandoval, M., Henry, P. R., Littell, R. C., Cousins, R. J., & Ammerman, C. B. (1997). Estimation of the relative bioavailability of zinc from inorganic zinc sources for sheep. *Animal Feed Science and Technology, 66*, 223–235.

Singh, K. K., Maity, S. B., & Maity, A. (2018). Effect of nano zinc oxide on zinc bioavailability and blood biochemical changes in pre-ruminant lambs. *Indian Journal of Animal Sciences, 88*, 45–47.

Smith, S. B., Lunt, D. K., Chung, K. Y., Choi, C. B., Tume, R. K., & Zembayashi, M. (2006). Adiposity, fatty acid composition, and delta-9 desaturase activity during growth in beef cattle. *Animal Science Journal, 77*, 478–486.

Sobhanirad, S., Carlson, D., & Kashani, R. B. (2010). Effect of zinc methionine or zinc sulfate supplementation on milk production and composition of milk in lactating dairy cows. *Biological Trace Element Research, 136*, 48–54.

Sonawane, S. N., & Arora, S. P. (1976). Influence of zinc supplementation on rumen microbial protein synthesis in in vitro studies. *Indian Journal of Animal Sciences, 46*, 13–18.

Song, H. J., Grant, I., Rotondo, D., Mohede, I., Sattar, N., Heys, S. D., et al. (2005). Effect of CLA supplementation on immune function in young healthy volunteers. *European Journal of Clinical Nutrition, 59*, 508–517.

Spears, J. W. (1996). Organic trace minerals in ruminant nutrition. *Animal Feed Science and Technology, 58*, 151–163.

Suttle, N. F. (2010). *Mineral nutrition of livestock*. Wallingford, Oxfordshire, UK: CABI.

Tofalo, R., Schirone, M., Fasoli, G., Perpetuini, G., Patrignani, F., Manetta, A. C., et al. (2015). Influence of pig rennet on proteolysis, organic acids content and microbiota of Pecorino di Farindola, a traditional Italian Ewe's raw milk cheese. *Food Chemistry, 175*, 121–127.

Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: Seven dietary factors. *Lancet, 338*, 985–992.

Van Soest, P. V., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*, 3583–3597.

Wang, R. L., Liang, J. G., Lu, L., Zhang, L. Y., Li, S. F., & Luo, X. G. (2013). Effect of zinc source on performance, zinc status, immune response, and rumen fermentation of lactating cows. *Biological Trace Element Research, 152*, 16–24.