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The status of essential elements and associations with milk yield and the occurrence of mastitis in organic and conventional dairy herds

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ABSTRACT

There is a lack of detailed information on the impact of organic feeding regulation on the health and well-being of cows. This has become especially important since January 2008 when the EU required 100% organic ration for organic dairy herds. The aim of this investigation was to determine and compare the levels of essential elements in organic and conventional dairy herds, and to associate them with milk yield and the occurrence of mastitis. The field study was carried out in 10 organic and 10 conventional herds from 2005 to 2010. This period included the point in time when the ration became 100% organic in organic dairy herds. Essential element concentrations (Cu, Co, Se, Zn, Mn, Mo, I and Fe) were determined by inductively coupled plasma–mass spectrometry in 158 serum samples. Associations between concentrations of elements and milk yield and mastitis were determined with mixed linear and logistic regression models, respectively. No significant differences in metal levels between organic and conventional herds were found. No severely deficient concentrations of essential elements were observed in organic herds, either before or after the change in regulation. Cows with low serum concentrations of Se had lower somatic cell counts. Daily milk yield was significantly influenced by deficient concentrations of Cu. For the evaluation of clinical mastitis occurrence, herds were classified for each element, based on the individual values of the sampled cows. Low levels of some elements (Se, I) were associated with a reduced risk of mastitis occurrence. However, other elements indicated a protective effect against mastitis.

1. Introduction

Regulation of organic dairy production results in general in different feeding regimens compared to conventional systems (Fall and Emanuelson, 2009). There is a lack of detailed information on the impact of organic feeding practices on the health and well-being of cows. Since January 2008 the responsible body of the EU has required the ration for organic herds to be 100% organic (EEC 889/2008). While in conventional dairy production mineral deficiencies can be corrected through the inclusion of mineral diet supplements, organic production can only give organic feed; 60% of the food ration must consist of on-farm-produced roughage, and the use of mineral supplements is restricted (EEC 889/2008). Organic herds require strict management of the feed ration to avoid mineral imbalances. Organic management claims to improve animal health and welfare (Sundrum, 2012), but if the diet does not contain sufficient essential elements it

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may actually have the opposite effect (Mitchell and Gray, 2003; Thamsborg et al., 2004; Zollitsch et al., 2004), and it has been reported that organic cows could be prone to some mineral deficiencies (Govasmark, 2005; Govasmark et al., 2005; Kupiainen et al., 2004).

Essential elements are important for a well-functioning immune system (Suttle and Jones, 1989). Their biological function has been underestimated in the past, but has recently been emphasised, clearly showing how necessary they are to stimulate immune response and thereby maintain animals in good health (National Research Council, 2001; Suttle, 2010). Numerous studies have confirmed that the appropriate inclusion of, for example, Se, Mn, Cu, Zn and Co in diets is important for optimising the cows' health, particularly for prevention of mastitis and high somatic cell counts (SCCs) (Ali-Vehmas et al., 1997; Smith et al., 1997; Allison and Laven, 2000; Cebra et al., 2003).

The overall aim of the present study was to determine and compare the levels of essential elements in organic and conventional dairy herds and to evaluate the potential associations between low levels of essential elements and milk yield and the occurrence of mastitis. The study also focused on different conditions that may affect the levels of essential elements, such as feeding regulations for organic herds and cow physiological state.

2. Material and methods

2.1. Study design

This study is one part of an extensive longitudinal survey performed during 2005 to 2010 with the goal of investigating the metabolic status and general health of Swedish organic and conventional dairy herds (Blanco-Penedo et al., 2012; Fall et al., 2008; Fall and Emanuelson, 2009). Originally, 40 farms, half of them organic, with >40 lactating cows and participating in the Swedish Official Milk Recording Scheme, were selected (see Fall et al., 2009, for details about the selection process). For this part of the study the original 40 farms were contacted again by letter and asked to participate. Of these, 13 conventional and 13 organic farms agreed to participate; four declined and one was excluded for technical or time-limiting reasons. Of the 26 farms that were visited, we finally analysed 20 farms to fit the number of samples to the analytical kits. The 26 herds were all visited twice to collect samples during the barn periods November 2005 to March 2006 (first visit) and October 2009 to February 2010 (second visit), with Regulation No 889/2008 *in vigour* during the last period. All herd visits were carried out by a veterinarian. The study was approved by the Ethics Committee for Animal Experimentation in Uppsala, Sweden (C240/9).

All organic farms were managed according to the standards of the Swedish organisation for organic production (www.krav.se) and had been certified for at least three years when the study started. All the components of the diet were in accordance with the practices and legislation associated with each farming system at the time of the visits (CEC, 2005; EEC 889/2008).

Concerning the feeding at the selected herds, there was a large variation in feeding management for lactating cows, from individual feeding of roughage and concentrate to total mixed rations. For dry cows, most farms presented two different feeding regimens: they typically fed roughage *ad lib* with the minerals supplied in a mineral bucket, or in some cases with the minerals spread on top of the roughage once daily.

2.2. Sampling and analyses

Blood samples were taken from approximately 8 randomly chosen healthy cows in each farm to obtain serum from early-lactating (0–6 weeks in lactation) ($n\approx5$) and dry cows (from dry-off until parturition) ($n\approx3$). The total number of samples corresponded to 60 cows at the first visit and 100 cows at the second visit, resulting in 160 serum samples of which two were rejected due to haemolysis (Supplementary material 1). Blood from the coccygeal vein or artery of each cow was collected in evacuated test tubes with gel for serum (tube of 8 ml Z Serum Sep Clot Activator, Vacuette®; Greiner Bio-One, Germany). Blood samples were refrigerated and transported to the laboratory of the Department of Clinical Sciences at Swedish University of Agricultural Sciences or to a field station (in the case of far-off farms). Samples were centrifuged at 2000 g for 10 min and serum was frozen within 6 h of sampling. Samples were stored at $-20\,^{\circ}C$ before analysis. Essential (Cu, Co, Fe, I, Mo, Mn, Se and Zn) and non-essential (Cd and Pb) element concentrations were determined in 158 serum samples by inductively coupled plasma–mass spectrometry (ICP-MS; Thermo X7, Thermo Elemental, Winsford, UK). A sample volume of 250 μL was diluted 10 times with an alkaline solution according to Bárány et al. (1997). The analytical accuracy was checked against reference material (SERONORM Trace Elements Serum LOT. 0903106; SERO AS, Billingstad, Norway). The obtained values for the quality samples showed good agreement with the recommended and certified concentrations. All determinations were made in duplicate preparations, and the method imprecision, calculated as the coefficient of variation for the duplicate measurements, were for all elements but Cd and Pb below 10%. The values for Cd and Pb were 22% and 13%, respectively.

Chemical composition of the diets in the organic and conventional herds at the second visit is presented in Supplementary material 2. Detailed information on the diets at the first visit was not available. Briefly, daily feed consumption on each farm was recorded during a 24 h period by weighing individual feed allocations, or, when cows were fed in groups, by weighing the feed allocated to the group and dividing it by the number of animals, as described in Nordqvist et al. (2014). For estimation of nutrient content the samples were analysed using the NIR method, or, for Ca and Mg, the atomic absorption spectrophotometry method (NMKL, 1998; Eurofins, Lidköping, Sweden). Data on the composition of the concentrates were taken from the feed declarations by the manufacturers.

2.3. Data analysis

To calculate mean concentrations, non-detectable concentrations were assigned a value of half the quantification limit. A Kolmogorov–Smirnov test was used to check whether the data were normally distributed. Problems with non-normally distributed variables were solved by logarithmic transformations. Levels of essential and non-essential elements were examined by the coefficient of variation (CoV) with mean and standard deviation from individuals in the herd to assess intra-herd variability.

Herd data on housing type, as well as individual cow data, such as age, breed, calving date, parity, individual test-day milk yield and SCC from test-milking occasions were retrieved from the Swedish Dairy Association (2007). From 1 January 2005 to 28 February 2010 (last period of data recording), data from all cows were used, except the three months before and after the activation of Regulation no 889/2008, to avoid bias in the analyses caused by the adaptation time to the legal feeding framework practices in organic herds. Data on veterinary treatments were retrieved from the National Animal Disease Recording System.

Cd and Pb concentrations were below levels usually associated with biological disturbances (Puls, 1994), so only essential elements were included in the multivariable analysis. Mixed linear regression model analyses of the concentrations of essential elements, with herd as a random effect, were performed using three predictors: (1) sampling period accounting for changes in EU regulation (period 2005–2006/period 2009–2010), (2) herd type (conventional/organic) and (3) cow physiological state (early lactation/dry period).

The relationships between daily milk yield and SCC and essential elements were evaluated by mixed linear regression models, with herd as a random effect. Variables of mineral concentration were classified as dichotomous variables as below or above a threshold for each essential element. The applied cut-off was based on the lower quartile of the actual analysed values (lower quartile; Supplementary material 3). The rationale was that reference values are mostly appropriate for theoretical orientation, whereas adaptation according to the situation at hand was considered more suitable in the study context (Spolder et al., 2010). Apart from the status of each essential element, other predictors were also included to adjust for their effects on milk yield and SCC, such as breed, parity, days in milk (DIM) and housing type. Breed was a categorical variable with three classes: Swedish Holstein, Swedish Red and Other. Parity was a categorical variable with three classes: first, second, and third or greater, and housing type a dichotomous variable with loose housing and tie stall. For the model of SCC, the variable was transformed to the natural log scale (lnSCC) to obtain normally distributed residuals. To model the lactation curve, a function of DIM was included (Ali and Schaeffer, 1987). The observations of daily milk yield and SCCs correspond to the milk-test occasion closest to the sampling date.

Classification of the herd for the evaluation of the nutritional status in cattle herds (Herdt and Hoff, 2011; Kincaid, 1999, 2008) was based on the lower quartile of individual values for each element of the sampled cows. The development of the herd classification is a modification of the methodology proposed by Enjalbert et al. (2006). A mixed logistic regression model was performed, with two hierarchical levels (herd and cow) in the data, to study the association between herd classification and cases of clinical mastitis. The outcome variable was defined by the presence of veterinary-treated cases of mastitis (yes=1) or not (no=0) in individual cows. A re-treatment within 21 days was not counted as a new case. The approach for Zn and Co herd status was not valid, because all herds were finally classified as adequate 'Zn' and 'Co' herds, and these were consequently dropped from the analysis.

In all statistical analyses, two-way interactions were investigated once a main-effects model had been achieved. Collinearity among predictor variables was investigated using the Spearman rank correlations test and considered if correlation was $\geq 60\%$. No highly correlated variables were detected. Modelling was done manually, both by backwards elimination of non-significant variables and by forward selection. For each eliminated or entered variable, confounding was assessed by comparing the coefficient change of included variables. Potential confounders were considered as present if a coefficient changed $>20\%$ when the variable was excluded from the model. A Wald's test was used to check whether predictors in the final model were associated with the outcomes. All statistical analyses were performed using STATA Software v. 11 (Stata Corporation, College Station, TX, USA).

3. Results

Concentrations of essential and non-essential element are displayed in Fig. 1 and Supplementary material 3. Results of the statistical analyses of the associations between predictor variables and essential elements in serum are presented in Table 1. Organic cows had higher concentrations of Mo ($P<0.001$), Co ($P=0.028$) and Se ($P=0.047$) in the period 2009–2010 than organic cows in the period 2005–2006, and higher concentrations of Fe during the early lactation ($P=0.005$). The mean Cu concentration was significantly higher for early-lactating cows ($P<0.001$) compared to dry cows, while Mo and Se concentrations were significantly higher in dry cows. Fe and Zn concentrations were significantly higher during the period 2009–2010. For non-essential elements, a large number of serum samples did not contain detectable amounts of Cd (88.6%), and 8.8% of serum samples did not contain detectable Pb.

Intra-herd variability (examined by coefficient of variation, CoV) is presented in Fig. 2. For most of the elements CoVs were below 50%, but tended to be higher during the period 2009–2010 for some elements. There was no clear pattern for herd type. Regarding non-essential elements, non-detected and detected levels alone created the higher CoVs for Cd and lesser for Pb.

Results of the analyses of individual daily milk yield and SCC are presented in Table 2. Concentrations of Cu below the cut-off were associated with lower milk yield ($P=0.039$). Cows from second or greater parity and Swedish Holsteins were associated with higher milk yields. Concentrations of Se below the cut-off were associated

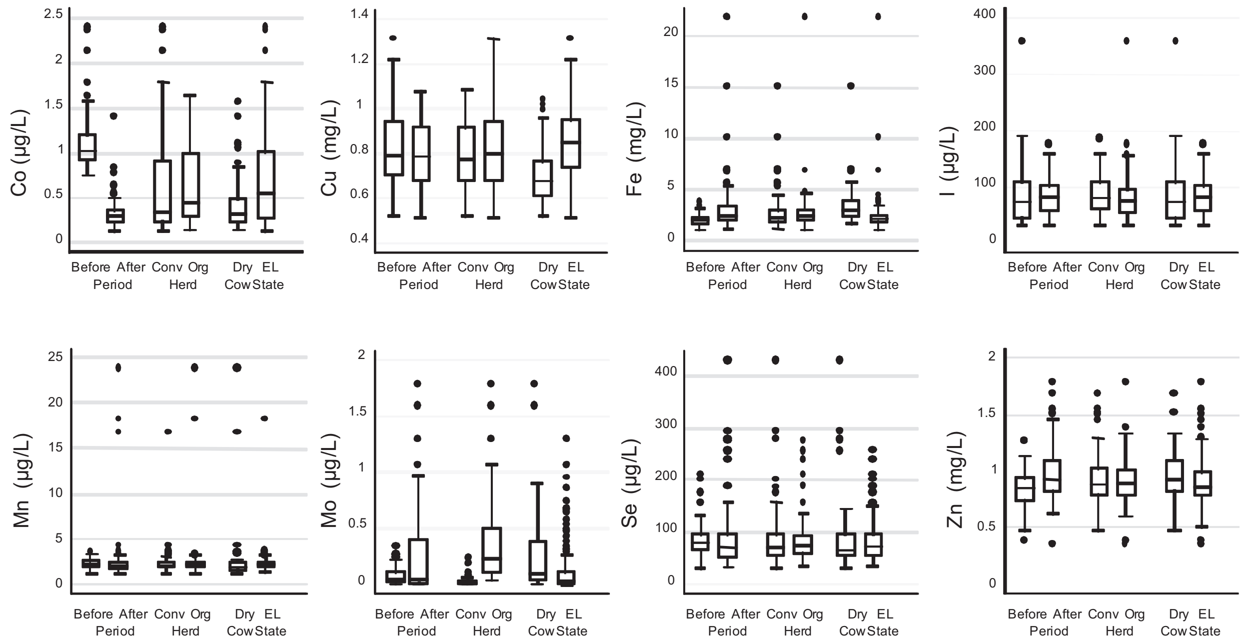

Fig. 1. Box plot showing concentrations of essential elements in serum of dairy cows in 10 organic and 10 conventional Swedish herds. The total number of samples per sub/group and abbreviations are *before* (N=59; years 2005–2006) and *after* (N=99; years 2009–2010) for the period; *Conv* (N=88; conventional) and *Org* (N=70; organic) for herd type; and *Dry* (N=44; dry period) and *EL* (N=114; early lactation period) for cow state.

Table 1
Fixed effect coefficients (and *p*-values) of the mixed linear regression models for the effects of predictor variables on log-transformed essential elements in serum concentrations of the sampled cows from 10 organic and 10 conventional Swedish herds (n=158). Only the statistically significant models are shown.

Predictor variable		Co	Cu	Fe	Mo	Se	Zn
Intercept		0.055	2.81	3.50	1.40	1.95	2.91
Period	2005–06	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
	2009–10	−0.607	0.020	0.093	−0.111	−0.064	0.067
		(<0.001)	(0.129)	(0.001)	(0.088)	(0.011)	(<0.001)
Herd type[a]	Conv	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
	Org	−0.016	0.012	−0.124	0.844	−0.029	−0.001
		(0.745)	(0.336)	(0.019)	(<0.001)	(0.752)	(0.948)
Physiol. State[b]	Dry	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
	EL	−0.023	0.089	−0.216	−0.264	−0.042	−0.002
		(0.434)	(<0.001)	(<0.001)	(<0.001)	(0.049)	(0.875)
2009/10*Org		0.114			0.584	0.074	
		(0.028)			(<0.001)	(0.047)	
EL*Org				0.170			
				(0.005)			

[a] Herd type: conventional (Conv) and organic (Org).
[b] Physiological state: dry period (Dry) and early lactation period (EL).

with lower SCCs (P=0.025). Also, cows in third or greater parity had higher SCCs.

Results from the analysis of clinical mastitis are presented in Table 3. Herds with low Mn and Mo levels had increased odds of clinical mastitis, while herds with low I and Se levels had reduced odds. Low Cu levels were associated (P=0.026) with an increase in cases of clinical mastitis for cows in second parity. Cows of the Swedish Holstein breed and cows kept in loose housing were both at higher odds for suffering mastitis, but the housing type was only marginally significant. In contrast, cows in organic herds were at lower odds (P=0.021).

4. Discussion

4.1. Concentration, status and role of essential elements

The current organic guidelines may influence the conditions for the mineral supply of the organic cows, but our study did not indicate any deficiency of essential elements in organic herds, although significant differences between herd type, legislation and physiological state were found for Co, Fe, Mo and Se. Analysing in detail the significant differences due to herd type and period of sampling, it may be reasonable to assume that the ban on use of feed of

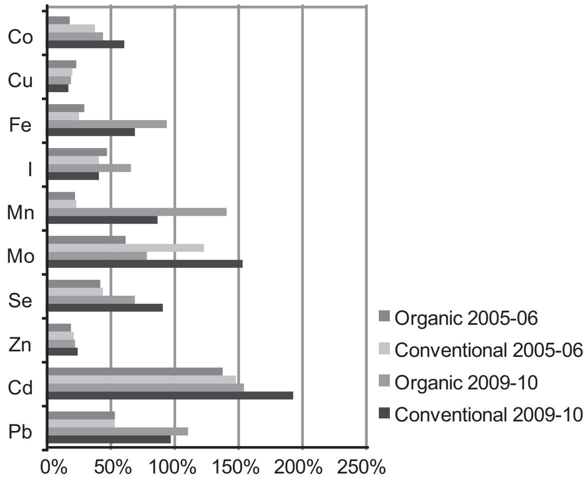

Fig. 2. Coefficients of variation for concentrations of non-essential and essential elements for the two periods 2005–2006 and 2009–2010 in the 10 organic and 10 conventional herds considered in this study.

conventional origin in the recent past has not impaired the trace element status of dairy cows in organic herds. In general, there is a lack of information on differences in feeding regime for conventional and organic herds. Large-scale epidemiological studies may therefore be useful to investigate possible differences in mineral status (of individual animals) in spite of the difficulties of such studies. To our knowledge, there are a rather limited number of earlier studies of essential elements focusing on cows of organic dairy systems. These suggested that organic dairy cows were at a greater risk of deficiency of Cu and Zn compared to animals kept in conventional herds (Govasmark, 2005; Govasmark et al., 2005; Tomza-Marciniak et al., 2011), but in our study we found no significant differences in metal levels. However, the differences in study design and the country context make it difficult to compare results.

The physiological state of the cow was also a significant factor in the variation of element concentrations. Differences in serum concentrations between early-lactating

Table 2

Fixed effect coefficients of the mixed linear regression model for the effects of essential elements on daily milk yield ($n=148$) and log-transformed somatic cell counts (lnSCC; $n=140$) of sampled cows from 10 organic and 10 conventional Swedish herds.

		Daily milk yield		SCC	
		Coef.	*p*-value	Coef.	*p*-value
Intercept		208.5		−4.55	
Threshold_Co[a]	Above cut-off	Baseline		Baseline	
	Below cut-off	−1.48	0.346	0.055	0.874
Threshold_Cu[a]	Above cut-off	Baseline		Baseline	
	Below cut-off	−2.81	0.039	−0.426	0.166
Threshold_Fe[a]	Above cut-off	Baseline		Baseline	
	Below cut-off	−0.042	0.975	0.343	0.242
Threshold_I[a]	Above cut-off	Baseline		Baseline	
	Below cut-off	−1.01	0.532	0.518	0.149
Threshold_Mn[a]	Above cut-off	Baseline		Baseline	
	Below cut-off	1.92	0.146	0.155	0.590
Threshold_Mo[a]	Above cut-off	Baseline		Baseline	
	Below cut-off	2.48	0.167	0.637	0.073
Threshold_Se[a]	Above cut-off	Baseline		Baseline	
	Below cut-off	0.777	0.657	−0.805	0.025
Threshold_Zn[a]	Above cut-off	Baseline		Baseline	
	Below cut-off	−1.21	0.338	0.048	0.864
Period	2005–06	Baseline		Baseline	
	2009–10	−1.69	0.258	0.071	0.833
Herd type	Conventional	Baseline		Baseline	
	Organic	0.095	0.967	−0.121	0.703
Stage of lactation	DIM/305	−349.6	< 0.001	13.8	0.538
	log(305/DIM[d])	−204.4	0.002	10.2	0.505
	(DIM[d]/305)^2	154.6	0.002	−4.55	0.676
	(log(305/DIM[d]))^2	57.4	0.006	−2.93	0.560
Parity	First	Baseline		Baseline	
	Second	10.6	< 0.001	−0.293	0.381
	Greater	11.6	< 0.001	0.937	0.002
Breed	SR[b]	Baseline		Baseline	
	SH[c]	6.63	< 0.001	0.053	0.867
	Other	0.433	0.833	−0.106	0.804
Housing type	Tie stall	Baseline		Baseline	
	Loose housing	−1.31	0.539	−0.140	0.618

[a] Considering a threshold of deficiency (below) or safety (above) status for each essential element. The applied cut-off was based on the distribution of the actual analysed values (see Supplementary material 2).

[b] SR: Swedish Red.

[c] SH: Swedish Holstein.

[d] DIM: Days in milk.

Table 3
Fixed effect coefficients of the mixed logistic regression model for the effect of herd-level status of essential elements on the occurrence of clinical mastitis in 10 organic and 10 conventional Swedish herds ($n=2947$ observations).

		Coef.	Odds ratio	*p*-value
Intercept		−3.77		
Herd_Cu status[a]	Adequate	Baseline		
	Low	−0.292	0.7	0.410
Herd_I status[a]	Adequate	Baseline		
	Low	−0.899	0.4	0.010
Herd_Fe status[a]	Adequate	Baseline		
	Low	0.090	1.0	0.705
Herd_Mn status[a]	Adequate	Baseline		
	Low	0.598	1.8	0.017
Herd_Mo status[a]	Adequate	Baseline		
	Low	1.86	6.2	< 0.001
Herd_Se status[a]	Adequate	Baseline		
	Low	−1.23	0.3	0.002
Parity	First	Baseline		
	Second	−0.183	0.8	0.610
	Greater	0.502	1.7	0.088
Low herd Cu status – Second parity		0.946	2.6	0.026
Low herd Cu status – Greater parity		0.906	2.5	0.011
Herd type	Conventional	Baseline		
	Organic	−0.480	0.6	0.021
Breed	SR[b]	Baseline		
	SH[c]	0.713	2.1	< 0.001
	Other	0.363	1.5	0.094
Housing type	Tie stall	Baseline		
	Loose housing	0.662	1.8	0.058

[a] With > 2 (low) or ≤ 2 (adequate) observations per herd within the lower quartile of data distribution.
[b] SR: Swedish Red.
[c] SH: Swedish Holstein.

and dry cows were probably related to physiological variations (Grace and Knowles, 2012). The higher serum levels of Cu at the beginning of lactation could be explained by the elevation of ceruloplasmin synthesis, which depends on oestrogen levels, which increase during pregnancy (Yocus et al., 2004). Lower serum Mo concentrations after parturition could vary with stage of pregnancy and level of milk production, since both affect mineral requirements. In our study, there was a low serum Fe concentration in early pregnancy, which might be caused by the high demand of Fe by the foetus during early gestation (Yocus et al., 2004). But higher Fe concentrations in early-lactating organic cows in our study might be explained by high levels of Fe from forage, possibly contaminated with soil (Blanco-Penedo et al., 2009). Finally, seasonal variations were not expected to contribute to the variation in this study, because all sampling was performed during the stable period.

The use of serum chemical parameters, interpreted with care, provides information that may serve as a basis for the diagnosis, treatment and prognosis of diseases (Herdt and Hoff, 2011). However, tissue samples may prove more useful for the detection of some trace element deficiencies (e.g. liver for subclinical Cu deficiency; Mulligan et al., 2006). Serum concentrations of Mn and I in our study were predominantly within marginal and deficient ranges according to Puls (1994), but comparable to those of other studies in dairy cows (Erdongan et al., 2004; Guyot et al., 2009). Regarding Mo levels, observations within marginal and deficient ranges according to Puls (1994) are of minor relevance, since cattle are known to be a ruminant species very sensitive to molybdenosis (Radostits et al., 2006), and the Swedish context is characterised by situations of molybdenosis (Frank, 2004). Soil type, soil mineral content, the use of resources (fertilisers) and mineral supplementation were not examined in our study. So, finding differences in Mo and Se between herd types (predominance of cases of deficiency in conventional cows) should be interpreted with caution.

4.2. Animal performance and udder health

In our study, animal performance, measured by daily milk yield of individual cows, was seriously compromised at lower levels of Cu. This effect could be explained by the necessity of Cu for the maintenance of normal productivity in animals (NRC, 2001). Whilst the level required to overcome a deficiency symptom does not necessarily promote productivity (Trewavas and Stewart, 2003), lower concentrations at least imply milk losses (NRC, 2001), as we partly observed in this study.

Associations between concentrations of trace elements and occurrence of mastitis are hard to interpret, because many compounds have both health-improving and health-debilitating effects, depending on the concentration (Trewavas and Stewart, 2003). From the classification of our herds we hypothesise that the association between trace element concentration and the occurrence of a disease could be explained by the lack of optimum concentration (according to a defined cut-off) to develop the full function of the element with respect to its potential to limit occurrence of mastitis. This supports what we found for Mn, Mo and Cu.

Most of the literature indicates a positive association between Se and udder health (Andrieu, 2008), for instance, as a reduced incidence of clinical mastitis. So far, only one study is in line with our results (Giadinis et al., 2011), indicating a possible predisposing role of high Se in ovine mastitis. Regarding I, studies merely report its effectiveness against mastitis as a teat sanitiser (Lopez-Benavides et al. 2009). It is difficult to compare previous studies to our study, due to different experimental settings. The evaluation of SCC also found that low Se in serum was associated with lower SCC, which is consistent with the results of clinical mastitis. The mechanisms of these associations remain unknown; however, they may be explained by the biological activity of different species, bioavailability of Se (Scaletti et al., 2003), and body compartments, but we measured only total content of Se (Kincaid, 1999). To some extent, the imbalances of Se and I status (highly correlated in our study: $R_s = 0.422$, $P < 0.001$) may reflect a concomitant necessity for an adjusted mineral supplementation while the cow is suffering an episode of mastitis.

Complexity arises, because interactions between individual animals and the herd system are complex and site specific, making it difficult to draw conclusions that can be generalised and transferred directly from one level (herd) to the other (cow). Further studies are needed to establish a threshold at which an early warning and application of necessary preventive measures to reduce clinical mastitis would be triggered. This issue is especially important in organic management, where strategic mineral supplementation should possibly also target periods when mastitis occurs most frequently.

5. Conclusions

The main conclusion of our study is that the new European feeding regulation for organic management did not impair the range of essential element levels necessary to secure animal health. We also identified that low levels of some elements (Se, I) were associated with an increased risk of mastitis occurrence, whilst others may improve biological processes and offer protective effects against mastitis.

Conflict of interest statement

There is no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.livsci.2014.07.016.

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