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ORIGINAL ARTICLE

Effects of different strategies of mineral supplementation (marine algae alone or combined with rumen boluses) in organic dairy systems

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Summary

This study was designed to evaluate the effect of marine algae supplementation alone or in combination with a regular mineral supplement (rumen boluses) to improve the mineral status in organic dairy cattle and their effect on the milk mineral composition, milk production, composition (% of fat and protein) and quality (SCC). Thirty-two Holstein Friesian lactating cows were randomly selected and assigned to the algae (A), boluses (B), algae+boluses (AB) and control group (C). For the algae groups (A, AB), a supplement composed of Sea Lettuce (80%), Japanese Wireweed (17.5%) and Furbelows (2.5%) was formulated to be given to the cows at the rate of 100 g/animal per day (A1) for the length of 4 weeks. In the second half of the experiment (weeks 5–8), the algae mixture was reformulated and the proportion of Furbelows was increased from 2.5% to 5.0% with a subsequent decrease of Lettuce to 77.5% (A2). In the boluses group (B), each cow received 2 boluses after calving. Blood (serum) and milk samples were collected at 2 and 4 week intervals, respectively, and analysed for trace element concentrations by ICP-MS. Information related to the milk composition and SCC during a 305-day lactation for each animal were obtained from the Dairy Records Management System. The supplementation with algae, boluses or the combination of both treatments showed a statistically significant effect on the iodine (algae), selenium (boluses) and cobalt (algae+boluses) status of the animals. In milk, treatments had a statistical significant increase on iodine, and a tendency to increase selenium concentrations. The assayed algae mixture combined with another source of selenium could be an effective tool to improve the mineral status in serum and milk.

Keywords dairy cattle, minerals, milk, algae, rumen boluses, organic systems

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Introduction

Organic and other sustainable production systems are highly dependent on the environment. Mineral disturbances can appear if concentrations in the soil are low, unbalanced or unavailable (López-Alonso, 2012).

A recent study in Northern Spain has identified mineral deficiencies, especially of iodine (I) and selenium (Se), in organic dairy systems associated to a low content of these elements in the diet (Miranda et al., 2015). As mentioned in previous studies in different countries (Rasmussen et al., 2000; Dahl et al., 2003; Bath et al., 2012; Köhler et al., 2012), organic milk from this region of Spain has a lower mineral content compared to the conventional dairy systems (Rey-Crespo et al., 2013). All these studies have pointed out that the general mineral restrictions in organic farming leads to a significantly lower I content in organic milk, which could pose a problem for populations at risk including pregnant women and children (Bath et al., 2012).

However, it is also possible that the low Se and I status of cattle under organic systems in Spain could lead to a lower production in these farms compared to other pastured-based farms in this area, which can lead to reproductive disorders and other diseases (Cook and Green, 2007, 2010; Spears and Weiss, 2008; Borucki-Castro et al., 2011; Overton and Yasui, 2014).

In a previous experiment, we have assessed the suitability of using a mixture of several algae species from the Galician coast in organic dairy production (Rey-Crespo et al., 2014). The results have showed beneficial effects, as the serum and milk I concentration was improved and I milk concentration was similar to cows under non-organic dairy systems (Rey-Crespo et al., 2014). However, although Se concentration in the seaweed mixture was higher than in other feedstuffs, its low rate of inclusion in the diet was not enough to correct the low Se status in the cow, and consequently the low milk Se concentration. The capacity of the marine algae to improve the mineral status of cows could indirectly have a positive effect in milk production because it was reported a positive relationship between feeding micronutrients and increase milk yield and decrease somatic cell count (SCC) (Cook and Green, 2010; Overton and Yasui, 2014). In addition to minerals, marine algae are an important source of antioxidants and have antimicrobial and immunomodulatory activities (Allen et al., 2001), which could have important benefits for dairy production. We have the hypothesis that marine algae supplementation in cattle under organic systems can lead to increase the mineral intake in cows and thus to improve the overall mineral status in cows. This study was designed to evaluate the effect of marine algae supplementation alone or in combination with a regular mineral supplement (rumen boluses) to improve the mineral status in organic dairy cattle and their effect on the milk mineral composition, milk production, composition (percentage (%) of fat and protein) and quality (SCC).

Materials and methods

All the experiments followed the Spanish standards for the protection of animals used for experimental and other scientific purposes, and permission for developing the experiment was granted by the Bioethical Committee of the University of Santiago de Compostela (Spain).

Farm of study

This study was conducted in a certified organic dairy farm located in the Province of A Coruña, Spain (latitude north: 43.08, longitude west: −8.25). The farm was managed according to the standards of organic farming (EC 889/2008) and has been certified as organic for the last 10 years. The farm was involved in a big project to evaluate the nutritional and productive status of organic farming in North Spain (Spanish Government Ref. AGL 2010-21026). It was selected because of its records of low I (<40 μg/l; Alderman and Stranks, 1967) and Se (<40 μg/l deficiency, 40–70 marginal; Gerloff, 1992) status based on results from previous experiments (Miranda et al., 2015).

The farm had 153 Holstein Friesian cows in lactation with an average 305-d-corrected milk yield of 7505 kg/cow. Cows remained at pasture (a mixture of white clover and perennial ryegrass mixture) all day and received a complementary feeding (11.9–15.5 kg of total DMI depending on the seasonal availability) as total mixed ration (TMR). Diet was composed of preserved forages, purchased forages and concentrates. Using our own calculations inferred from the Spanish organic dairy cattle population (Milk (L) = 1.709*DMI (kg)−10.391; R^2 = 0.939, p < 0.001), DMI during the experiment was 20.2 kg/cow/day, pasture representing 28.4%. All the components of the diet were in accordance with the practices and legislation for organic production (EC 889/2008). Chemical and mineral composition of the diet was presented in Tables 1 and 2 respectively.

Treatment groups

The experiment was carried out on four groups of animals: Algae (A), boluses (B), algae plus boluses (AB) and control group that remained unsupplemented (C).

Algae supplement

Two different algae mixtures were assayed. During the first half of the experiment (weeks 1–4), the animals received 100 g/animal/day of an algae mixture (A1) composed by Sea Lettuce (*Ulva rigida*) (as flakes, 80%), Japanese Wireweed (*Sargasum muticum*) (as flakes, 17.5%) and Furbelows (*Saccorhiza polyschides*) (powder, 2.5%) that were satisfactorily administered in another organic farm (Rey-Crespo et al., 2014). According to the requirements for dairy cattle

Table 1 Chemical composition of the pasture and the total mixed ration (TMR)

	Pasture	TMR
Chemical composition (% DM)		
Dry matter (DM)	30.6	56.2
Crude protein (CP)	19.3	11.0
Neutral detergent fibre (NDF)	46.6	40.1
Acid detergent fibre (ADF)	26.5	25.8
Organic matter (OM)	89.1	92.7
Ingredient (% DM)		
Corn silage		44.3
Hay		23.4
Prairie silage		14.7
Concentrate		17.6

Table 2 Trace element concentrations in the basal diet (mg/kg DM), algae mixtures (A1: weeks 1–4, A2: weeks 5–8), ruminal boluses and physiological requirements (NRC, 2001)

		Treatment			
Element	Basal diet (mg/kg DM)	Algae mixture A1 (mg/kg DM)	Algae mixture A2 (mg/kg DM)	Boluses* (g/treatment)	Requirements (mg/kg DM)
Co	0.206	0.298	0.304	0.480	0.1
Cr	2.67	3.54	3.51		
Cu	4.39	2.77	2.77	33.00	16
Fe	285	473	468	48	24
I	0.192	188	302	0.500	0.45
Mn	61.2	17.4	17.3	17.6	17
Mo	0.528	0.222	0.223		
Ni	2.02	3.28	3.26		
Se	0.09	1.15	1.16	0.48	0.3
Zn	45.5	29.5	30.6	27.0	43

Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; I, iodine; Mn, manganese; Mo, molybdenum; Ni, nickel; Se, selenium; Zn, zinc.
*Corresponding to 2 boluses of 107 g/unit. Details of the algae mixtures composition and rumen boluses are given in the text.

(National Research Council (NRC), 2001), the algae mixture satisfied the mineral requirements of the animals with the exception of Se, which was still deficient (Table 2). However, as detailed in the results section, milk mineral analysis during the experiment revealed that I concentration in the groups receiving the algae supplement was lower than expected. Therefore, in the second half of the experiment (weeks 5–8), the algae mixture was reformulated (A2): furbelows were increased from 2.5% to 5.0% with a subsequent decrease of Lettuce to 77.5%. Except for I (62% increase) differences in the mineral content between both algae mixtures were very low (<3%). Algae were supplied by a local manufacturer (Porto Muiños S.L., Cerceda, A Coruña, Galicia, Spain) and were harvested along the Galician coast (Northwestern Spain). Algae were washed with fresh water and dried with forced air at <30 °C. Leaves were cut into small flakes and Furbelows were pulverized with an automatic mortar to obtain dry powder.

Boluses

Megabric ® Grass Neolait (Tregueux, France), two boluses of 107 g each were used per cow. As indicated by the manufacturer, they allow a precise individual long-term delivery of mineral (8 months) very suitable for animals on pasture-based systems. The mineral composition given by the supplier is presented in Table 2.

Experimental design

This study was conducted with 32 Holstein Friesian cows lasting for a full lactation. The animal selection was carried out on the basis of including all cows with an estimated parturition date after 15 February 2013 until the desired number of animals was obtained. Before beginning the experiment, the selected cows were randomly assigned to an experimental group (A, B, AB or C) ensuring that there were no statistically significant differences ($p > 0.05$) in the number of lactations, milk production, composition (% of fat and protein) and quality (SCC) during the previous lactation based on the data of the Dairy Records Management System (DRMS). The treatment started at parturition. Immediately after calving, cows were identified with collars of different colours to facilitate the identification within the herd and the animals assigned to groups B and AB received the boluses treatment. The algae supplement (groups A and AB) was daily administered to the animals in a basket mixed with the TMR feed at their morning milking. Cows in all treated groups received the same type and amount of feeding with the exception of the mineral supplements.

Sampling and sample processing

Blood samples (10 ml from the coccygeal vein) from each cow were collected fortnightly for mineral analysis, and milk samples (25 ml) were collected at 4-week intervals. Samples were collected immediately after the morning milking. Information related to the milk production, composition (% of fat and protein) and SCC for each animal was obtained from the DRMS. Two of the experimental cows did not finish their lactations, and two other cows had incomplete data. Therefore, these cows were excluded from the analysis.

Samples (serum and milk) were digested with acid (all elements except I) and alkaline (I) methods. Ten trace minerals were determined by inductively coupled plasma mass spectrometry (ICP-MS; VG Elemental PlasmaQuad SOption): Cobalt (Co), chromium (Cr), copper (Cu), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn). Detailed information about sampling processing was previously reported (Rey-Crespo et al., 2014).

An analytical quality control programme was followed throughout the study. All samples were above the quantification limits except for Cr (0.126 μg/l) in milk. Analytical recoveries were determined from certified reference materials (Serum: standard reference material 1598a, bovine serum. Milk: non-fat milk powder NIST-1549), which were analysed alongside the samples. Mean recoveries were between 92% and 108% for serum and 89–114% for milk in all cases.

Statistical analysis

All statistical analyses were carried out using the program SPSS for Windows (v.21.0; Chicago, IL, USA), and the criterion for statistical significance was established at $p < 0.05$. Normal distribution of data was checked using a Kolmogorov–Smirnov test. The effect of treatment (A, B, AB or C) on trace element concentrations in serum, milk production (l/day), composition (% of fat and protein) and quality (SCC) was evaluated using repeated measures ANOVA, whereas the effect of treatment on milk trace element concentrations was evaluated using one-way ANOVA. Differences between groups were tested using a *post hoc* Tukey test.

Results and discussion

Trace mineral concentrations in the basal diet (Table 2) satisfied the physiological requirements (according to National Research Council (NRC), 2001; Suttle, 2010) except for I (0.45 mg/kg DM) and Se (0.3 mg/kg DM). The inclusion of the algae supplementation in the diet (100 g in 20.2 kg DMI) allowed us to increase the dietary I concentration to adequate values (1.12 and 1.69 mg/kg DM for A1 and A2 respectively), although Se concentrations were still marginal (0.096 mg/kg DM) (Table 2). The boluses nearly satisfy the physiological requirements of Se (estimated daily release from boluses: 3.4 mg; Sprinkle et al., 2006), although I concentrations were still marginal due to its low concentration in the boluses. The combination of the two treatments (group AB) allowed to correct Se and I intake without exceeding the maximum allowed concentration for trace elements established by the EU (Commission Regulation (EC), 2003).

Table 3 Serum trace element concentrations at the beginning of the experiment in the whole experimental animals (n = 32) and reference values

	Mean ± SE	Range	Reference values
Co (μg/l)	0.227 ± 0.011	0.119–0.356	0.17–2.0*
Cr (μg/l)	2.07 ± 0.06	1.63–3.30	–
Cu (mg/l)	0.693 ± 0.018	0.517–0.959	0.6–1.1*
Fe (mg/l)	1.73 ± 0.07	0.87–2.47	1.1–2.5*
I (μg/l)	29.8 ± 2.0	7.5–53.1	>40†
Mn (μg/l)	2.96 ± 0.12	1.92–4.37	0.9–6.0*
Mo (μg/l)	14.3 ± 2.27	5.1–53.7	2.0–35*
Ni (μg/l)	3.21 ± 0.12	1.77–4.87	1.2–5.6‡
Se (μg/l)	27.5 ± 0.9	17.1–37.0	>70§
Zn (mg/l)	0.725 ± 0.032	0.472–1.099	0.6–1.9*

Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; I, iodine; Mn, manganese; Mo, molybdenum; Ni, nickel; Se, selenium; Zn, zinc.
*Herdt and Hoff (2011); †Alderman and Stranks (1967); ‡Puls (1994); §Gerloff (1992).

Dietary mineral concentrations were in agreement with the animal mineral status at the beginning of the experiment (Table 3). Overall serum trace elements concentrations were within the adequate range except for I and Se, and no statistically significant differences ($p > 0.05$) were observed for any trace element concentrations among the treatment groups (data no shown).

The supplementation with algae (A), rumen boluses (B) or the combination of both treatments (AB) showed a statistically significant effect on the I and Se serum levels of the animals. Serum I concentrations were statistically higher in the groups that received the algae supplement, even though no differences were observed between them (A and AB). There was an increase of approximately 40% in the serum I concentration in groups A and AB from week 5 up to the end of the experiment (Fig. 1). Serum I concentrations of cows treated with rumen boluses (B) increased to reach adequate values (67.0 ± 13.3 μg/l) in week 2 but progressively decreased to the basal values (C) at the end of the experiment. For Se, as expected, the treatment groups that showed the higher increase in serum Se concentrations were those receiving the rumen boluses (B and AB) (Fig. 1). Similarly to I, the higher effect of the rumen boluses on the serum Se concentration was observed at week 2, when animals showed a mean serum concentration slightly above the adequate level (71.5 ± 8.8 μg/l), to then decrease to concentrations close to the basal range. A shorter effect of ruminal boluses than expected was also found in other studies. In fact, Cook and Green (2010) administering similar boluses (500 mg Se/treatment) at dry-off to dairy cattle only

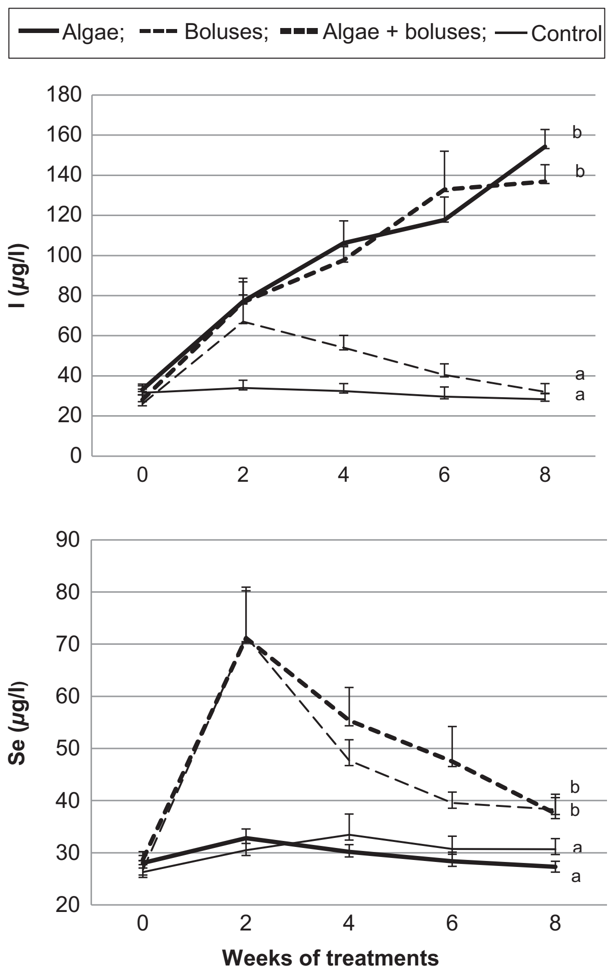

Fig. 1 Scatterplots showing blood I and Se serum concentrations in the algae (*n* = 8), rumen boluses (*n* = 7), algae+boluses (*n* = 8) and control (*n* = 8) groups during the experiment. Different letters indicate statistically significant differences between groups for the whole experiment.

found a significant effect (based on milk production) until 100 days of lactation, suggesting than a second application of boluses during lactation may be beneficial. Finally, the algae groups (A, AB) showed significantly higher serum Co concentrations compared to the control group (36% and 51% respectively); even though, the serum Co concentration was always within the reference ranges. Iodine, Se and Co serum concentrations show a good response to dietary supplementation (Franke et al., 2009; Schöne et al., 2009; Cook and Green, 2010). The homoeostatic control of these elements is mainly mediated by urinary excretion: they are absorbed from the intestinal tract at high rates without a homoeostatic control (Kirchgessner et al., 1994, 1997, 1999).

Mineral concentrations in milk are presented in Table 4. Overall, mineral concentrations in the control group were within the reference ranges except for I and Se that were in the deficient and marginal range respectively (according to Puls, 1994). As expected, the algae and boluses supplementation had a statistical significant effect on milk I concentrations ($p < 0.001$). This was especially relevant during the second half of the experiment for the groups that received algae, which reached 122-158 μg/l. For Se, a tendency to increase milk Se concentration was observed (Table 4; $p < 0.1$). This increase was enough to reach adequate values in the groups receiving rumen boluses (Table 4), although Se milk concentration was only close to the lower reference limit in cows receiving the algae (8 weeks; 18.6 and 19.8 μg/l for groups A and AB respectively). It is well known that both I (Franke et al., 2009; Schöne et al., 2009) and Se (mainly organic compounds; Ceballos et al., 2009; Stockdale et al., 2011) excretion in milk is related to dietary intake (based on their renal homoeostatic control as previously indicated) which makes it technically feasible to increase both elements in milk supplying the diet of the animals (Ceballos et al., 2009; Schöne et al., 2009; Stockdale et al., 2011).

Data of milk production (l/cow/day), milk composition (% of fat and protein) and quality (SCC) are summarized in Table 5. Overall, there were no statistically significant effects of treatments on any of these parameters. Both Se and I are very important trace elements in dairy farming, their supplementation has been demonstrated to increase milk production and to reduce SCC by improving immunity (Cook and Green, 2010; Overton and Yasui, 2014). In addition to trace elements, marine algae are an important source of different micronutrients that could have important benefits for dairy production and the supplementation of marine algae to the diet is an effective strategy to enhance quality and milk production (Franklin et al., 1999; Boeckaert et al., 2008; Hostens et al., 2011). However, the beneficial effects of algae supplementation have not always been proved and differences between studies could be related to, besides the type and quantity of supplement, other nutritional and management factors (Friggens et al., 2010; Hostens et al., 2011). In example, SCC (the main udder health maker) are affected not only by pathogens presence but by management and animal factors (related to parity and stage of lactation) (Friggens et al., 2010).

Conclusion

Under the experimental conditions of our study, which are similar in most organic farms in Spain, the

Table 4 Trace element concentrations in milk (μg/l) in the algae (A, $n = 8$), bolus (B, $n = 7$), algae+bolus (AB, $n = 8$) and control (C, $n = 8$) groups

		Treatment group					
	Week	C	A	B	AB	RMSE	p
Co	4	0.459	0.455	0.462	0.558	0.152	0.474
	8	0.759	0.456	0.419	0.534	0.507	0.559
Cu	4	53.6	71.6	92.0	68.2	51.7	0.563
	8	45.1	73.2	74.2	64.3	34.8	0.335
Fe	4	512	428	442	468	156	0.723
	8	394	406	385	420	81	0.851
I	4	12^{a}	38^{bc}	22^{ab}	46^{c}	15	0.001
	8	25^{a}	158^{b}	37^{a}	122^{b}	55	0.000
Mn	4	38.1	34.8	35.1	34.1	11.7	0.906
	8	39.3	51.3	42.6	33.8	14.9	0.148
Mo	4	44.8	39.4	38.0	36.6	10.0	0.398
	8	40.7	46.0	42.3	47.9	9.6	0.439
Ni	4	5.06	4.69	5.59	3.43	4.17	0.774
	8	5.73	5.57	10.00	7.23	7.10	0.613
Se	4	15.1	17.6	20.1	22.8	6.0	0.094
	8	15.5	18.6	17.2	19.8	3.4	0.086
Zn	4	5422	5907	6233	6239	1777	0.777
	8	5336	6138	5786	6671	1620	0.423

RMSE, root mean square error; Co, cobalt; Cu, copper; Fe, iron; I, iodine; Mn, manganese; Mo, molybdenum; Ni, nickel; Se, selenium; Zn, zinc. Results are expressed as mean values in wet weight. Different letters within the same row indicate statistically significant differences between groups ($p < 0.05$). For chromium all samples were below the detection limit.

Table 5 Summarized data of milk parameters related to milk production, composition and quality in the algae (A, $n = 6$), bolus (B, $n = 6$), algae+bolus (AB, $n = 7$) and control (C, $n = 8$) groups for 305-day lactation. Results are given as estimated marginal mean values

	Treatment group					
	C	A	B	AB	RMSE	p
Milk (l/cow/day)	23.7	25.7	22.1	23.8	9.7	0.368
Fat (%)	4.19	3.94	3.79	4.00	1.44	0.460
Protein (%)	3.31	3.25	3.28	3.27	0.47	0.898
SCC (Log_{10}cel/μl)	5.41	4.95	5.42	5.07	1.33	0.149

RMSE, root mean square error.

algae mixture combined with other source of Se could be an effective tool to improve mineral concentrations both in the animal and milk. New studies that accurately monitor feed and nutrient intake in other organic farms should be carried out to corroborate our results and to properly evaluate the possibly effect of the supplement on milk production and composition. Even though ruminal boluses fit the principles of organic farming (and would be a good supplementation option for animals maintained exclusively at pasture), their effect is not maintained in the time and a second application during the lactation would be beneficial. Alternatively, the addition of a natural source of Se (preferably organic) to the algae mixture deserves further investigation.

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