Þang sem fóðurbætir fyrir mjólkurkýr

Lokaskýrsla

Ásta Heiðrún Pétursdóttir

Corentin Beaumal

Gunnar Ríkharðsson

Helga Gunnlaugsdóttir

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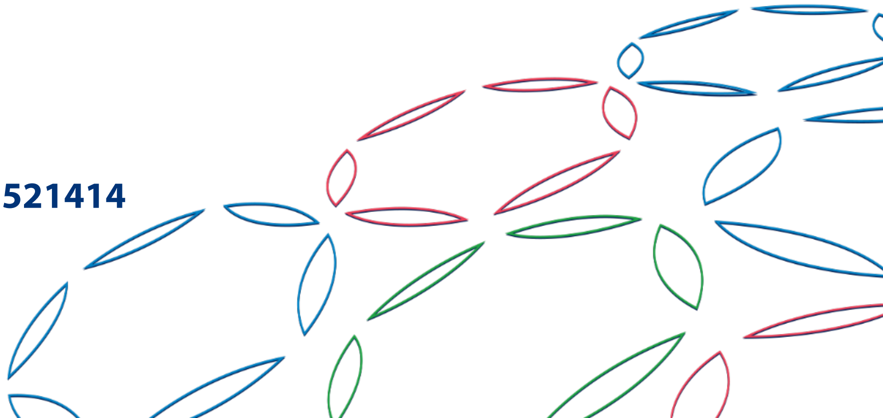

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Report summary

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Titill:	**Þang sem fóðurbætir fyrir mjólkurkýr – aukin nyt og gæði?**		
Höfundar:	Ásta Heiðrún Pétursdóttir, Corentin Beaumal, Gunnar Ríkharðsson, Helga Gunnlaugsdóttir		
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Verknr.:	62481		
Styrktaraðilar:	Framleiðnisjóður landbúnaðarins, Nýsköpunarsjóður námsmanna		
Ágrip á íslensku:	Lagt var upp með að kanna hvort hægt væri að auka nyt mjólkurkúa með þanggjöf og kanna efnainnihald og gæði mjólkurinnar. Einnig hvort hægt væri að nýta þanggjöf sem steinefnagjafa, t.d. fyrir lífrænt fóður sem gæti leitt af sér nýja afurð á borð við joðríka mjólk og því hvatað nýsköpun í nautgriparækt. Niðurstöður leiddu í ljós að þanggjöf gæti haft jákvæð áhrif á mjólkurframleiðni þar sem hóparnir sem fengu þanggjöf sýndu lítilsháttar aukningu á mjólkurframleiðslu miðað við samanburðarhópinn, en breytingin var ekki marktæk. Niðurstöður á safnsýnum sýndu að snefilefnasamsetning breyttist. Fóðurbæting með þangi gæti t.d. verið áhugaverður kostur fyrir bændur sem hafa hug á eða stunda nú þegar lífræna framleiðslu en áhugi á lífrænni ræktun er að aukast hjá nautgriparæktendum.		
Lykilorð á íslensku:	*Þörungar, mjólk, þungmálmar, heilnæmi, joð, arsen, kýr, þang, þari*		
Summary in English:	The project's main aim is examining whether it would be possible to increase milk production of dairy cows by using seaweed as a feed ingredient and to examine the chemical content and quality of the milk. Also whether seaweed could be used as a mineral source, e.g. for organic feed that could lead to new products such milk high in iodine. The results showed that seaweed feeding may have a positive effect on milk production, as the groups that received seaweed feeding showed a slight increase in milk production compared to the control group, but the change was not significant. The results of pooled samples showed that the trace elemental composition changed. Seaweed feed supplementation could e.g. be an interesting option for farmers who are interested in or already engaged in organic production.		
English keywords:	*Seaweed, feed, algae, macroalgae, macrominerals, milk, trace elements, iodine, cattle, cows, quality, safety*		

Fóðurtilraun – stutt yfirlit á íslensku

Rannsóknaáætlun var unnin í samstarfi við Gunnar Ríkharðsson og Svein Sigurmundsson (hjá Búnaðarsambandi Suðurlands) auk bændanna á Stóra-Ármóti. Einnig var ráðfært við Jóhannes Sveinbjörnsson hjá Landbúnaðarháskóla Íslands snemma í ferlinu og seinna í ferlinu við Dr. Sokratis Stergiadis hjá Háskólanum í Reading, Bretlandi, sem mun koma að mælingum á joði.

Tilgátur

Hefur þaragjöf áhrif á magn af þungmálmum og joði í mjólk? Getum við dregið ályktun um nyt kúa? Eykst hún, stendur í stað, minnkar? Er hægt að nýta þang/þara sem steinefnagjafa í lífrænt fóður?

Þangið

Notuð var blanda af þaramjöli og þangmjöli (*Laminaria digitata* + *Ascophyllum nodosum*) pantað frá Þörungaverksmiðjunni Reykhólum. Fýsileikakönnun framkvæmd í lok maí/byrjun júní 2018 sýndi að kýrnar fúlsa ekki við þaranum.

Prósenta af þangi/þara er byggð á efnamælingum á þanginu og þaranum til að uppfylla reglugerðir um magn arsens í fóðurþætti. Þangi verður blandað við í 1.5% við kjarnafóður. Fiskimjöli var sleppt og notað kjarnfóðrið Feitur Robot. Fiskimjöli var sleppt í 2 vikur á undan tilraun.

Tilraunasnið

37 kúm á Stóra-Ármóti var skipt í 3 hópa. Kúnum var raðað saman m.a. eftir stöðu kúa á mjaltaskeiði og raðað í þessa 3 hópa:

- A: kontról hópur (hefðbundið fóður (án fiskimjöls))
- B: hópur með þaragjöf (0.75%)
- C: hópur með þaragjöf (1.5%)

Fóðurtilraun fór fram í 13 vikur en mælingar framkvæmdar frá viku 2.

Sýnataka og mælingar

Sýni tekin úr hverri kú. Þau fryst á Stóra-Ármóti og þeim var komið til Matís í byrjun mars 2019 og voru geymd frosin, u.þ.b. 900 sýni. Mjólkursýnum var blandað eftir hlutföllum morgun/kvöldmjólkur og sýni tekið fyrir safnsýni, og einstaklingssýni geymd fyrir joðmælingar. Í heildina voru 3 safnsýni (A, B, C) af mjólk per viku í 12 vikur, eða 36 sýni af mjólk í þungmálmamælingar. Tekin voru sýni vikulega af gróffóðrinu. Samdóma álit sérfræðinga (hjá BSSL, Háskólanum í Reading og Landbúnaðarháskóla Íslands), sem rætt var við í gerð tilraunasniðs áður en fóðurtilraun fór í gang, var að bæta þyrfti við mælingum á meltanleika, trénis ofl sem ekki hafði verið gert ráð fyrir í kostnaðaráætlun.

Framkvæmdar mælingar í verkefninu:

- Stóra-Ármót: Nyt, hlutfall kvöld og morgunmjólkar.
- SAM: Hefðbundnar mælingar (frumutala, fita, protein, kasein, laktósi, FFS, úrefni).
- Matís: Mjólkursýnin voru frostþurrkuð og undirbúin fyrir mælingar. Sýnin voru mæld m.t.t. þungmálma og steinefna (Al, Zn, Fe, Cr, Co, Ni, Cd, Sn, Hg, Pb, Na, Mg, P, Ca, K).
- Efnagreining Hvanneyri: Gróffóður og kjarnfóður mæld m.t.t. þurrefnis, ösku, meltanleika, próteins, trénis, fitu, sykurs, ammóníum, sterkju og vatnsleysanlegra kolvetna.

Ekki reyndist vera nægt fjármagn fyrir mælingum á joði í sýnunum, m.a. þar sem styrksupphæð var lægri en sótt var um, og senda þyrfti þau erlendis í mælingar þar sem joð er ekki mælt hjá Matís. Hins vegar var komið á fót samstarfi við Háskólann í Reading og sýnin voru send til Reading í ágúst 2019. Mjólkursýnin og fóðursýnin verða mæld m.t.t. joðs í sýnunum á næstu mánuðum. Hluta Matís í þessari tilraun sem styrkurinn frá Framleiðnisjóði nær til er hins vegar lokið. Niðurstöður og umræður hér fyrir neðan eru á ensku m.a. vegna samstarfs við Háskólann við Reading.

Stutt samantekt á niðurstöðum
Lítill munur reyndist vera á mjólkursamsetningu hvað varðar t.d. prótein, fitu og laktósa. Þanggjöf hefur hugsanlega jákvæð áhrif á mjólkurframleiðni þar sem hóparnir sem fengu þanggjöf sýndu vísi að lítilsháttar aukningu á mjólkurframleiðslu miðað við samanburðarhópinn. Hins vegar er lítið hægt að álykta útfrá þessum niðurstöðum og þyrfti lengri rannsókn þyrfti til að sannreyna þetta.

Nokkur munur sást á þungmálmum og steinefnum. Sérstaklega bentu niðurstöður um selen í mjólk til að þrátt fyrir hærra magn af seleni í fóðrinu með þanggjöf reyndist styrkur selens lægri í mjólkinni. Þetta vekur áhuga þar sem selen getur annars vegar verið eitrað í of miklu magni og er á sama tíma nauðsynlegt frumefni. Þess vegna gæti of hár styrkur Se í fóðrinu haft skaðleg áhrif en einnig of lágur styrkur Se í mjólkinni þar sem hún er veruleg uppspretta Se fyrir neytendur. Önnur snefilefni sýndu vísi að svipaðri þróun (Cu, Zn, Fe) og selenið, þ.e.a.s. lægri styrkur á fóðurgjafatímabili tilraunarinnar. Til að fara betur í saumana á þessari þróun þyrfti að magngreina einstaklingssýni af mjólk fyrir hverja kú.

Arsen, eitrað snefilefni, virtist vera til staðar í lítillega hækkuðu magni í mjólk kúnna sem fengu þanggjöf, en fannst engu að síður í lágum styrk. Aðrir þungmálmar fundust einnig í lágum styrk.

Sótt verður til Framleiðnisjóðs 2019 um styrk til að geta mælt einstaklingssýni af mjólk m.t.t. þungmálma og steinefna til að sannreyna og skilja betur niðurstöður verkefnisins.

Contents

Abbreviations (sorted in alphabetical order)

AN: *Ascophyllum Nodosum*
CRM: Certified reference materials
HPLC: High performance liquid chromatography
iAs: Inorganic arsenic
ICP-MS: Inductively coupled plasma – mass spectroscopy
LD: *Laminaria Digitata*
LOD: Limit of detection
LOQ: Limit of quantification
RDI: Reference daily intake
SD: Standard deviation
UHMI: Ultra-high matrix introduction

Introduction

This project aims to investigate whether seaweed supplementation has a negative or a positive impact on the chemical composition of milk, and whether if it can have an impact on the production of a healthier milk.

Iodine deficiency is one of the world's greatest single cause of preventable brain damage and responsible for poor school performance, reduced intellectual ability and IQ points, and impaired work capacity[1]. It is also the cause of a lot of thyroidal diseases, like goitre. In Europe, an increasing number of countries are reporting iodine deficiency[2]. Salt fortified with iodine has been on the market since early in the 19th century, however, high salt intakes increase blood pressure and the risk of cardiovascular and renal diseases. Milk and dairy products are the second biggest source of iodine available in the food, thus making milk the most nutritious and reliable source of iodine. In 2010, 187 million people around the world (2.7% of the population) suffer from goitre as a result of iodine deficiency[3]. Therefore, by increasing the concentration of iodine in liquid milk may possibly safely increase iodine supply to consumers without requiring changes in dietary habits and choices.

Seaweed is a currently an underexploited resource where it may have a beneficial effect on cattle due to bioactive compounds and minerals, e.g. amelioration of the health, reduction of E. Coli, modification of the intestinal flora, etc.[4–8]. Seaweeds are a good source of minerals[10], and can be used as food and feed supplements to supply minerals. Further, research has indicated that seaweed supplementation may increase milk production. Nevertheless, the concentration of heavy metals, and especially arsenic, is also higher in seaweed than in other feed. This element is present as different species: inorganic arsenic, arsenosugars, arsenolipids and a lot of organoarsenicals. Inorganic arsenic (iAs) is the most toxic form of arsenic in food and feed, and is classified as carcinogen by the International Agency for Research on Cancer[9].

The aim of the study is to investigate the effect of a seaweed supplementation on cow feed intake, productivity, milk composition (fat, protein, lactose, etc.) and heavy metal and mineral profile of the feed and milk. Additionally, the milk iodine content will be determined at the University of Reading (UK), however, this data will not be accounted for here.

The project will aim to assess whether the seaweed chosen, widely available in the North Sea, can be used as cow diet supplement without compromising milk productivity and toxicity.

Materials and methods

Chemicals and Reagents

All calibration standards solutions for total trace elements were prepared from 1000 µg/L single element standard solutions (CPI International Peak Performance, USA) by dilution with 2% (v/v) HNO_3 in ultrapure deionized water. Analytical reagent grade concentrated HNO_3 (69%) was obtained from Fluka, Germany and hydrogen peroxide solution (≥ 30%) was obtained from Sigma Aldrich, Germany. Ultrapure deionized water with a resistivity of 18.2 MΩ.cm was obtained from a Milli-Q Plus water purification system (Millipore, France). For the Certified Reference Material (CRM), milk powder was obtained from Fapas, UK (Test Materials 07201 and 07172), seaweed extract (Hijiki) was obtained from the National Metrology Institute of Japan (CRM 7405-a) and fish proteins (DORM-4) was obtained from the National Research Council of Canada.

Feeding trial and analysis of samples

1. Feeding trial design, sample collection and preparation

Feed trial and samples

A total of 888 milk samples were collected from 37 dairy cows from the Stóra-Ármót farm, Selfoss, Iceland (Appendix 2) during 12 weeks between November 2018 and February 2019. Milk samples were collected once a week, during morning and evening milking on adjoining days for each dairy cow in 50 mL vials and frozen directly in the farm at -18°C. Cows were separated into three different groups, with a different diet (Table 1): group A was the control group, and group B and C were experimental groups.

Table 1: Division of cows and feed in each group

Group (n° of cows)		A (11)	B (13)	C (13)
FEED	Week 2-4	Normal diet	Normal diet	Normal diet
	Week 5	Normal diet	Adaption to 0.75% diet	Adaption to 1.5% diet
	Week 6-9	Normal diet	0.75% seaweed diet	1.5% seaweed diet
	Week 10	Normal diet	Adaption to normal diet	Adaption to normal diet
	Week 11-13	Normal diet	Normal diet	Normal diet

Hay samples were collected once a week during the twelve weeks of the experiment and frozen directly at the farm at -18°C.

Concentrated feed samples of several weeks were collected at the farm and stored at -18°C. Samples collected were concentrate feed without seaweed from weeks 3, 5, 7, 9 and 12 and concentrate feed with seaweed from weeks 7 and 9.

Ascophyllum nodosum and *Laminaria digitata*, which were mixed with concentrate, were also analysed to check their heavy metals and minerals concentration.

Milk sample preparation for heavy metals and minerals analysis

Milk samples of the same week and coming from the same cow were mixed together, according to the proportion of milk yield in the morning and evening. Then, three subsamples were taken from the mixed morning/evening milk (Appendix 2): 1) Pooled samples: 10 mL went to a tray, where 10 mL of mixed milk of each cow of the same group were mixed to make a pooled sample of each group (heavy metal & mineral analysis). 2) 30 mL were transferred into a 50 mL polypropylene tube to be sent to University of Reading (iodine). 3) The remaining milk was kept. All the samples were stored at -18°C. After 24 hours in the freezer, all samples were put in a freeze dryer (Christ, Germany) for 72 hours except for the milk for iodine analysis which was kept as frozen liquid. After freeze-drying they were homogenized into powder manually. The samples for each individual cow were homogenized and a subsample taken for analysis of fatty acid profile of the milk to be carried out at University of Reading. Only the pooled samples were analysed for heavy metals and minerals.

Pooled samples were digested with closed vessel acid digestion. Briefly, 0.150 g to 0.200 g of the freeze-dried samples were weighed in quartz digestion vessel and 1 mL of nitric acid and 1 mL of hydrogen peroxide were added. Samples were digested in Ultra wave Acid Digestion System (Milestone Inc., Italy), according to method described in Table 2. Digested samples were quantitatively transferred to 50 mL polypropylene tubes and diluted to 50 mL with Milli-Q water.

Table 2: Digestion method (Ultra wave Digestion System, Milestone Inc., Italy)

Power (W)	**Time (min)**	**Pressure (bar)**	**Temperature (°C)**	**Agitation**
1500	0.00	130	20	No
1500	20.00	130	250	No
1500	30.00	130	250	No

For minerals analysis, 200 µL of diluted digested samples were diluted in 10 mL vials with 2% (v/v) HNO_3.

Hay and feed samples preparation for heavy metals and minerals analysis

Hay samples were freeze-dried for 24 hours before being homogenised into powder using a laboratory grinder (Janke and Kunkel, Germany). Feed samples were directly milled and homogenised. Subsequently, the samples were digested with closed vessel acid digestion, following the same protocol used for milk samples digestion.

For minerals analysis, 200 µL of diluted digested samples were diluted in 10 mL vials with 2% (v/v) HNO_3.

2. Analysis by ICP-MS

An Agilent 7900 quadrupole inductively coupled plasma mass spectrometer (ICP-MS) (Agilent Technologies, Singapore) was used. It was combined with an ultra-high matrix introduction (UHMI) system with a quartz cyclonic spray chamber and MicroMist nebulizer (Glass Expansion, UK) operating with peristaltic pump.

The helium and argon gas utilized were of spectral purity (> 99.999%). Before each experiment, the instrument was tuned for daily performance with an aqueous multi-element standard solution of Li, Mg, Co, Y, Ce and Tl used to check consistent sensitivity (^{59}Co, ^{89}Y and ^{205}Tl) and minimum doubly charged and oxide species levels (^{140}Ce). The instrumental settings and operative conditions are reported in Table 3.

Table 3: ICP-MS conditions

Spectrometer	7900 ICP-MS (Agilent)	***Argon gas flow rates (L/min)***	
Nebulizer	MicroMist	Plasma	15.0
Spray chamber	Quartz cyclonic	Auxiliary	0.90
Interface	Pt cones	Nebulizer	1.05
Mass analyzer	Quadrupole	Lens voltage (V)	6.25
Tune mode	He	Sweeps/replicate	100
He gas flow rate (mL/min)	5.0	Sample uptake rate (mL/min)	0.4
RF power (kW)	1.55	No. of replicates per sample	3

3. Calibration procedure

For the quantitative analysis of the samples, external calibration technique was followed. Calibrations curves were built on nine different concentrations, from 0.05 µg/kg to 200 µg/kg, for Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sn, on six different concentrations, from 0.05 µg/kg to 10 µg/kg, for Hg and Pb, and on seven different concentrations, from 5g/kg to 1500 g/kg, for Na, Mg, P, and Ca prepared by diluting standard solutions at 1000 µg/L in 2% (v/v) HNO_3 (the same percentage of acid present in the samples). Indium (^{115}In) was used as internal standard. All the measurements were carried out using the full quantitative mode analysis. The correlation coefficients for all the curves were higher than 0.999, showing good linear relationship throughout the ranges of concentrations studied. Samples were analyzed in duplicates.

4. Quality control of chemical analysis

The capacity of the method as a routine method was evaluated via the limit of detection (LOD) and the limit of quantification (LOQ) of each element (Appendix 3). LOD was calculated as 3 SD of the digestion blanks divided by the slope of the calibration curve. LOQ was calculated as 10 SD of the digestion blanks divided by the slope of the calibration curve. An average dilution factor was used to calculate the LOD and LOQ in the sample. Instrumental LOD/LOQ determined everyday was lower than the LOQ reported in Appendix 3.

At the beginning of the analysis, blanks prepared by completion of the full analytical procedure without samples were analysed. At regular intervals during the analysis, blanks (2% (v/v) HNO_3) were analysed between samples to check for any loss or cross contamination.

In order to check the accuracy of the method, CRMs, seaweed (Hijiki, CRM 7405-a), fish proteins (DORM-4) and milk powder (Fapas 07172 and Fapas O7201) were analysed. Certified values (mean ± SD), observed values (mean ± SD) and recovery percentage are shown in Table 5. The measured concentrations were in good agreement with certified values with recoveries between 84% and 125% for all the elements, except for Pb in Fapas 07172 and 07201, where the recoveries are respectively 130% and 210%. This is likely due to the low concentrations of Pb in the Fapas CRMs and low contamination of the samples. This contamination was not investigated further.

During the first analysis, contamination appeared for some samples. Subsequently, selected samples were prepared and analysed again, in triplicates (list of the samples in Appendix 4). The results for Al were still contradictory with high RSD. Since Al was not an element of high importance for this study, this was not pursued further.

Table 4: Analysis of certified reference materials Hijiki, DORM-4, Fapas 07172 and Fapas 07201

Elements	Hijiki (CRM 7405-a) (n = 10)			DORM-4 (n = 10)		
	Certified value (mg/kg)	Observed value (mg/kg)	Recovery (%)	Certified value (mg/kg)	Observed value (mg/kg)	Recovery (%)
Heavy metals						
^{27}Al	147 ± 7	135 ± 15	92	NA	NA	NA
^{52}Cr	3.4 ± 0.1	3.78 ± 0.43	111	1.87 ± 0.18	2.34 ± 0.77	125
^{55}Mn	14.1 ± 0.7	15.5 ± 1.9	110	3.17 ± 0.26	3.30 ± 0.18	104
^{56}Fe	311 ± 11	302 ± 33	97	343 ± 20	321 ± 20	94
^{59}Co	1.07 ± 0.06	1.22 ± 0.15	114	NA	NA	NA
^{60}Ni	2.2 ± 0.1	2.31 ± 0.27	105	1.34 ± 0.14	1.36 ± 0.14	101
^{63}Cu	1.55 ± 0.07	1.73 ± 0.23	111	15.7 ± 0.5	15.2 ± 0.52	97
^{66}Zn	13.4 ± 0.5	13.8 ± 1.4	103	51.6 ± 2.8	49.3 ± 1.5	96
^{75}As	35.8 ± 0.9	38.0 ± 4.3	106	6.87 ± 0.44	6.59 ± 0.27	96
^{78}Se	NA	NA	NA	3.45 ± 0.40	3.80 ± 0.29	110
^{111}Cd	0.79 ± 0.02	0.788 ± 0.092	100	0.299 ± 0.018	0.288 ± 0.013	96
^{201}Hg	NA	NA	NA	0.412 ± 0.036	0.346 ± 0.020	84
^{208}Pb	0.43 ± 0.03	0.479 ± 0.103	111	0.404 ± 0.062	0.400 ± 0.017	99
Minerals						
^{23}Na	16 200 ± 200	14643 ± 6 520	90			
^{24}Mg	6 790 ± 100	6519 ± 367	96			
^{31}P	1 010 ± 30	901 ± 93	89			
^{39}K	47 500 ± 700	44 271 ± 1564	93			

	Fapas 07172 (n = 8)			Fapas 07201 (n = 11)		
	Certified value (µg/kg)	Observed value (µg/kg)	Recovery (%)	Certified value (µg/kg)	Observed value (µg/kg)	Recovery (%)
Heavy metals						
^{75}As	56.4	49.5 ± 9.7	88	38.3	35.7 ± 4.6	93
^{111}Cd	18.6	20.8 ± 3.7	112	12.5	12.0 ± 1.8	96
^{201}Hg	25.3	22.8 ± 4.0	90	9.69	8.91 ± 3.18	92
^{208}Pb	66.2	85.9 ± 27.1	130	27.0	56.7 ± 34.0	210

Results and discussion

1. Chemical composition of the hay and concentrate feed

The composition of the feed was analysed by “Efnagreiningar” in Hvanneyri Iceland who provide analytical services to farmers.

Table 5. Composition of the hay and concentrate feed during the experiment in %.

Samples	Dry matter	Dry matter after drying*	Ash	NCGD (Neutral Cellulase Gammanase Digestibility)	Crude protein	Neutral detergent fibre (NDF)	Acid Detergent Fibre (ADF)	Soluble crude protein (sCP)	Indigestible neutral detergent fibre (iNDF)	Sugar	Fat	Amm-onium (NH4H)	Starch	WSC (Water soluble carbohydrate)
Hey W2	31.1	89.7	7.0	78	16.4	49.6	28.1	11.3	9.5	4.9	6.1	0.08		
Hey W3	37.7	90.1	7.3	74	16.0	51.2	31.0	10.3	9.4	4.7	5.8	0.09		
Hey W4	30.5	90	7.2	76	16.2	52.8	29.6	10.9	9.6	3.2	5.3	0.07		
Hey W5	29.4	89.8	7.4	75	14.3	52.5	32.8	10.1	11.3	5.2	5.8	0.08		
Hey W6	30.9	88.9	6.9	78	16.8	50.8	28.5	11.4	8.0	4.4	6.5	0.05		
Hey W7	30.1	89.8	7.2	76	16.7	51.4	30.2	11.0	8.7	6.2	5.4	0.09		
Hey W8	30.7	89.6	7.0	76	16.1	48.6	30.2	10.8	8.6	5.1	5.5	0.08		
Hey W9	30.6	88.7	6.7	78	17.6	48.3	29.0	11.8	7.9	4.3	6.5	0.06		
Hey W10	29.0	90.2	6.8	78	17.8	49.1	30.1	11.7	7.8	3.0	5.5	0.06		
Hey W11	29.7	91.5	8.0	76	14.8	51.2	30.2	10.2	10.0	4.8	5.5	0.05		
Hey W12	41.6	91.6	7.0	74	15.1	58.7	31.4	9.7	9.4	5.5	6	0.08		
Hey W13	34.7	91.4	7.4	76	17.4	50.8	28.0	10.9	9.1	3.1	6.1	0.06		
Average	**32±4**	**90±1**	**7.2±0.3**	**76±1**	**16±1**	**51±3**	**30±1**	**10.9±0.6**	**9±1**	**5±1**	**5.8±0.4**	**0.07±0.01**		
Conc. V3	89.9	89.9	8.9		21.1	10.8	64.7				2.6		32.4	12.1
Conc. V5	89.7	89.7	9.3		21.1	12.9	64.7				2.7		28.6	11.5
Conc. V7	89.6	89.6	8.3		21.3	11.9	58.3				2.8		26.8	13.0
Conc. V9	89.7	89.7	8.7		22.0	10.3	52.8				2.4		27.3	16.0
Conc. V12	89.4	89.4	8.6		21.0	10.9	46.6				2.6		28.7	13.1
Conc.Sw.V7	89.4	89.4	8.9		21.6	11.5	52.9				2.4		28.3	14.9
Conc.Sw.V9	89.3	89.3	8.7		20.0	11.9	49.5				2.7		24.8	12.7
Average	**89.5±0.2**	**89.5±0.2**	**8.8±0.3**		**21.1±0.6**	**11±1**	**56±7**				**2.6±0.2**		**28±2**	**13±1**

The dry matter in the hay was on average 32 ± 4% and 89.5 ± 0.2% for the concentrate. The frozen hay samples were dried and used for the analysis, Table 5. In general, the composition of hay and concentrate was similar between weeks, Table 5.

2. Seaweed supplement

Heavy metals and minerals concentrations were determined in order to check the composition of the AN and LD added to the concentrate was fed to the cattle.

Table 6: Concentrations of total arsenic, inorganic arsenic and iodine in AN, LD and seaweed mix (mg/kg). Results from the Eurofins, Germany.

	Laminaria Digitata	*Ascophyllum Nodosum*	Legislation	Mix (91% AN + 9% LD)
Total As	55 ± 11	21 ± 4	40	32.7
iAs	19 ± 4	0.3 ± 0.1	2	1.98
I	6300 ± 1300	1200 ± 240		1659

Results found for total arsenic at Matís are reasonably close to the values found by the contracting laboratory for both AN and LD, Table 6 &Table 7. The As concentration is moderately higher at Matís, however, the certified reference materials were excellent and very similar concentrations were found even though the samples were analysed with different operators using different instruments at Matís. Other heavy metals and minerals concentrations are shown in Table 7. It can be clearly seen that some elements, like Fe, Al or K, are present at high concentrations.

Table 7: Heavy metals and minerals concentration measured in Ascophyllum Nodosum *and* Laminaria Digitata*, and calculated with the proportions of the mix, in mg/kg (n = 2).*

	Laminaria Digitata	***Ascophyllum Nodosum***	**Mix (91% AN + 9% LD)**
Heavy metals			
^{27}Al	3 262 ± 255	1 829 ± 32	1 96
^{52}Cr*	3.18 ± 0.65	4.36 ± 1.37	4.25
^{55}Mn	93.3 ± 5.7	75.4 ± 0.9	77.0
^{56}Fe*	5 459 ± 631	1954 ± 198	2 27
^{59}Co	2.39 ± 0.13	1.83 ± 0.01	1.88
^{60}Ni*	3.79 ± 0.45	4.31 ± 0.31	4.27
^{63}Cu*	12.2 ± 3.9	4.02 ± 0.41	4.76
^{66}Zn*	23.5 ± 13.3	15.3 ± 1.6	16.0
^{75}As*	72.2 ± 3.2	28.8 ± 0.3	32.7
^{78}Se*	0.87 ± 0.45	0.475 ± 0.006	0.51
^{97}Mo	0.322 ± 0.005	0.782 ± 0.029	0.74
^{111}Cd*	0.339 ± 0.025	0.813 ± 0.046	0.77
^{118}Sn	0.299 ± 0.056	0.058 ± 0.003	79.7
^{201}Hg* (µg/kg)	8.75 ± 3.50	11.3 ± 1.6	11.1
^{208}Pb*	0.290 ± 0.190	0.110 ± 0.024	0.13
Minerals			
^{23}Na	29 256 ± 579	23 352 ± 696	28 724
^{24}Mg	7 726 ± 50	9 541 ± 249	7 890
^{31}P	2 663 ± 11	1 408 ± 21	2 551
^{39}K	93 907 ± 337	14 999 ± 408	86 806
^{43}Ca	26 503 ± 397	64 236 ± 904	29 900
^{44}Ca	24 311 ± 213	63 981 ± 317	27 881

*: n = 5

3. Concentrate feed and hay analysis

First, it is essential to see the concentrations of heavy metals and minerals in the feed provided to the cows. They were fed by hay and by pellets of concentrate feed which were either with or without seaweed depending on their group.

The concentrations of minerals in the hay are stable during the twelve weeks of the study, apart from deviations in the mineral concentrations in week 3 and high quantities of phosphorus for week 9 (Appendix 7). Heavy metal concentrations in the hay are also stable during the study, except for week 9, which shows different results (Appendix 8). In general, the concentrations of heavy metals and minerals in hay are similar between weeks during the study.

Heavy metal and minerals concentrations were analysed in concentrate feed. Figure 1 shows that the mineral concentrations are the same for concentrate feed with and without seaweed.

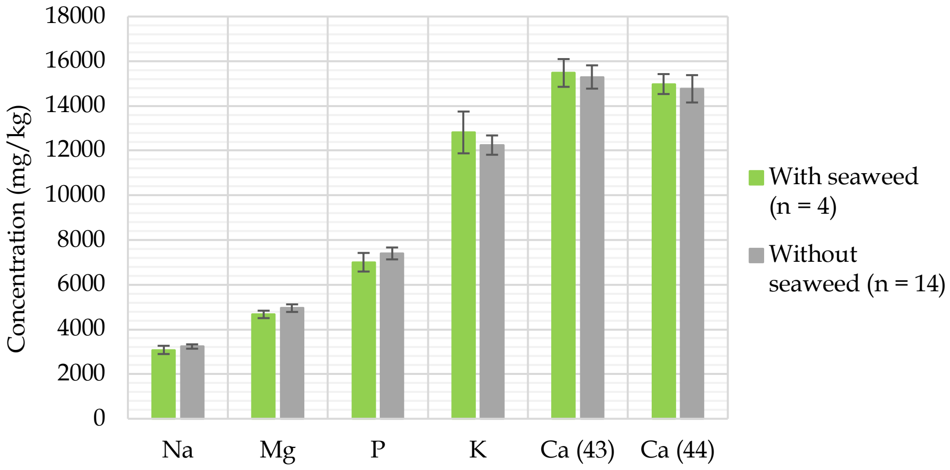


Figure 1: Minerals concentrations in concentrate feed, in mg/kg.

The heavy metal concentrations was mostly independent on the presence of seaweed in the concentrate except for a few elements. As revealed in Figure 2, concentrations are significantly higher in concentrate with seaweed than in concentrate without seaweed for arsenic (respectively 1.05 ± 0.11 mg/kg and 0.644 ± 0.126 mg/kg) and cadmium (respectively 0.112 ± 0.004 mg/kg and 0.0637 ± 0.0142 mg/kg). However, the concentration of lead is more than two times higher in the concentrate without seaweed (0.412 ± 0.177 mg/kg) than in the concentrate with seaweed (0.201 ± 0.001 mg/kg).

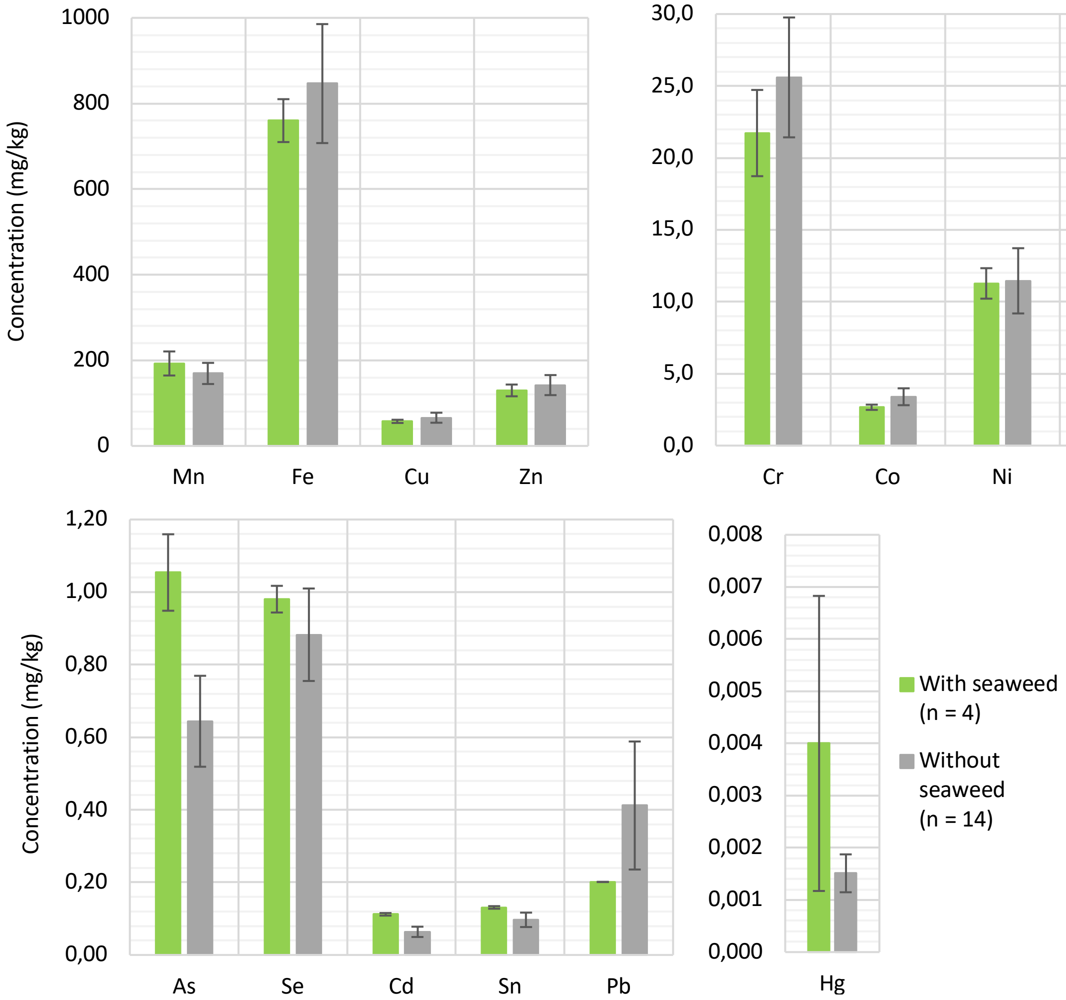


Figure 2: Heavy metals concentrations in concentrate feed, in mg/kg.

4. Heavy metals and minerals analysis in the milk

This section aims to address and discuss the main results of the study, focusing on elements that may have an impact on the health. Further, a subsection is dedicated to the analysis of the other compounds of the milk (protein, lactose, fat, urea and casein).

Minerals

Minerals are essential for human health hence they were investigated in the milk. Figure 3 shows the average concentration of each mineral in the milk of each group according to their diet, normal (from weeks 2 to 5 and from weeks 11 to 13) or adapted (from weeks 6 to 10). For each mineral, concentrations are in the same range, independently of the group and the weeks. A little difference can be seen for group C, where concentrations are approximately 10% lower during the adapted diet period than during the normal diet one, but this affirmation can't be verified for group B where the concentrations are almost the same. It can also be observed that concentrations of group A during the adapted diet are approximately 5% lower than during the normal diet. These differences are small and should not be interpreted as a clear influence of the diet. Moreover, as it is shown in the previous section (Figure 1) the mineral intake is the same, regardless of the diet. In addition, it is important to note that there is no retention effect, i.e. the concentrations after the adapted diet (weeks 11 to 13) directly revert to the same as before the adapted diet.

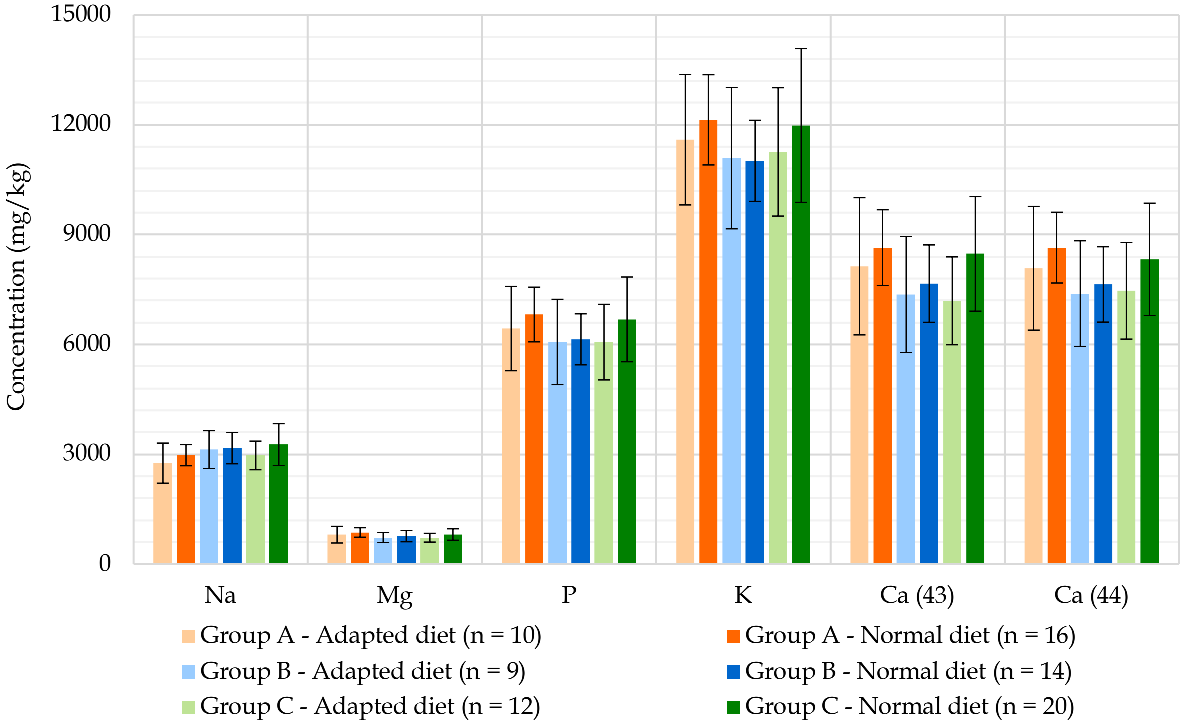


Figure 3: Minerals concentrations in milk (mg/kg) during normal diet (weeks 2 to 5 and 11 to 13) and adapted diet (weeks 6 to 10). Group A: control, Group B: 0.75% seaweed inclusion, Group C: 1.5% seaweed inclusion.

Heavy metals

In this section, results for heavy metals are summarized. All the results are given as mean ± SD, for dry matter.

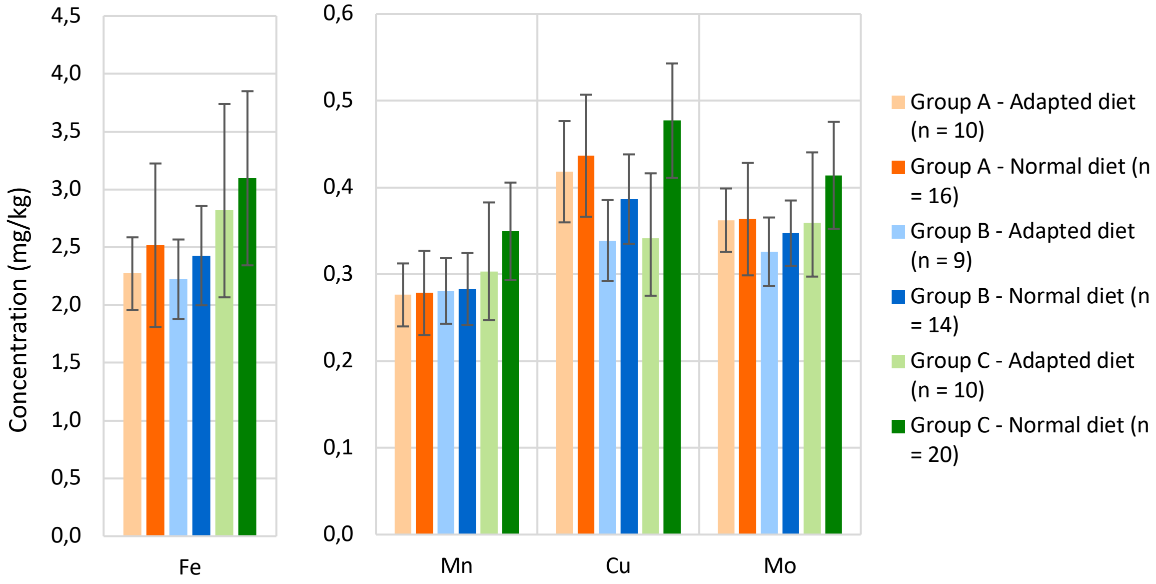


Figure 4 Heavy metals concentration in dry milk (mg/kg). Group A: 1.5% seaweed inclusion, Group B: control, Group C: 0.75% seaweed inclusion.

The concentrations of heavy metals for group A, the control group, are stable during all the study. Results from groups B and C indicate a decrease of Mn, Cu and Mo during the adapted diet (Figure 4 & Appendix 7). Because the concentrations are the same before and after the adapted diet, an average value of concentrations from weeks 2 to 5 and 11 to 13 was considered, to simplify the graphics.

Results for Al, Cr, Co, Ni, Sn, Hg, Cd and Pb were very low or below LOQ and are not further discussed, Appendix 8.

Results for zinc and selenium

Zinc is an important trace element for humans, animals and plants. It has many biological roles in the metabolism of RNA and DNA, but also in gene expression and interacts with a lot of proteins[45]. First, the zinc concentration in the milk is much higher than the other compounds analysed. The amount of zinc in the milk is more than enough needed to guarantee the RDI of zinc, which is 11 mg/day[11]. Secondly, Figure 5 indicates that zinc concentration decreases during the adapted diet for both groups B (approximately 8% lower) and C (approximately 16%), Figure 5a, while staying constant for group A.

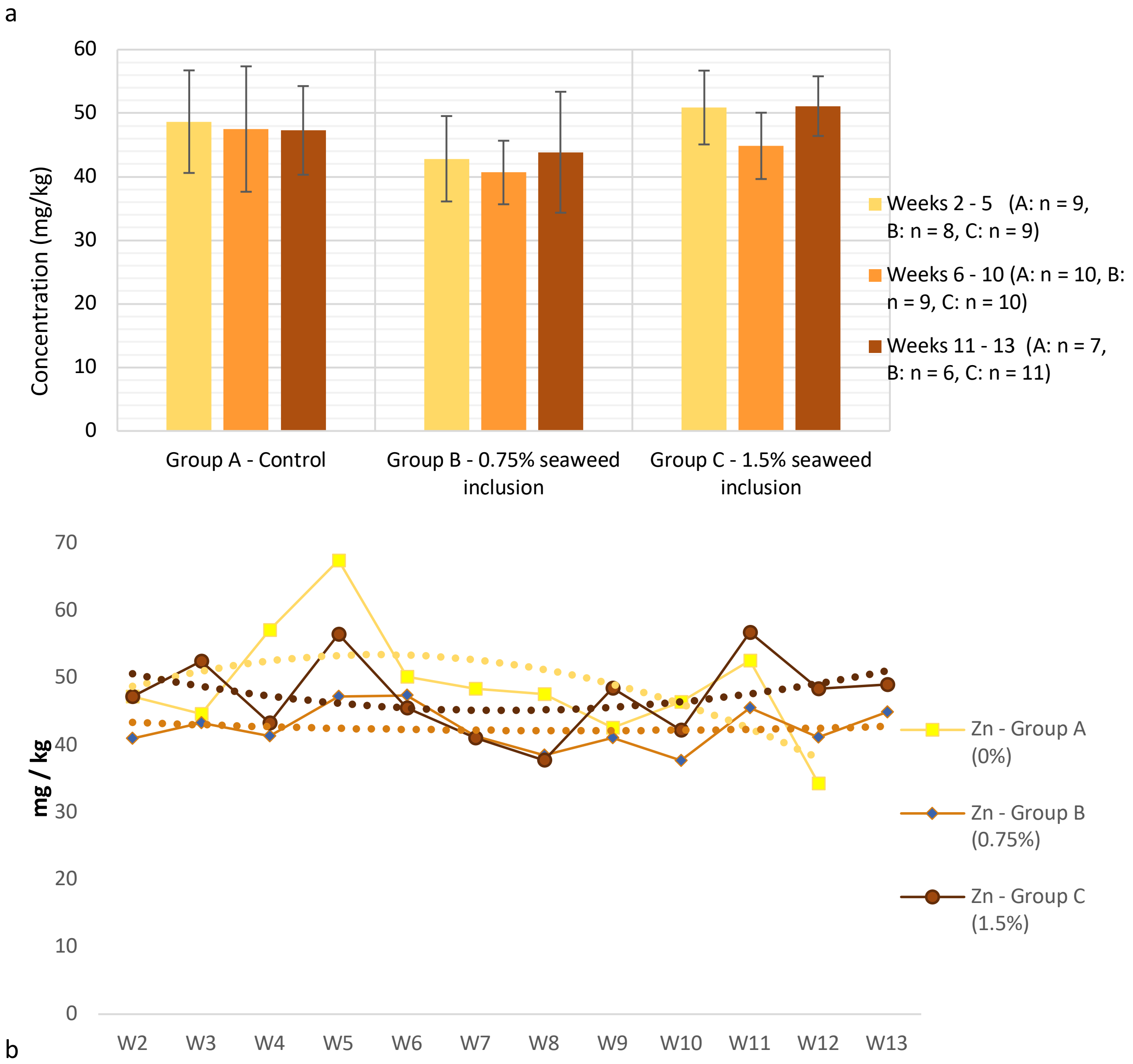


Figure 5: Zinc concentration in the milk (dry weight), depending on the groups and the weeks. A) Average Zn concentrations for three periods. B) Timeline representation of the Zn concentration.

Selenium is toxic in large doses but is useful in the human body because it has a role in the thyroid functioning and is used for reduction of antioxidant enzymes[12-13]. Its RDI is 0.055 mg/day[12-13]. It can be seen that the concentration of selenium decrease for both groups B and C during the adapted diet, whereas it stays stable for group A (Figure 6). As zinc, the difference is more pronounced for group C (approximately 30%) than group B (approximately 20%), Figure 6a. The amounts found in the milk are high compared to the RDI. However, this value is low compared to the maximum safe dietary intake, which is 0.8 mg/day.

Selenium was higher in the concentrate with seaweed compared to the control, whereas Zn was very similar. This potential decrease of Se and Zn in the milk may therefore indicate a biological mechanism at play, where the Selenium is e.g. taken up or excreted differently.

For both zinc and selenium, it can be observed that there is no effect of retention. The concentration is the same before and after the adapted diet, which means that the seaweed diet impact directly the quality of the milk, but there is no influence when the diet ends. This can be clearly seen in the timeline representation, Figure 6b.

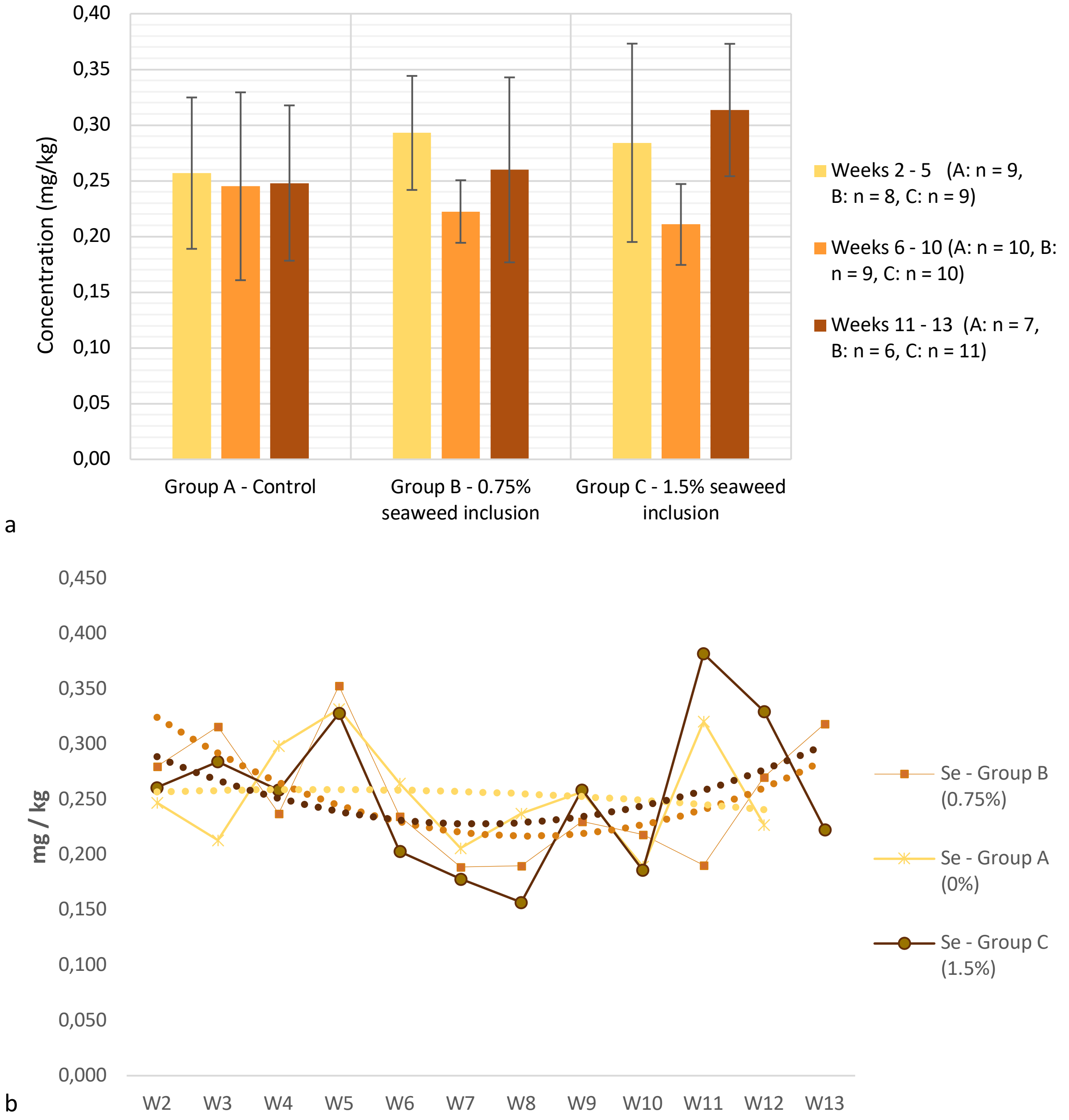


Figure 6: Selenium concentration in the milk, depending on the groups and the weeks. A) Average Zn concentrations for three periods. B) Timeline representation of the Zn concentration.

Comparing the results of Se and Zn to data from a Spanish study where the milk of seaweed supplemented cows was compared to a control group the opposite trend was seen where the supplemented cows showed slightly higher Se and Zn conc. compared to the control group[14]. The difference was however not significant. The conc. of Se and Zn in the Icelandic milk was approximately 2 times higher compared to the Spanish milk, or 27-40 mg/kg Se here (Appendix 8) compared to 18-20 mg/kg in the study[14], and 5300-6700 mg/kg Zn here (Appendix 8) compared to 3280-3690 mg/kg in the study[14].

Results for arsenic

Arsenic is a toxic and carcinogenic element. Its more toxic species are inorganic arsenic (As (III) and As (V)). In China, the maximum level of total arsenic is 0.5 mg/kg in milk powder[32]. The arsenic was found to be under the method LOQ but was over the instrumental LOQ. When calculated per wet weight the As concentration is found to be 0.6 ppb and 0.9 ppb in groups without and with seaweed in the diet, respectively. This is below the maximum level of arsenic in water which is 5 ppb. These results are very comparable with Rey-Crespo et al[14] where they found the same values for non-supplemented vs supplemented cows.

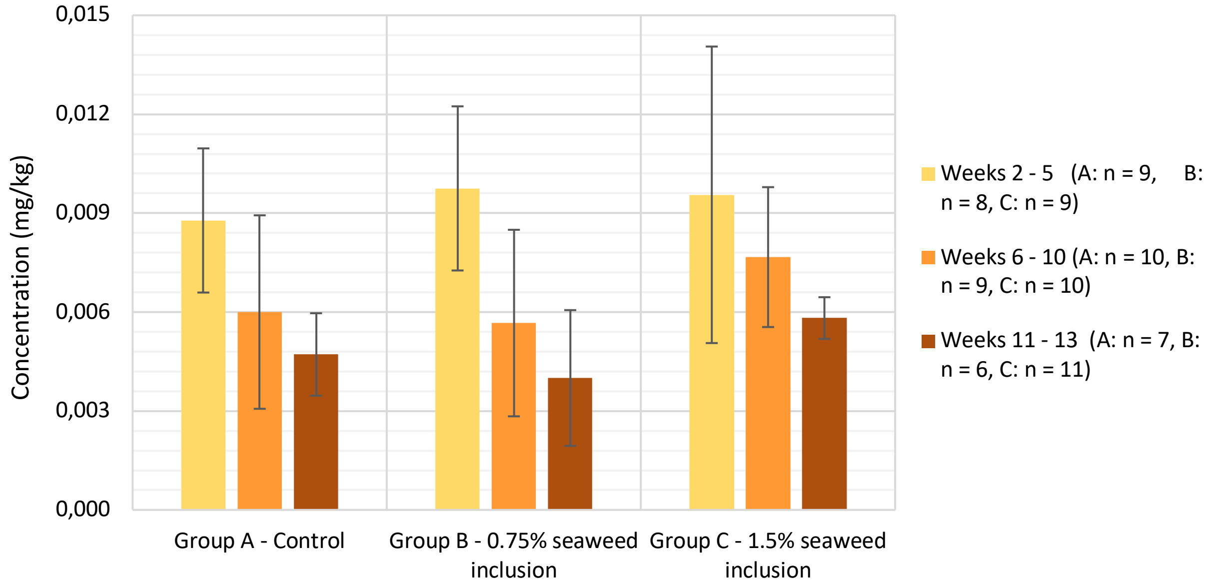


Figure 7. Arsenic concentration in the milk, depending on the groups and the weeks.

5. Milk composition analysis and productivity

Analysis on milk composition was carried out on the milk samples, in order to check the variation of e.g. fat, proteins, casein, urea and lactose. They were all analyzed by Auðhumla (part of MS dairy products company, Selfoss) using a Combifoss 6000. The results of these analysis show that the concentration of all the compounds are stable in the time, independently of the group and the diet (Figure 8). This is in accordance with a recent study where supplementation of AN to grazing cattle showed limited effects on milk yield, concentrations and yields of milk components, and stress- and animal health-related parameters such as blood cortisol, body temperature, and respiration rate[ref].

The milk productivity was also investigated during the study as records were kept at the farm. Generally speaking there seemed little difference between the groups for fat, proteins, casein, urea or lactose, Figure 8.

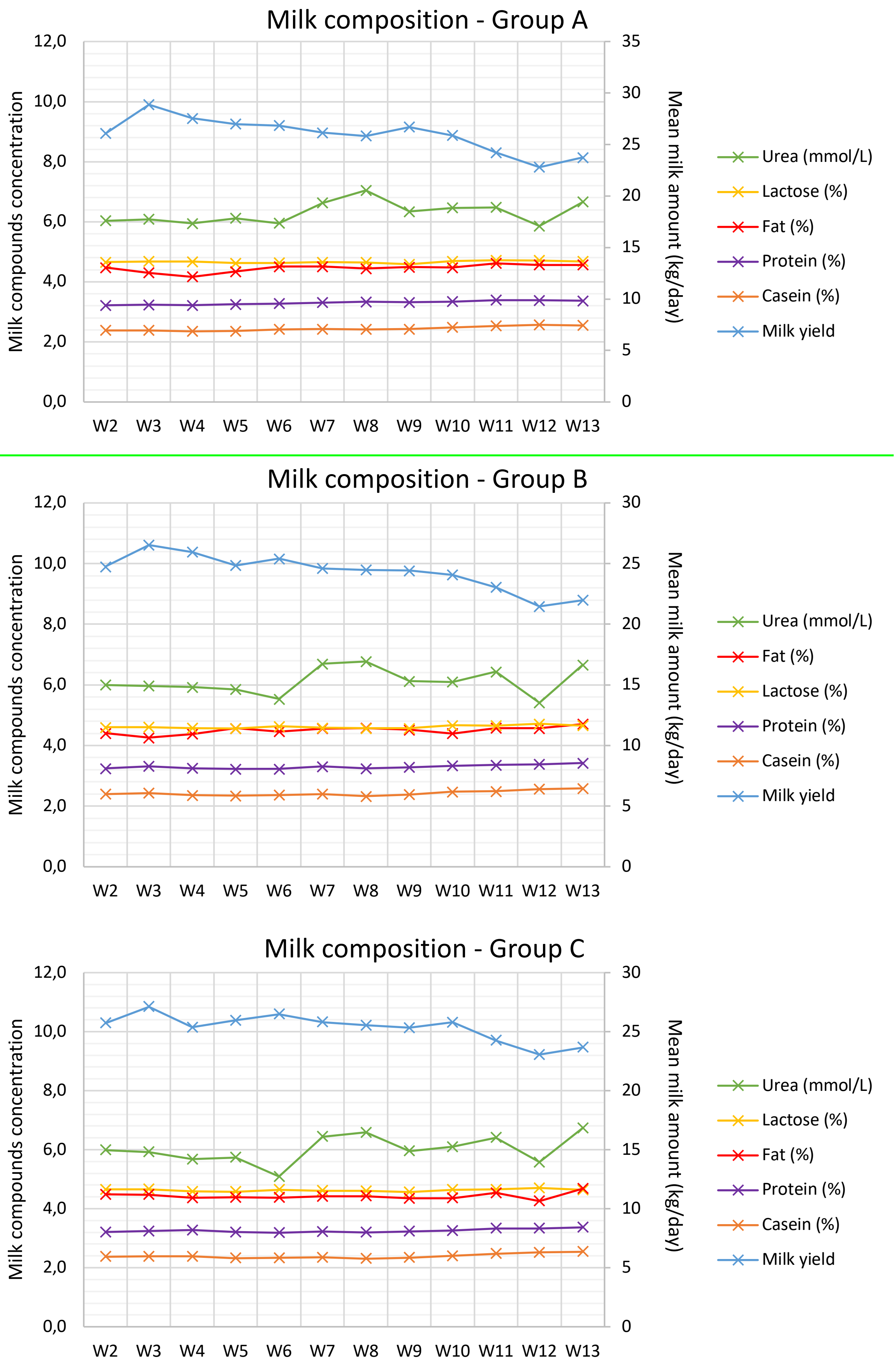
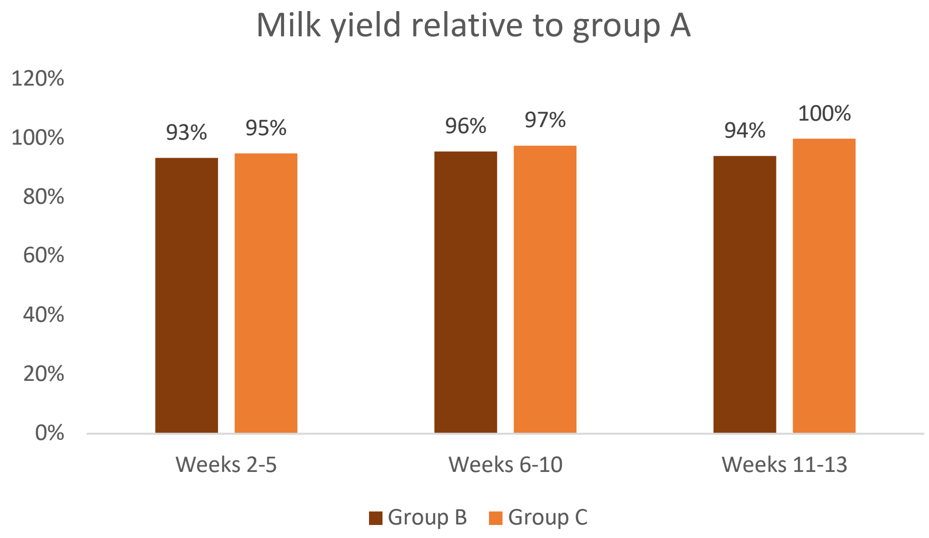


Figure 8. Milk productivity of each group and milk composition in fat, proteins, casein, lactose and urea during the study.

When looking closely at the milk yield there may have been a moderate increase during and after the seaweed intake for the group receiving 1.5% seaweed. Group A milked best of the three groups independent of diet. When the milk yield is calculated relative to group A it can be seen that when the three groups receive the same diet groups B & C milk only 93% and 95% of what group A milks respectively. However, during the adapted diet they milk 96% and 97% relatively, hence a 2-3% increase compared to the control group. The milk yield for the group with lower seaweed inclusion reverts to a similar milk yield as before (94%) but the milk yield continues to increase for group C (100% relative to control).



The milk values are taken as averages for the morning and evening milking for the whole groups.

This study indicates that the seaweed supplementation does not have a negative impact on milk yield and may moderately increase it. However, longer studies are needed to verify this.

Conclusions

Some differences were observed for heavy metals and minerals. In particular for the mineral selenium the results indicated that despite a higher level of selenium in the feed when supplemented with seaweed the selenium concentration in the milk was lower in the supplemented cattle. This is of significant interest since selenium is an essential micronutrient but can also be toxic in too high quantities. Therefore, too high concentrations of Se in the feed could have detrimental effects but also too low concentrations of Se in the milk as it is a significant source of Se for consumers.

Other elements showed an indication of a similar trend (Cu, Zn, Fe), i.e. with lower levels during the supplemented period of the experiment. The trend was not further analysed for statistical significance as the grant did not allow for analysis of samples of milk from individual cows.

Arsenic, a toxic element, seemed to be moderately elevated in the milk of cattle that received seaweed supplementation, but still found at very low levels. Other heavy metals were also found at very low levels.

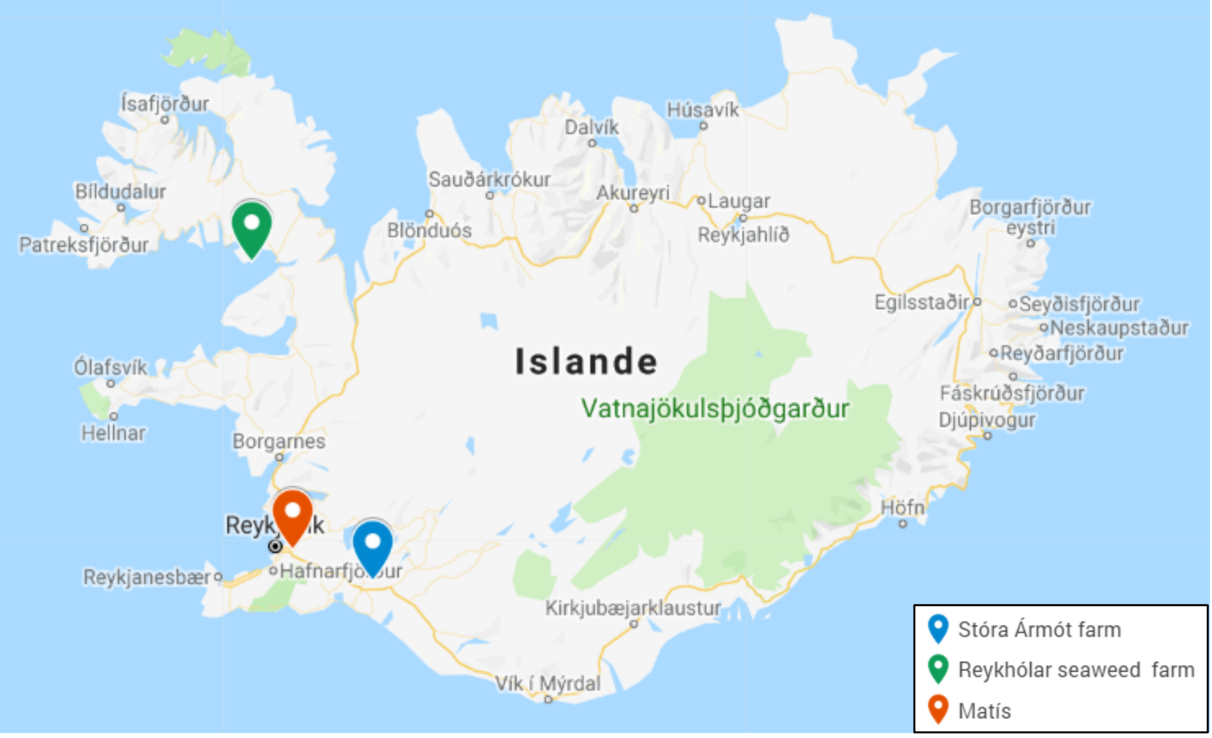
For further investigation of these trends it would be possible to either run a longer trial or alternatively analyse the milk samples from each individual cow of this trial for better understanding and in order to obtain statistical significance. Collaborating statistical scientists at University of Reading have proposed that the milk samples need to be analysed for heavy metals and minerals per each individual cow to be analysed with a statistical model of a linear mixed effect model using date, diet and their interaction as fixed factors and cow ID as random factor. Matís will apply for additional funding with Framleiðnisjóður to carry out these analyses to fully understand the effect of the seaweed supplementation on the heavy metal and mineral profile of the milk samples.

The funding amount was not sufficient to analyse the iodine concentration of the samples, however, a collaboration with the University of Reading was established and the samples will be analysed there as part of a PhD project carried out at University of Reading. This will provide valuable additional input to the results of this project.

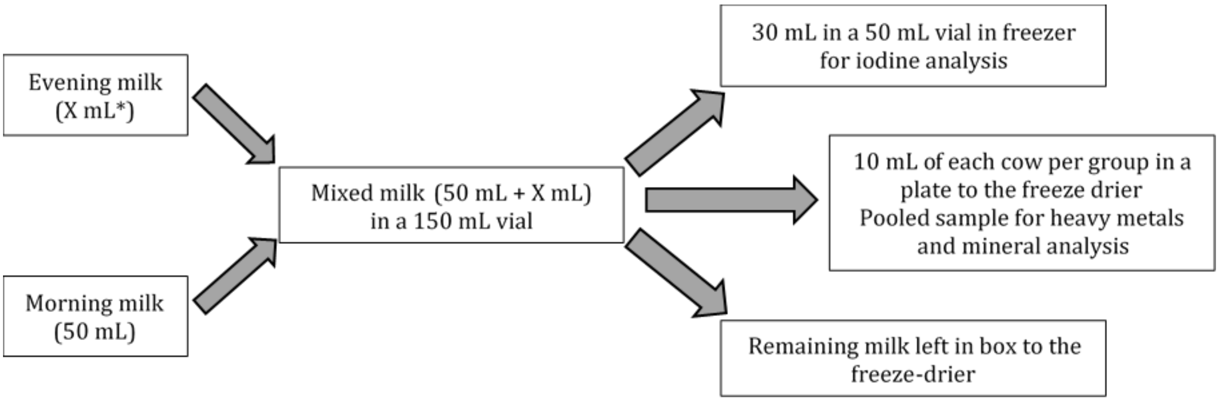
Little difference was found in the milk composition regarding e.g. protein, fat and lactose. The seaweed supplementation potentially has a beneficial effect on milk yield since the groups fed with seaweed showed a relative small increase in milk yield compared to the control group. A longer study would be needed to verify this.

Appendix

Appendix 1: Map of Iceland



Appendix 2: Milk samples preparation protocol



*: Quantity proportional to the ratio of evening milk produced

Appendix 3

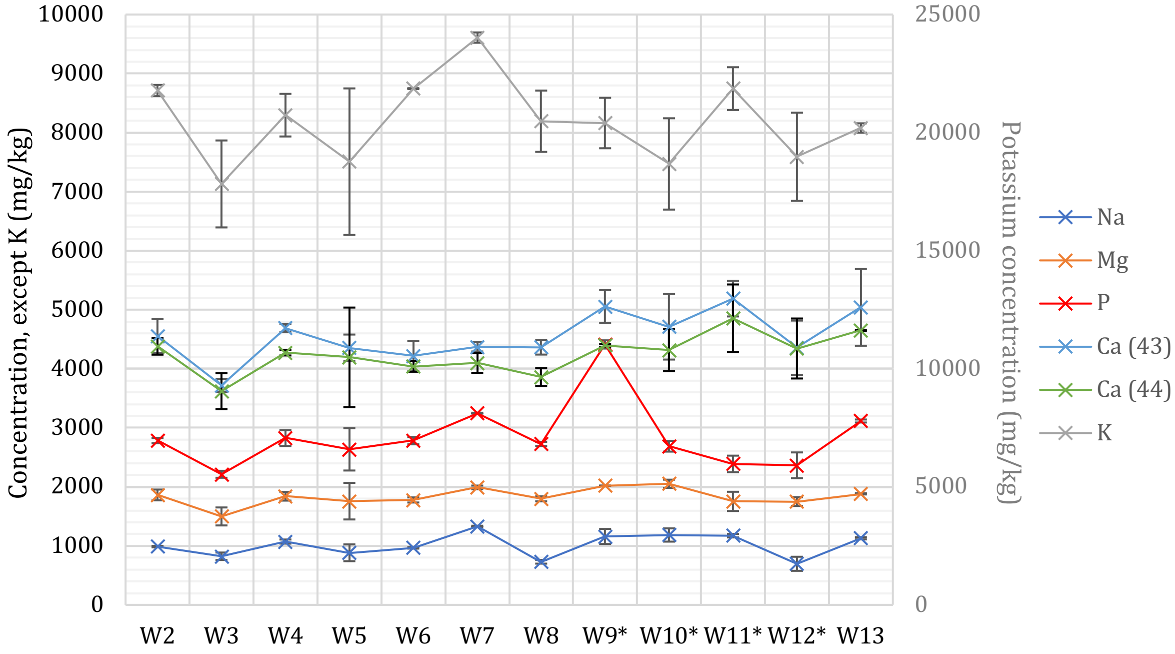
Table 8: LOD and LOQ of the complete method for each element analyzed in the samples

Heavy metals (n = 5)	**LOD (mg/kg)**	**LOQ (mg/kg)**	**Minerals (n = 3)**	**LOD (µg/kg)**	**LOQ (µg/kg)**
^{27}Al	1.2	3.9	^{23}Na	0.873	2.91
^{52}Cr	0.009	0.030	^{24}Mg	1.14	3.79
^{55}Mn	0.039	0.13	^{31}P	1.06	3.54
^{56}Fe	0.22	0.75	^{39}K	1.12	3.72
^{59}Co	0.002	0.006	^{43}Ca	6.92	23.1
^{60}Ni	0.015	0.050	^{44}Ca	6.22	20.8
^{63}Cu	0.022	0.072			
^{66}Zn	0.21	0.70			
^{75}As	0.013	0.047			
^{78}Se	0.090	0.30			
^{95}Mo	0.006	0.021			
^{111}Cd	0.003	0.008			
^{118}Sn	0.010	0.033			
^{201}Hg	0.008	0.025			
^{208}Pb	0.023	0.077			

Appendix 4: Number of replicates for each sample

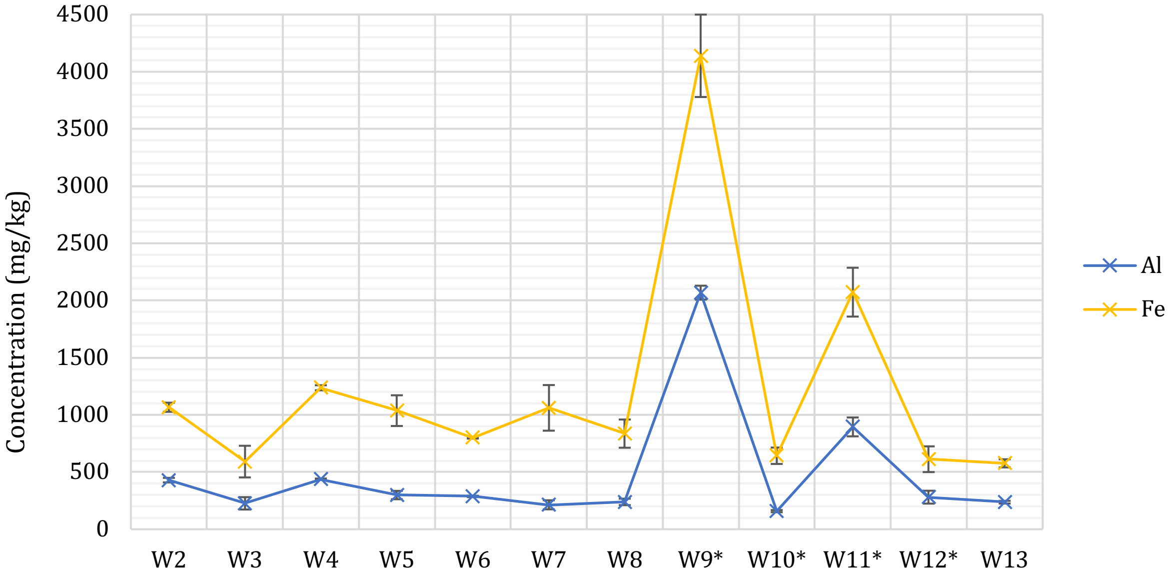
Week	**Milk group A**	**Milk group B**	**Milk group C**	**Concentrate**	**Hay**
2	2	2	2	X	2
3	3	2	2	2 w/o seaweed	2
4	2	3	2	X	2
5	2	2	2	4 w/o seaweed	2
6	2	1	2	X	2
7	2	2	1	2 w/ seaweed; 4 w/o seaweed	2
8	2	3	2	X	2
9	4	2	2	2 w/ seaweed; 2 w/o seaweed	5
10	2	2	2	X	5
11	2	3	2	X	5
12	5	2	2	2 w/o seaweed	5
13	4	2	2	X	2
Total	**31**	**26**	**23**	**4 w/ seaweed; 14 w/o seaweed**	**36**

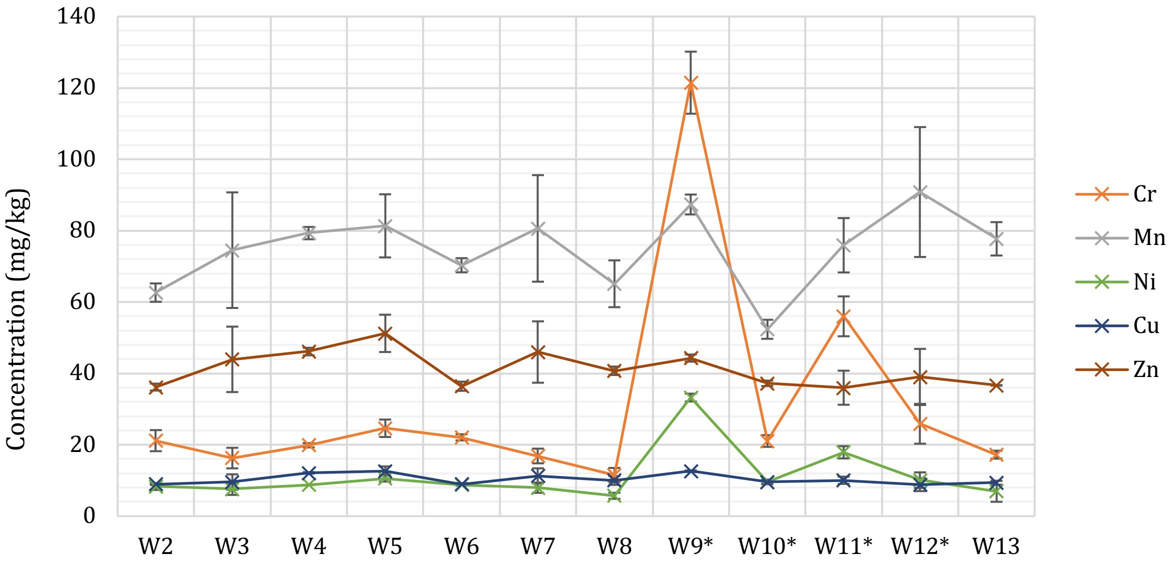
Appendix 5: Minerals concentrations in dry hay, in mg/kg (n = 2)

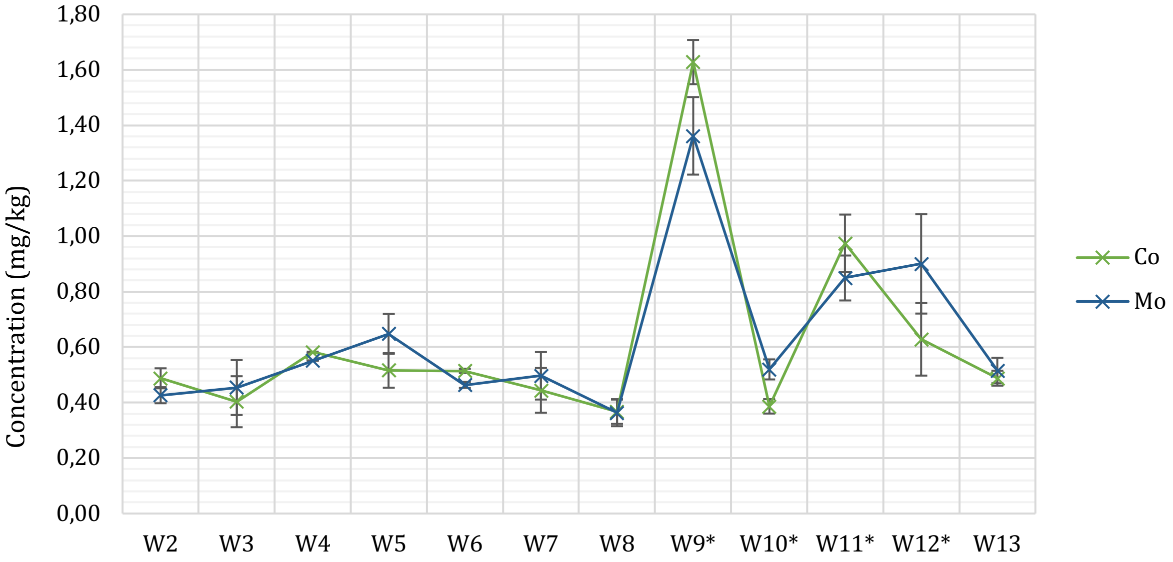


*: n = 5

Appendix 6: Heavy metals concentrations in dry hay, in mg/kg (n = 2)




140
120
100
80
60
40
20
0
Concentration (mg/kg)
W2
W3
W4
W5
W6
W7
W8
W9*
W10*
W11*
W12*
W13
Cr
Mn
Ni
Cu
Zn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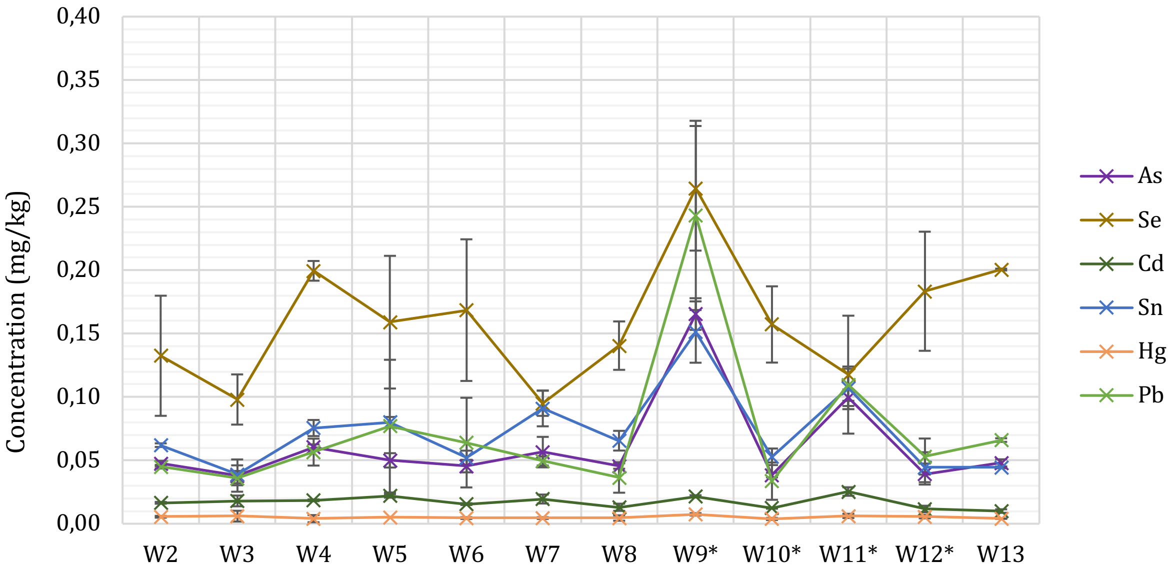
1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
Concentration (mg/kg)
W2
W3
W4
W5
W6
W7
W8
W9*
W10*
W11*
W12*
W13
Co
Mo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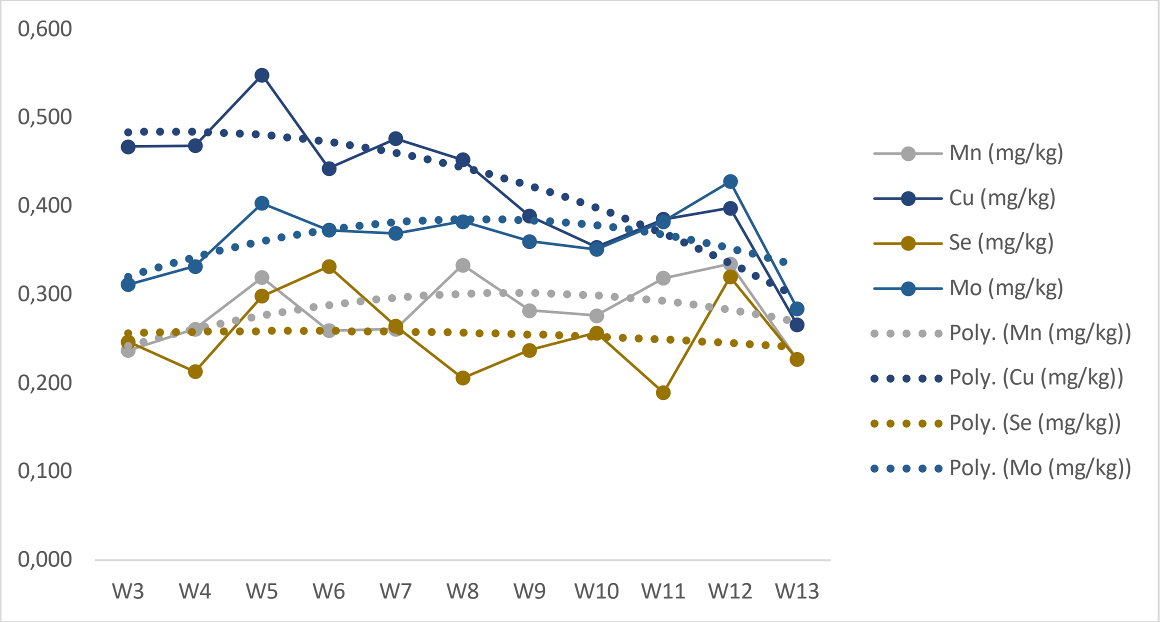
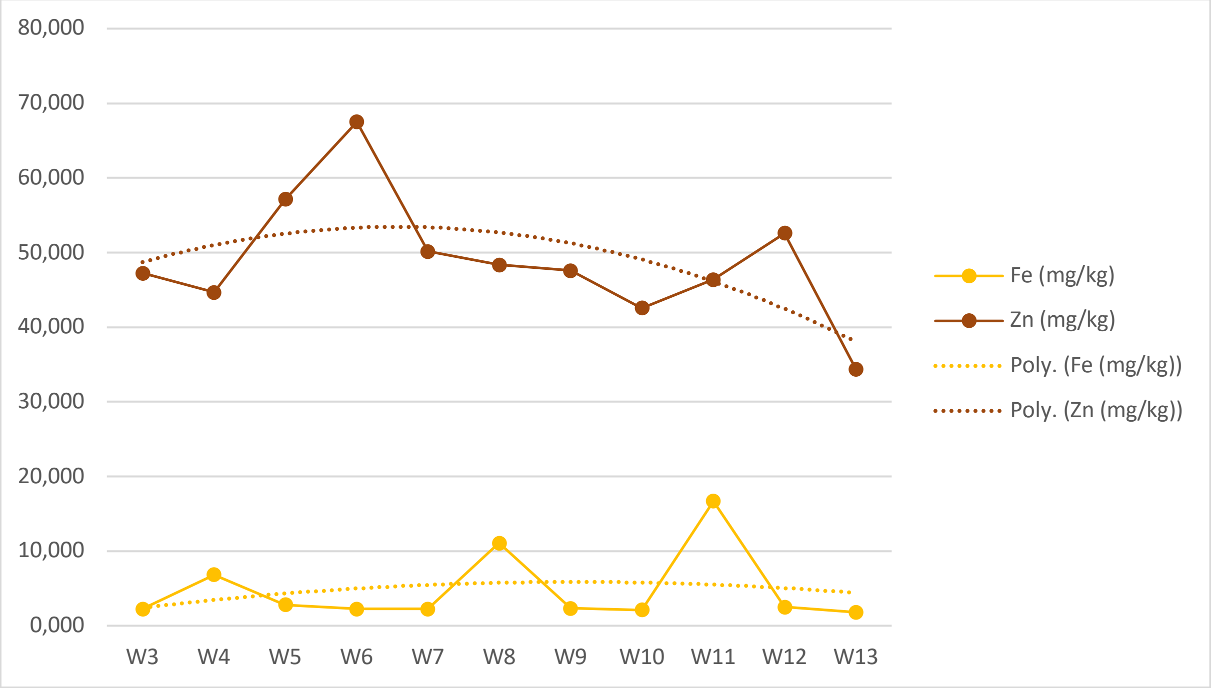
0,30
0,25
0,20
0,15
0,10
0,05
0,00
Concentration (mg/kg)
W2
W3
W4
W5
W6
W7
W8
W9*
W10*
W11*
W12*
W13
As
Se
Cd
Sn
Hg
Pb

*: n = 5

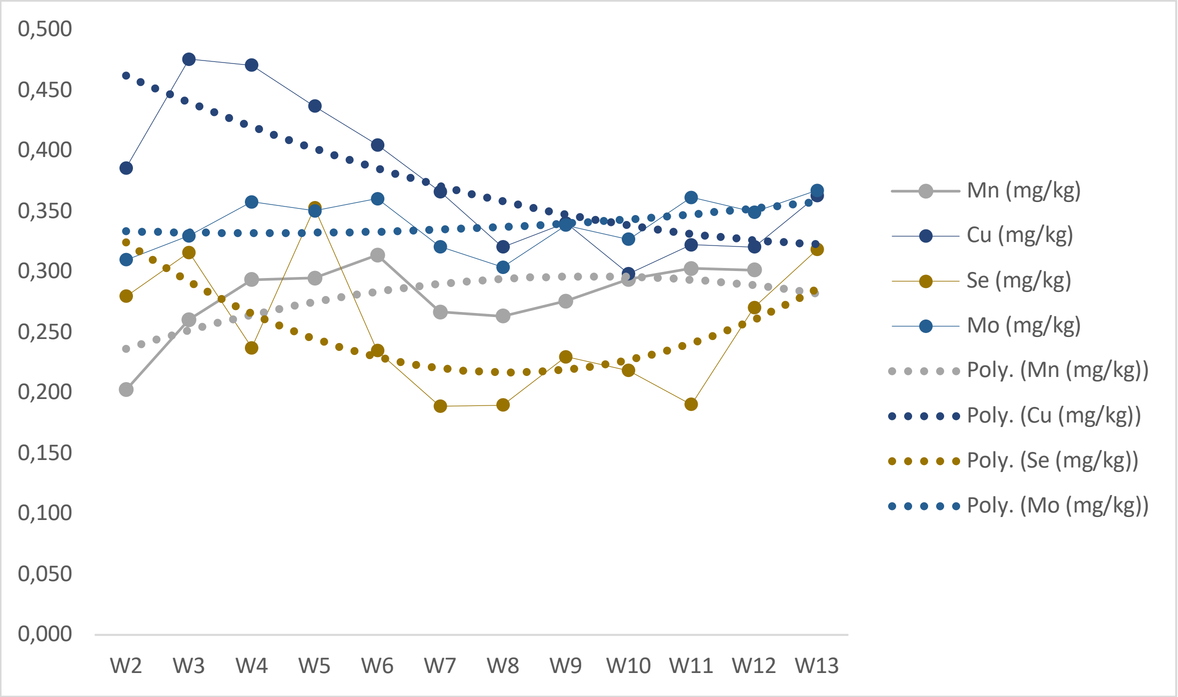
Appendix 7: Heavy metals concentration in dry milk (mg/kg). Group A: 0% seaweed inclusion, Group B: 0.75% seaweed, Group C: 1.5% seaweed inclusion. Polynomial trendline included to indicate whether there is a change in concentration during the feeding trial.

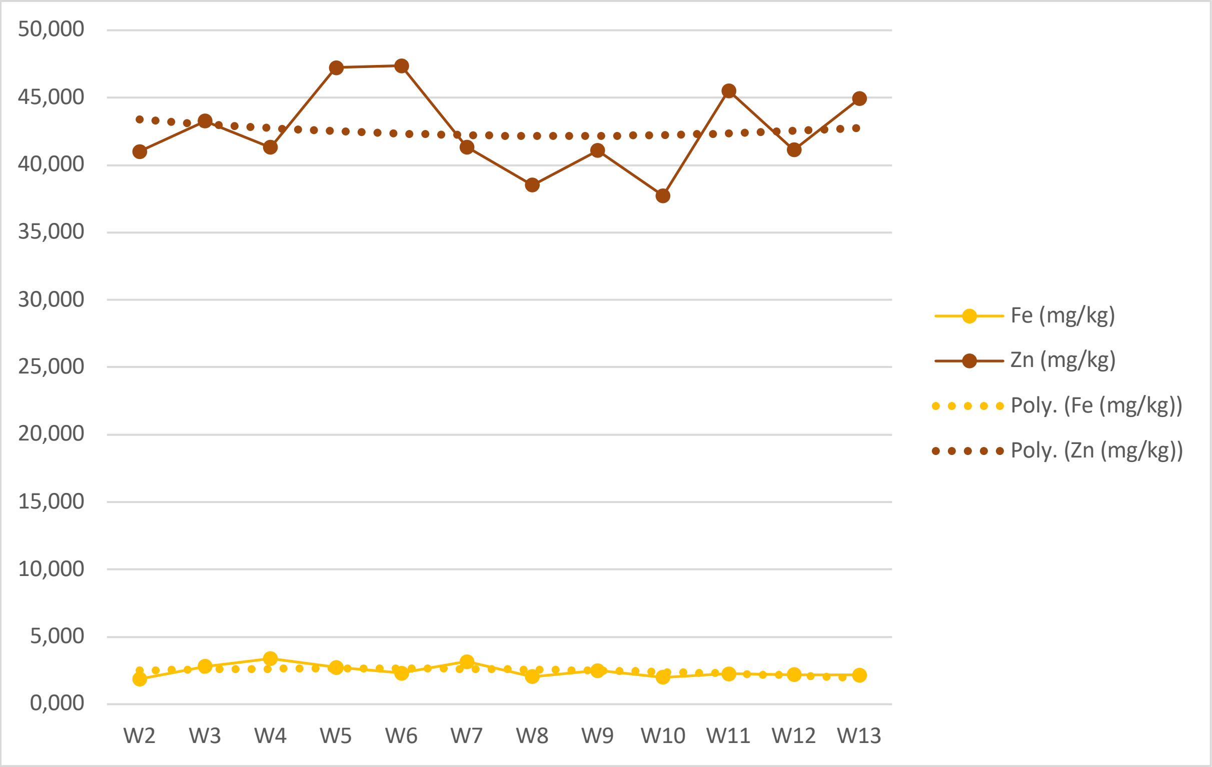
Group A



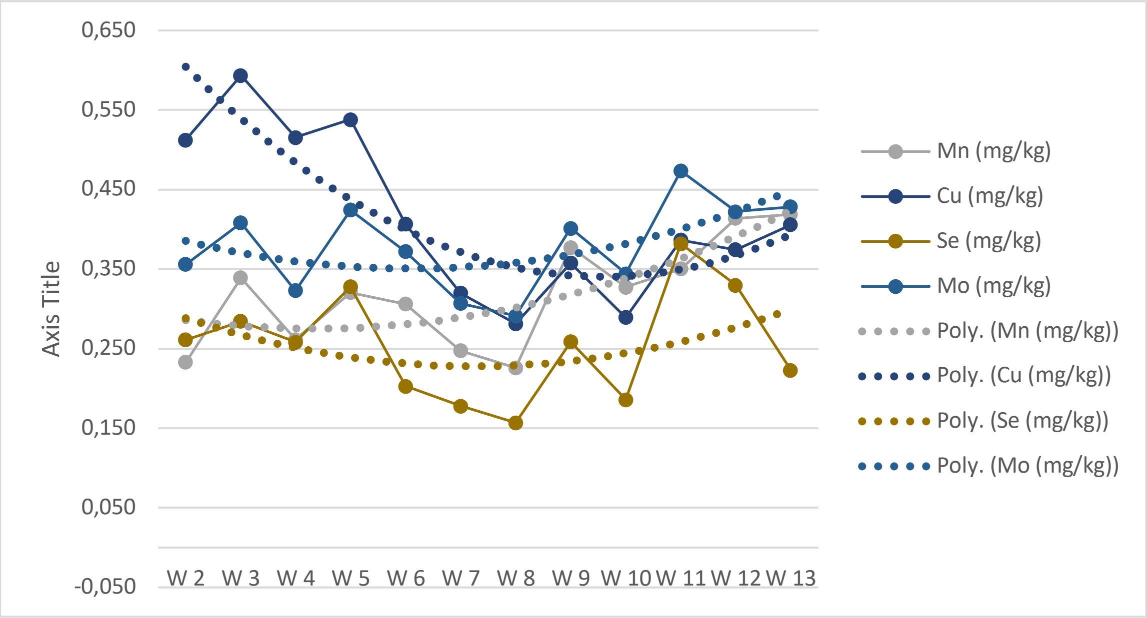


Group B





Group C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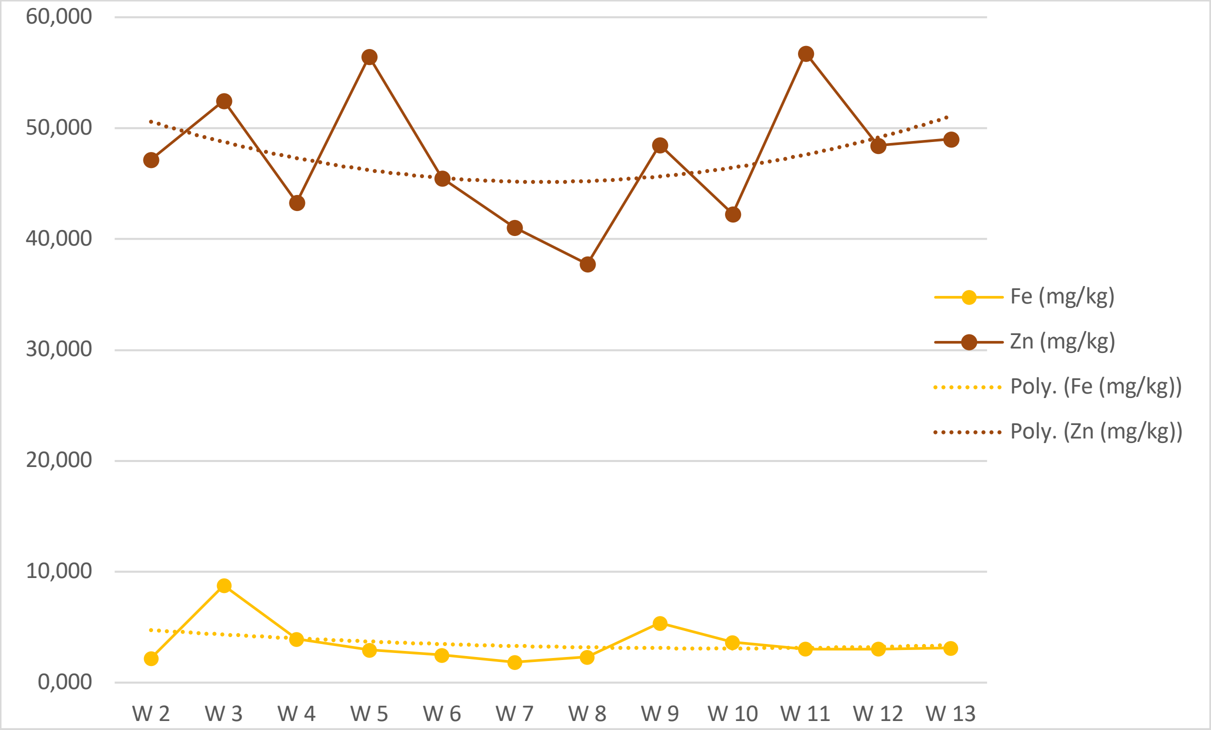
0,550
0,450
0,350
0,250
0,150
0,050
-0,050
Axis Title
W 2
W 3
W 4
W 5
W 6
W 7
W 8
W 9
W 10
W 11
W 12
W 13
Mn (mg/kg)
Cu (mg/kg)
Se (mg/kg)
Mo (mg/kg)
Poly. (Mn (mg/kg))
Poly. (Cu (mg/kg))
Poly. (Se (mg/kg))
Poly. (Mo (mg/kg))


60,000
50,000
40,000
30,000
20,000
10,000
0,000
W 2
W 3
W 4
W 5
W 6
W 7
W 8
W 9
W 10
W 11
W 12
W 13
Fe (mg/kg)
Zn (mg/kg)
Poly. (Fe (mg/kg))
Poly. (Zn (mg/kg))

Appendix 8 Conc. Of heavy metals in the milk

Concentration of heavy metals in the fresh milk in mg/kg.

		Mn	Fe	Co	Cu	Zn	As	Se	Mo
A	Weeks 2 - 5 (n = 9)	34.4	353.8	0.62	66.5	6429	1.16	33.9	45.3
	SD	5.8	126.8	0.09	8.2	887	0.33	6.8	7.5
	Weeks 6 - 10 (n = 10)	36.4	299.5	0.59	55.1	6262	0.79	32.3	47.8
	SD	4.8	41.3	0.13	7.7	659	0.37	3.7	4.8
	Weeks 11 - 13 (n = 7)	39.3	311.6	0.55	49.0	6255	0.62	32.8	50.8
	SD	7.1	60.2	0.16	10.4	1259	0.27	11.0	9.7
B	Weeks 2 - 5 (n = 8)	35.0	345.0	0.69	57.2	5590	1.27	38.2	43.8
	SD	7.1	93.8	0.14	8.2	758	0.59	11.6	5.2
	Weeks 6 - 10 (n = 9)	37.0	293.3	0.56	44.7	5364	0.75	29.3	43.0
	SD	5.0	45.3	0.11	6.2	688	0.28	4.8	5.2
	Weeks 11 - 13 (n = 6)	39.6	294.1	0.58	44.6	5837	0.53	34.6	47.9
	SD	3.8	18.7	0.07	5.3	625	0.08	7.9	4.7
C	Weeks 2 - 5 (n = 9)	37.0	400.9	0.72	73.2	6706	1.26	37.4	50.8
	SD	6.5	143.0	0.19	11.6	1063	0.29	9.0	8.8
	Weeks 6 - 10 (n = 10)	39.3	365.0	0.54	44.2	5807	0.99	27.3	46.5
	SD	10.3	118.9	0.14	9.7	1277	0.38	10.9	10.6
	Weeks 11 - 13 (n = 9)	54.7	412.1	0.73	52.2	6685	0.76	41.0	57.8
	SD	8.3	55.1	0.11	5.8	913	0.16	9.1	7.4

Concentration of heavy metals in the dry milk in mg/kg.

		Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Sn	Hg	Pb
Group A	Weeks 2 - 5 (n = 9)	0.71	0.015	0.26	2.68	0.005	0.002	0.50	48.7	0.009	0.257	0.343	LOD	0.005	LOD	0.010
	SD	0.62	0.006	0.04	0.96	0.001	0.004	0.06	6.7	0.002	0.051	0.057		0.005		0.006
	Weeks 6 - 10 (n = 10)	0.51	0.014	0.28	2.27	0.005	0.007	0.42	47.5	0.006	0.245	0.362		0.005		0.009
	SD	0.78	0.005	0.04	0.31	0.001	0.004	0.06	5.0	0.003	0.028	0.037		0.006		0.008
	Weeks 11 - 13 (n = 7)	0.29	0.008	0.30	2.36	0.004	0.005	0.37	47.3	0.005	0.248	0.384		0.005		0.014
	SD	0.41	0.004	0.05	0.46	0.001	0.004	0.08	9.5	0.002	0.083	0.073		0.005		0.016
Group B	Weeks 2 - 5 (n = 9)	0.70	0.021	0.28	3.04	0.005	0.004	0.56	50.9	0.010	0.284	0.386	LOD	0.004	LOD	0.023
	SD	0.68	0.016	0.05	1.09	0.001	0.006	0.09	8.1	0.002	0.068	0.066		0.004		0.026
	Weeks 6 - 10 (n = 10)	0.51	0.014	0.30	2.82	0.004	0.006	0.34	44.9	0.008	0.211	0.359		0.007		0.017
		0.45	0.009	0.08	0.92	0.001	0.004	0.08	9.9	0.003	0.084	0.082		0.006		0.015
	Weeks 11 - 13 (n = 9)	1.13	0.024	0.42	3.15	0.006	0.014	0.40	51.1	0.006	0.314	0.442		0.004		0.028
	SD	1.44	0.017	0.06	0.42	0.001	0.009	0.04	7.0	0.001	0.070	0.057		0.003		0.028
Group C	Weeks 2 - 5 (n = 8)	0.74	0.019	0.27	2.64	0.005	0.007	0.44	42.8	0.010	0.293	0.335	LOD	0.006	LOD	0.013
	SD	0.58	0.013	0.05	0.72	0.001	0.006	0.06	5.8	0.004	0.089	0.040		0.006		0.009
	Weeks 6 - 10 (n = 9)	0.33	0.014	0.28	2.22	0.004	0.003	0.34	40.7	0.006	0.222	0.326		0.004		0.006
	SD	0.38	0.009	0.04	0.34	0.001	0.002	0.05	5.2	0.002	0.036	0.039		0.004		0.005
	Weeks 11 - 13 (n = 6)	1.12	0.009	0.30	2.21	0.004	0.004	0.34	43.9	0.004	0.260	0.360		0.004		0.017
	SD	2.56	0.004	0.03	0.14	0.001	0.006	0.04	4.7	0.001	0.059	0.035		0.002		0.015

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