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Nutrients and toxic elements in semi-domesticated reindeer in Norway

Nutritional and food safety aspects

Ammar Eltayeb Ali Hassan

A dissertation for the degree of Philosophiae Doctor
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A dissertation for the degree of Philosophiae Doctor (PhD)
Center for Sami Health Research
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To my family, to everyone who taught me a letter and supported me along the way

"Do not write so that you can be understood; write so that you cannot be misunderstood."
Epictetus, AD 55 - 135.

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SUMMARY

Semi-domesticated reindeer (*Rangifer tarandus tarandus L.*) is an important part of the Sami culture and a main constituent of Sami traditional diet. Limited data are available on reindeer as a human foodstuff compared to domestic animals.

The main objective of this thesis was to increase knowledge on reindeer as human foodstuff. In details, to study concentration of specific fatty acids, total lipids, vitamins, essential and toxic elements in meat, liver, tallow and bone marrow from semi-domesticated reindeer and to study this in relation to recommended dietary allowances (RDA) or maximum levels (ML) and provisional tolerable weekly/monthly intake limits (PTWI/PTMI). Differences between concentrations in meat and the other edible tissues from reindeer, as well as effects of geographical variation and animal population density on these concentrations were studied. This thesis is based on samples from 131 semi-domesticated reindeer originating from 14 districts distributed across four counties (Finnmark, Troms, Nordland and Sør-Trøndelag) in the mid- and northern Norway.

We have found that semi-domesticated reindeer meat contains higher vitamin B12, iron, zinc and selenium concentrations when compared to Norwegian beef, lamb, mutton, pork and chicken meat. Reindeer meat is lean, but a good source of docosapentaenoic acid (DPA) and α-linolenic acid (ALA). Concentrations of DPA and ALA in reindeer meat are comparable to those formerly reported in crab, scampi, mussels, oysters and DPA in code. In addition, reindeer liver contains concentrations of vitamins A, B9, B12, iron and selenium that are high enough to cover the recommended dietary allowance (RDA). The ratios of ∑PUFA n-6/ ∑PUFA n-3 in meat, tallow and bone marrow are within the RDA ratios of 3-9. The tallow

contains a high concentration of vitamin B12, while bone marrow contains the highest concentrations of vitamin E and calcium.

Further, this study has shown that the vast majority of nutrient and toxic element concentrations in reindeer liver, tallow and bone marrow were significantly higher than those found in meat. Concentrations of the toxic elements detected in this study were generally low and below the provisional tolerable weekly/monthly intake limits. Most vitamin concentrations in liver, tallow and bone marrow were significantly positive correlated with the concentrations in meat. Positive correlations were revealed between iron and calcium, and vitamin B12 and zinc. Cadmium and arsenic were the only toxic elements positively correlated between liver and meat.

Geographical variations in nutrients and toxic element concentrations between some districts were revealed, with vitamin E, selenium, arsenic and cadmium demonstrating the largest geographical differences. No clear geographical trend was observed except for the east-west gradient for arsenic, with the highest concentrations measured in the east (near the Russian border). Districts with low animal population density had on average higher selenium than those with medium and high population densities.

The measured concentrations of the studied nutrients demonstrate that reindeer intake (meat, liver, tallow and bone marrow) could contribute significantly to the recommended dietary intakes set for consumers. Furthermore, the consumption of meat, liver, tallow and bone marrow from reindeer is not associated with any health risk for consumers when toxic elements are the issue of concern. The geographical differences revealed in this study were not large and will most likely have no impact for consumers.

SAMMENDRAG

Reinsdyr (*Rangifer tarandus tarandus L.*) er en viktig del av samisk kultur og en viktig bestanddel av samisk tradisjonell kost. Data på rein som næringsmiddel for mennesker har vært mangelfulle i forhold andre husdyr.

Hovedmålet med denne avhandlingen var å øke kunnskapen om reinsdyr som matvare for mennesker, for å studere konsentrasjoner av fettsyrer, lipider, vitaminer, essensielle elementer og tungmetaller i kjøtt, lever, talg og benmarg fra rein i relasjon til anbefalt inntak. Vi har studert forskjeller mellom konsentrasjonene i kjøtt og de andre spiselige vev, samt effekter av geografisk variasjon og dyretetthet på disse konsentrasjonene. Denne avhandlingen er basert på prøver fra 131 rein fra 14 beitedistrikter fordelt på 4 fylker (Finnmark, Troms, Nordland og Sør-Trøndelag) i Midt- og Nord-Norge.

Reinkjøtt inneholder høyere vitamin B12, jern, sink og selen konsentrasjoner i forhold til norsk biff, lam, sau, svin og kylling kjøtt. Reinkjøtt er magert, men en god kilde til docosapentaenoic syre (DPA) og α-linolensyre (ALA). Konsentrasjoner av DPA og ALA i reinkjøtt er sammenlignbare med det som er rapportert i krabbe, scampi, blåskjell, østers og DPA i torsk. I tillegg, inneholder en porsjon reinlever konsentrasjoner av vitaminer A, B9, B12, jern og selen som er høye nok til å dekke det daglige anbefalte inntaket (RDA). Forholdet ΣPUFA n-6/ ΣPUFA n-3 i kjøtt, talg og benmarg er innenfor RDA ratio på 3-9. Reintalg inneholder høye konsentrasjoner av vitamin B12, mens benmarg inneholder de høyeste konsentrasjoner av vitamin E og kalsium.

Nivåene av de aller fleste næringsstoffene og tungmetaller i lever, talg og benmarg var betydelig høyre enn de som finnes i kjøtt. Konsentrasjoner av tungmetaller påvist i denne

studien var generelt lave. De fleste vitaminkonsentrasjoner i leveren, talg og benmarg var signifikant positiv korrelert med konsentrasjonene i kjøttet. Positive korrelasjoner ble funnet mellom jern og kalsium, og vitamin B12 og sink. Nivåene i lever og kjøtt av både kadmium og arsen var positivt korrelert.

Geografiske forskjeller i næringsstoffer og tungmetallkonsentrasjoner mellom enkelte beitedistrikter ble funnet. Vitamin E, selen, arsen og kadmium hadde størst geografiske forskjeller. Ingen klar geografisk trend ble observert, bortsett fra øst-vest gradient for arsen, med de høyeste konsentrasjonene målt i øst (nær den russiske grensen). Beitedistrikter med lav dyretetthet hadde i gjennomsnitt høyere selen enn de med middels og høy tetthet.

Funnene fra denne studien tilsier at rein (kjøtt, lever, talg og benmarg) kan bidra betydelig til å få dekket anbefalte næringsstoffinntak for konsumentene. Videre, er konsum av kjøtt, lever, talg og benmarg fra rein ikke forbundet med noen helserisiko for forbrukerne i forhold til risiko for høyt inntak av tungmetaller. De geografiske forskjellene avdekket i denne studien var ikke store, og vil mest sannsynlig ikke ha noen innvirkning for forbrukerne.

ČOAHKKÁIGEASSU

Boazu (*Rangifer tarandus tarandus L.*) lea deatalaš oassi sámi kultuvrras ja maiddái deatalaš sámi árbevirolaš biebmodoalus. Leat leamaš unnán dáhtát ja dieđut das makkár mearkkašupmin bohccos lea leamaš olbmuid biebmodoalus dan ektui go leat dieđut šibihiid birra.

Váldoulbmil dáinna dutkosiin lea háhkat eambbo dieđuid das makkár mearkkašupmi bohccos lea leamaš olbmo borramuššan, ja guorahallat buoidesuvrriid, lipiidaid, vitamiinnaid, deatalaš ávdnasiid ja lossametállaid čoahkkádusa bohccobierggus, vuoivasis, buoiddis ja ađđamiin, daid meriid ektui mat leat ávžžuhuvvon leat borramušain. Mii leat guorahallan čoahkkádusaid erohusaid bierggus ja eará borahahtti osiin, ja máid geográfalaš variašuvdna ja boazolohku mearkkaša dáid čoahkkádusaide. Dutkkus lea iskosiid vuođul 131 bohccos 14 orohagas 4 fylkkas Gaska- ja Davvi-Norggas (Finnmárkkus, Romssas, Nordlánddas ja Lulli-Trøndelágas).

Bohccobierggus lea eambbo vitamiidna B2, ruovdi, sink ja selen go Norgga oame-, lábbá-, sávzza-, spiinni- ja vuonccáčivgabierggus. Bohccobiergu lea guoirras ja das lea valjit docosapentaenoic suvri (DPA) og α-linolensuvri (ALA). DPA ja ALA mearit bohccobierggus leat seamma dásis go mii lea gávnnahuvvon reabbáin, alitskálžžuin, oistariin ja DPA mearri seamma dásis go dorskis. Dasa lassin leat ovtta borranmeari bohccovuoivasis vitamiinnaid A, B9, B12, ruovddi ja selen čoahkkádusat mat leat doarvái gokčat ávžžuhuvvon beaivemeari (RDA). ΣPUFA n-6/ ΣPUFA n-3 gorri bierggus, buoiddis ja ađđamiin lea siskkobealde RDA-ratio, mii lea 3-9. Bohcco buoiddis lea alla mearit vitamiidna B12, ja ađđamis ges leat alimus mearit vitamiidna E ja kalsium.

Eanas biepmusávdnasiid ja lossametállaid čoahkkádus vuoivasis, buoiddis ja ađđamiin lei mearkkašahtti alit go dat mii bierggus gávdnui. Lossametállaid čoahkkádusat dán guorahallamis ledje oppalaččat unnit. Eanas vitamiidnačoahkkádusat vuoivasis, buoiddis ja ađđamiin ledje signifikánta positiivat korrelerejuvvon čoahkkádusaiguin bierggus. Positiiva korrelašuvnnat gávdnojedje ruovddi ja kalsium gaskka, ja vitamiidna B12 ja sink gaskka. Sihke kadmium ja arsen čoahkkádusaid dásit vuoivasis ja bierggus ledje positiiva korrelerejuvvon.

Soames orohagaid gaskka gávdnojedje geográfalaš erohusat biepmusávdnasiid ja lossametállaid čoahkkádusain. Vitamiidna E, selen, arsen ja kadmium dáfus ledje stuorimus geográfalaš erohusat. Ii fuomášuvvon čielga geográfalaš erohus, earret nuorta-oarje gradieanta arsena dáfus, mas stuorimus čoahkkádusat gávdnojedje nuortan (Ruoššaráji lahka). Orohagain gos lea unnit boazolohku eatnamiid ektui, lei gaskamearálaččat alit selen-dássi go doppe gos ledje eambbo dahje ollu bohccot eananviidodaga ektui.

Gávdnosat dán guorahallamis čájehit ahte boazu sáhttá mearkkašahtti láhkái leat mielde deavdime ávžžuhuvvon meari biepmusávdnasiin maid olmmoš dárbbaša. Viidáseappot, de ii leat bohccobierggu, -vuoivasa, -buoiddi ja -ađđama borramis makkárge dearvvašvuođavahát olbmuide, ii ge riska ahte dain leat menddo ollu lossametállat. Geográfalaš erohusat maid dán guorahallamis gávnnaimet, eai leat stuorrát, ja dain ii dáidde leat makkárge mearkkašupmi geavaheddjiide.

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LIST OF PAPERS

The present thesis is based on following papers, referred to by their Roman numerals.

I. **Level of selected nutrients in meat, liver, tallow and bone marrow from semi-domesticated reindeer (*Rangifer t. tarandus L.*)**

Ammar Ali Hassan, Torkjel M. Sandanger and Magritt Brustad

Int J Circumpolar Health **2012**, 71: 17997

II. **Level of selected toxic elements in meat, liver, tallow and bone marrow of young semi-domesticated reindeer (*Rangifer tarandus tarandus L.*) from northern Norway**

Ammar Ali Hassan, Charlotta Rylander, Magritt Brustad and Torkjel M. Sandanger. Int J Circumpolar Health **2012**, 71: 18187

III. **Concentrations and geographical variations of selected toxic elements in meat from semi-domesticated reindeer (*Rangifer tarandus tarandus L.*) in mid- and northern Norway: Evaluation of risk assessment**

Ammar Ali Hassan, Magritt Brustad and Torkjel M. Sandanger

Int J Environ Res Public Health **2012**, 9 (5), 1699-1714.

IV. **Selected vitamins and essential elements in meat from semi-domesticated reindeer (*Rangifer tarandus tarandus L.*) in mid- and northern Norway: Geographical variations and effect of animal population density**

Ammar Ali Hassan, Torkjel M. Sandanger and Magritt Brustad

Submitted

ABBREVIATIONS

ADI	Acceptable Daily Intake
AI	Adequate intake
AM	Arithmetic Mean
AMAP	Arctic Monitoring and Assessment Programme
ANOVA	Analysis of Variance
As	Arsenic
ppb	part per billion (μg/kg = ng/g)
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
EC	European Commission
FAO	Food and Agriculture Organization
GM	Geometric Mean
HNO_3	Nitric Acid
H_2O_2	Hydrogen Peroxide
ICP-HRMS	Inductively Coupled Plasma High Resolution Mass Spectrometer
JECFA	Joint Expert Committee on Food Additives
Km	Kilometre
LOD	Limit of Detection
LOQ	Limit of Quantification

ML	Maximum Level
Max.	Maximum
Min.	Minimum
mg	Milligram (10^{-3} gram)
MUFA	Monounsaturated Fatty Acids
ng	Nanogram (10^{-9} gram)
Ni	Nickel
NIST	National Institute of Standards and Technology
NILU	Norwegian Institute for Air Research
pH	pondus Hydrogenii
Pb	Lead
PTWI	Provisional Tolerable Weekly Intake
PTMI	Provisional Tolerable Monthly Intake
PUFA	Polyunsaturated Fatty Acids
POPs	Persistent Organic Pollutants
QC	Quality Control
r	Pearson's correlation coefficient
RAE	Retinol Activity Equivalent
Re	Rhenium
RDA	Recommended Dietary Allowance
RDI	Recommended Daily Intake
r_s	Spearman's correlation coefficient
Se	Selenium
SD	Standard Deviation
SFA	Saturated Fatty Acids

UL	Tolerable upper intake level
WHO	World Health Organization
ww	Wet weight
V	Vanadium
Zn	Zinc
μg	Microgram (10^{-6} gram)

1. INTRODUCTION

1.1 Background

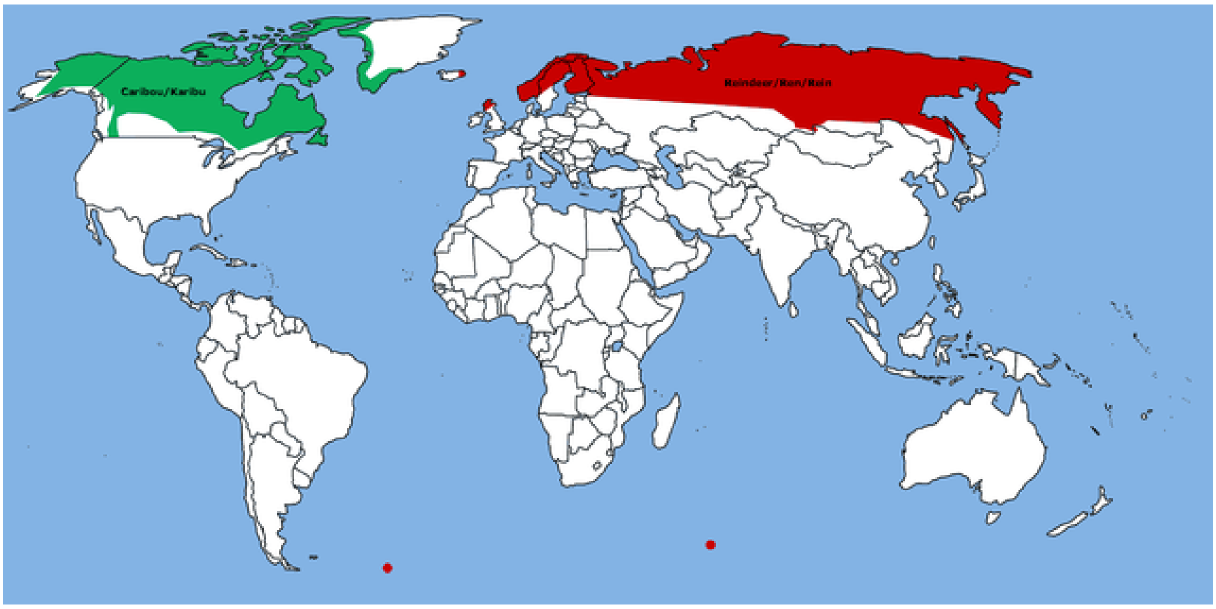
Despite the great effort that has been done in reindeer research during the last decades, there are still limited data that could be related to human consumption. Semi-domesticated reindeer belongs to the family Cervidae and the genus Rangifer, which also includes caribou. The scientific classification of semi-domesticated reindeer is presented below:

Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Class	Mammalia
Order	Artiodactyla
Family	Cervidae
Genus	Rangifer
Species	tarandus
Subspecies	tarandus Linnaeus

Source: Deer of the world [1]

The normal habitat of reindeer is Arctic and Sub-Arctic including both semi-domesticated and wild populations (Figure 2). Total world population of reindeer is approximately 5 million of which around 5% and 0.5% are found in Norway as semi-domesticated and wild reindeer, respectively [2-4]. Reindeer husbandry in Norway is restricted by law to the Sami indigenous people and is characterized by free range grazing and continuous movement all the year around [5]. The slaughter season starts in early September and lasts up to late January in which both stationary and mobile slaughterhouses contribute to total amount of approximately 2000 tons reindeer meat produced yearly in Norway [3].

Figure 2. Distribution of reindeer/caribou over the world



Source: Tom Bjornstad [6].

This thesis is about semi-domesticated reindeer as foodstuff and deals with nutritional and toxic elements aspects in meat and other edible parts of the animal. It is an attempt to enhance knowledge regarding reindeer as food item, and thereby contribute to the on-going discussion related to Arctic traditional food.

1.2 Arctic dilemma

Arctic food is known to be nutrient-rich and at the same time prone to environmental contamination from contaminants mainly produced elsewhere and transported to the Arctic via long range atmospheric transportation and ocean currents. The Arctic dilemma expresses the fact that the main source of nutrients is also a source of contaminants, particularly in cases in which accessibility to diverse food sources are limited. Further, it communicates a message regarding both the positive and negative aspects related to Arctic traditional food and the issue of how to get a balance consuming food considered to be a main nutrients source and at the same time a potential source of contaminants [7, 8].

1.3 Fatty acids and lipids

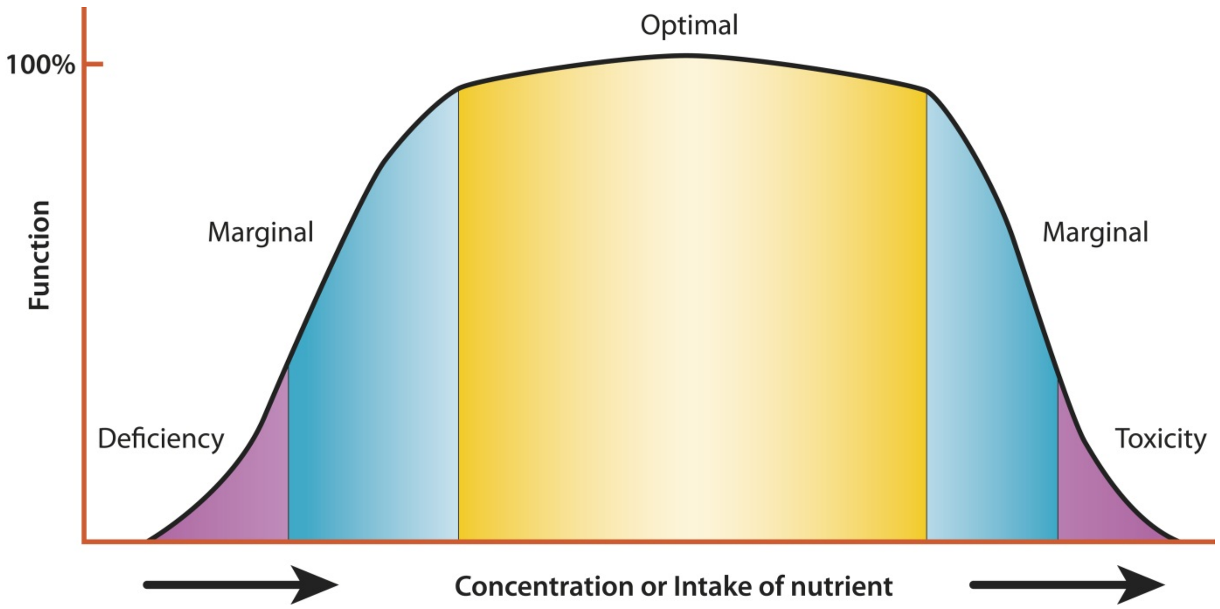
Fatty acids are carboxylic acids with un-branched hydrocarbon chains of 4-24 carbon atoms and they occur in all organisms as component of fats and membrane lipids [9]. These fatty acids are further classified into saturated (SFA), mono- (MUFA) and polyunsaturated (PUFA). The MUFA and PUFA are often referred to as healthy fats, whereas saturated (SFA), especially trans-fats, are considered as unhealthy fats. Some of the PUFA (*e.g.,* the C:20 arachidonic and the C:18 linoleic acids) are essential and need to be supplied in the diet. Fatty acids and lipids are important energy source in the diet, component of cell membranes and have special roles in human health [10-13]. Polyunsaturated fatty acids, especially long chained n-3 have been reported to be beneficial to human health due to their contribution in prevention of disease occurrences such as cardiovascular diseases [14-16].

Fatty acids composition in meat is influenced by the fatty acids in animals' diet and plays an important role in meat quality. Taste of cooked meat is influenced by the volatile flavor from different unsaturated fatty acids [17]. Moreover, unsaturated fatty acids are more susceptible to oxidation compared to saturated ones in meat [18, 19]. Studies have shown that reindeer meat contained higher concentrations of n-3 PUFA, total phenols and high anti-oxidant activity when compared to beef meat [20, 21]. Reindeer meat has also been reported to contain lower amount of total lipids compared to meat from domestic animals [21].

1.4 Vitamins

Vitamins are essential organic compounds that are required in small amount for normal cell function, growth, and development. Some are precursors of co-enzymes, hormones and some act as anti-oxidants [9]. Furthermore, they are divided according to their solubility into; water (vitamins B1, B2, B3, B5, B6, B7, B9, B12 and C) and fat (vitamins A, D, E and K) soluble vitamins. Vitamins deficiencies are most often caused by inadequate dietary intakes of these vitamins from foods containing these nutrients [9, 22]. Toxicity due excessive vitamin intake (hypervitaminosis) is less common, but has been described in the literature, Figure 3 [22, 23].

Figure 3. The concept of deficiency, optimality and toxicity for nutrients



Reindeer meat has been known of its high vitamin contents when compared to meat from other animal species, but data is limited particularly in Norway [24, 25]. The free ranging nature of reindeer herding allows the animal to get access to variety types of pasture compared to domestic animals. An example of this is the lichens which have been found to improve microbial activity inside reindeer rumen and count for the higher contents of vitamin B12 synthesis compared to other ruminants like cattle and sheep [26].

1.5 Essential elements

An element is essential when the deficiency of that element results in impairment of body physiological functions and the supplement of that element prevent or cure this impairment

[27, 28]. Essential elements are needed in small concentrations and their deficiencies are common and occur due to low intake, absorption disturbances and diseases [29]. Consumption of a diet deficient in essential elements could predispose people to toxicity from toxic elements [30]. Dietary deficiencies of calcium, iron, and zinc are likely to enhance the effects of lead on cognitive and behavioral development, and iron deficiency increases the gastrointestinal absorption of cadmium which competes with zinc for binding sites on metallothionein [31, 32]. Selenium protects against mercury and methyl mercury toxicity either by preventing damage from free radicals due to its antioxidant properties or by forming inactive selenium mercury compounds [31]. Presence of essential elements in higher concentrations than recommended causes toxicity to both humans and animals as shown in Figure 3 [29, 33, 34]. As an example, a dose of 5 mg/ day has been associated with Se poisoning in humans, whereas cattle fed on plants containing Se concentrations of about 5-50 mg/ kg have exhibited poisoning signs [35, 36]. Due to the association between essential and toxic elements as mentioned above, it is of great importance to identify important sources of these elements.

Reindeer meat contains significant amount of essential elements such as calcium, iron and zinc which has been reported to be higher in concentrations compared to meat from domestic animals [25, 37-39]. Data on essential elements in liver and other edible tissues from reindeer are limited.

1.6 Sources of toxic elements in the environment

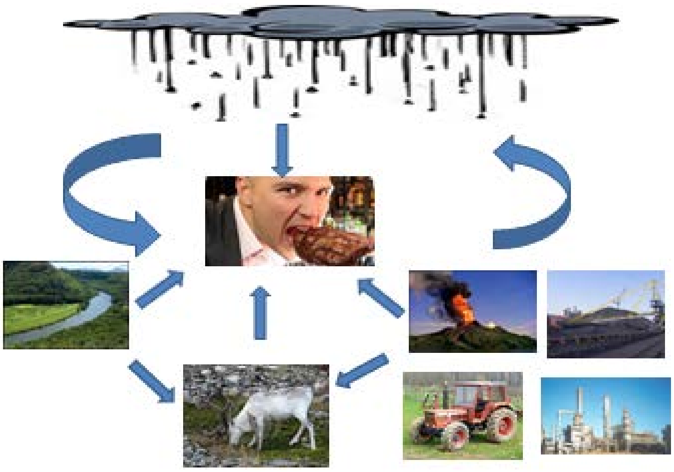
Toxic elements, also known as toxic or heavy metals are inorganic chemicals that have been loosely defined in literature according to many properties and different definitions of these properties [40]. Some of these properties are; atomic weight, density and toxicity to human, plants and animals [41]. Cadmium, lead, mercury and arsenic are examples of such elements. Sources of toxic elements in the environment can be natural or anthropogenic. The natural sources are earth crust, rocks and volcanic eruptions. The anthropogenic ones are agricultural activities, mining work, industry, combustion and waste disposal [42, 43]. Toxic elements are present in the atmosphere, water, soil, fuels, paints, electronic devices, tobacco, batteries, ammunition and many more [44, 45]. They are persistent, toxic in different chemical forms and some of them accumulate in plant, animal and human tissues [44, 46]. Both local sources and long range atmospheric transportation contribute to environmental toxic elements contamination as presented in Figure 4 [47, 48].

Reindeer liver and kidneys from Norway, Sweden, Finland, Russia, Greenland and Arctic Canada have been the main focus regarding toxic elements (due to their ability to accumulate such elements). Elevated concentrations of some toxic elements have previously been revealed in reindeer liver and kidneys originated from the above mentioned countries [49-55].

1.7 Human exposure to toxic elements

The most common route of human exposure to toxic elements is through diet (oral exposure). Moreover, exposures via lungs through inhalation of contaminated dust particles in the atmosphere and direct contact through skin and eyes contribute to a lesser degree [56]. Toxic elements accumulate in different human body tissues depending on their tissue preference and half-life. Cadmium has exceptionally long half-life (*e.g.*, 10-30 years in kidney) accumulates in liver and kidneys, lead in bones and arsenic in liver, kidneys and muscle tissues [29, 57]. However, in case of tissue saturation with the toxic elements, higher concentrations can also be found in tissues that are not regarded as targets.

Figure 4. Human exposure to toxic elements from the environment



1.8 Effect of toxic elements on human health

Toxicological effect of toxic elements on human depends on factors that are related to the toxic elements themselves, as well as to humans [44]. Factors related to toxic elements include abundance, chemical form, speciation, oxidation state, ionizability, particle size, magnitude/duration of exposure and irritant, corrosive, cytotoxic, mutagenic and carcinogenic properties of the specific toxic element. Human related factors include susceptibility, route of exposure, bioavailability, absorption, binding, metabolism, excretion, ability to penetrate blood-brain and placental barriers, target organ or tissue and nutritional and immune status. Furthermore, interaction of toxic elements with nutrients, alcohol, smoking and drugs is an important human factor [44, 58].

Toxic elements such as cadmium, lead, arsenic and nickel have the ability to penetrate the human cell and react with the deoxyribonucleic acid (DNA) causing chromosomal damage which leads to mutagenic and carcinogenic effects [59-61]. Target tissues such as liver, kidney and

bone in which cadmium, lead and arsenic accumulate can be damaged and lose their physiological functions [62]. Neurological symptoms have been seen in cases of mercury, cadmium, lead and arsenic toxicity [63]. Infertility has also been associated with exposure to toxic elements [64-67].

1.9 Animal population density and geography

There are considerable variations in animal population density among the different reindeer grazing districts in Norway which may result in various effects on forage quantity and quality [3]. These variations may possibly influence the level of nutrients in reindeer. The geography may also influence levels of both nutrients and toxic elements. Data is limited on quality and quantity of pasture across the different grazing districts extended from the northernmost Norwegian county (Finnmark) to Sør-Trøndelag County in the middle of Norway. Furthermore, differences in geology among the different grazing districts and presence of local contamination sources such as mining activities in some ones may also encounter differences in level of toxic elements in semi-domesticated reindeer.

1.10 Recommended dietary allowances/ intakes for nutrients

The recommended dietary allowances (RDAs) are defined as the levels of essential nutrients intakes considered to be adequate to meet the known nutrient needs of all healthy persons based on scientific knowledge [35]. Thus, persons with special nutritional needs are not included in the RDAs. Furthermore, the RDAs are categorized according to the needs for some nutrients based on age group (e.g., infants, children, adolescents, and adults), sex (male/ female) and physiological requirements (e.g., pregnancy, lactation).

1.11 Provisional tolerable weekly intake and provisional tolerable monthly intake of toxic elements

Provisional tolerable weekly intake (PTWI) and provisional tolerable monthly intake (PTMI) limits for some toxic elements such as cadmium and arsenic have been set by the Joint Expert Committee on Food Additives (JECFA) of World Health Organization (WHO) and, Food and Agricultural Organization (FAO) [68, 69]. Upper intake limits (UL) for toxic elements with no established tolerable intake limits (e.g., nickel and vanadium) have also been reported. The purpose is to ensure consumers a safe food when toxic elements are the issue of concern [70].

2. AIMS OF THE THESIS

The main aims of this thesis were to acquire knowledge on semi-domesticated reindeer as food substance and provide data that are relevant to human nutrition and food safety.

Specific aims:

I. Provide information about concentrations of vitamins, fatty acids, total lipids, essential and toxic elements in primarily meat, but also liver, tallow and bone marrow from reindeer.

II. Investigate whether there are differences in concentrations of the studied nutrients and toxic elements among the four type of tissues mentioned above.

III. Investigate whether concentrations of nutrients and toxic elements correlate between meat and the rest of the studied tissues, particularly correlation of toxic elements between liver and meat.

IV. Study geographical differences in concentrations of the studied nutrients and toxic elements in reindeer meat samples from northern and mid- Norway.

V. Study effect of animal population density on concentrations of vitamins and essential elements.

VI. Assess the possible impact of reindeer consumption on human nutritional and toxic element intakes.

3. MATERIALS AND METHODS

3.1. Sample collection

Samples were collected from semi-domesticated reindeer in northern (Finnmark, Troms and Nordland counties) and mid (Sør-Trøndelag county) Norway. The collection periods were from September 2004 to January 2005 (Paper I and II) and from October – December 2008 and September-December 2009 (Papers III and IV). A summary of each paper is presented in Table 1.

Table 1. Summary of variables, themes, matrices and geographical areas employed in the present study

Paper	Variables and themes	n	Matrices	Geographical areas
I	• Fatty acids, lipids, vitamins (A, B1, B2; B3, B5, B6, B7, B9, B12, C, D, E, and essential elements (Ca, Fe, Zn, Se). • Concentrations and differences between edible tissues. • Correlation of nutrients between meat and the other studied tissues.	31	Meat Liver Tallow Bone marrow	Finnmark and Nordland counties (n= 7 districts)
II	• Toxic elements: Cd, Pb, As, Ni, V. • Concentrations and differences between edible tissues. • Correlation of toxic elements between meat and liver.	31	Meat Liver Tallow Bone marrow	Finnmark and Nordland counties (n= 7 districts)
III	• Toxic elements: Cd, Pb, As, Cu, Ni, V • Geographical variations. • Risk assessment	100	Meat	Finnmark, Troms, Nordland and Sør-Trøndelag counties (n= 10 districts)
IV	• Vitamins A, B3, B7, B12, E, and the essential elements Ca, Fe, Zn, Se, Co and Cr. • Geographical variations. • Effect of animal population density on nutrient concentrations.	100	Meat	Finnmark, Troms, Nordland and Sør-Trøndelag counties (n= 10 districts)

Note: Details on missing and excluded observations are presented in the individual papers.

3.2. Fatty acids and total lipids analyses

The analyses of fatty acids and total lipids were undertaken by Unilab Analyse A/S in the Fram Centre, Tromsø, Norway according to a method for the isolation and purification of total lipids from animal tissues by Folch and colleagues [71]. The laboratory is accredited for the methods used in the analyses according to the European standard NS-EN ISO/ IEC 17025. Fatty acids are described by a shorthand nomenclature of chain length (number of carbon atoms): the number of double bonds and n-x which indicate the position of the last double bond related to the terminal methyl end. Additionally, common fatty acids names are used in polyunsaturated fatty acids.

3.3. Vitamins analyses

The analyses of vitamins were conducted by GBA-Food (Hamburg, Germany) according to methods approved by the German Food Act LMBG § 35, LFGB § 64 and the standard methods of the Association of Official Analytical Chemists [72, 73]. The laboratory is subcontracted by the Norwegian laboratory Unilab Analyse A/S, Tromsø, Norway and is accredited with the methods used in the analyses according to Staatliche Akkreditierungsstelle Hanover, AKS-P-20213-EU. The vitamin E concentration is composed of all tocopherols (α, β, γ and Δ tocopherols), whereas vitamins A and B3 concentrations refer to retinol and niacin, respectively. Measurement of uncertainty for vitamins analyses were given as extensive uncertainty measurement according to (Guide to the expression of uncertainty in measurement, ISO, Geneva, Switzerland) estimated by a covering factor of 2 (95% confidence interval).

3.4. Essential and toxic elements analyses

Meat, liver, tallow and bone marrow from semi-domesticated reindeer were separately digested using a microwave oven (Ethos Plus, Milestone Inc., Shelton, CT, USA). In short, concentrated supra-pure HNO_3 (5 ml) and H_2O_2 (3 ml) were added to the sample (0.6 – 0.7 g) before undergoing the microwave oven treatment. Hence, the following temperature regimes were used in the microwave: 20-50° C (5 min.), 50-100° C (10 min), 100-180° C (5 min.) and 180° C (15 min.). After cooling down the heated decomposed sample, the solution was diluted to 50 ml. The sample solution was analysed using an inductively coupled plasma high resolution mass spectrometer (ICP-HRMS), Bremen, Germany. All standards and calibration solutions contained 1 ppb Rhenium (Re) as an internal standard and 1% nitric acid (HNO_3). The calibration curve was verified by use of a standard quality control (QC) sample, (Spex Standard, Ultra Scientific, North Kingston, RI, USA) in compliance with ANSI/NCSLZ-540-1 and ISO 90001. The QC material SRM-1566a (Oyster tissue) was obtained from the National Institute of Standards and Technology (NIST), Maryland, USA. The resolutions used for essential and toxic elements were low (at 10) for (Zn, Cd and Pb), middle (at 20) for (Ca, Fe, Cu, Ni and V), and high (at 30) for (Se and As). The lens adjustment was optimized daily to ensure maximum intensity and top separation. The analyses were done by the NILU (Norwegian Institute for Air Research) Laboratory, Kjeller, Norway. The laboratory is accredited for the methods used in the analyses according to NS-EN ISO/IEC 17025, No. TEST008. The limits of detections (LODs) for the studied essential elements were three times standard deviation (SD) of the laboratory blanks, whereas the limits of quantifications (LOQs) were 10 times the SD of the blanks, decomposed simultaneously with the meat samples.

Precautionary measures, such as the use of closed cabinet, non-metal sampling devices, tools and containers, were taken when preparing the decomposed sample to avoid contamination by dust or from mineral alloys in laboratory tools.

3.5. Statistical analyses

All statistical analyses were performed using Stata/SE for Windows versions 11 and 12 (Stata Corp. College Station, TX, USA). Laboratory results for vitamins, essential elements and fatty acids below the limits of detection (LOD) were replaced by zero. Furthermore, toxic elements below the LOD were given a numeric value at half the detection limit (LOD/2) according to accepted statistical practice [74]. Dependent sample t-test was used in paper I, Wilcoxon matched-pairs signed-rank test in paper II and analysis of variance (ANOVA) in papers III and IV. Pearson's (Papers I, III and IV) and Spearman's (Paper II) correlations were used to test for possible significant correlations. Detailed statistical procedures were presented in the individual papers. The level of statistical significance was set at $p < 0.05$ for all the statistical analyses.

3.6. Ethical considerations

The study did not include any living animals, did not have any adverse environmental health effects, with samples collected from reindeers that had been slaughtered for human consumption. Animals were fixed prior to slaughter, made unconscious using a bolt pistol and put down under the inspection of an official veterinarian according to the Norwegian regulations on animal welfare in slaughterhouses [75].

4. MAIN RESULTS

Paper I

Level of selected nutrients in meat, liver, tallow and bone marrow from semi-domesticated reindeer (*Rangifer t. tarandus L.*) in northern Norway

Int J Circumpolar Health 2012, 71: 17997

The main purpose of this study was to obtain new knowledge on the nutrient value of semi-domesticated reindeer (n= 31) through the measurement of levels of selected vitamins, minerals, fatty acids and total lipids in the meat, liver, tallow and bone marrow. Our intent was to compare the nutrient value of reindeer meat, liver, tallow and bone marrow with data on corresponding tissues from other species. Additionally, we wanted to assess nutrient levels in these tissues in relation to recommended daily allowance (RDA).

We have found that semi-domesticated reindeer meat is lean, thus it suitably meets consumers' need for low-fat meat, and is also a good source of vitamin B12, docosapentaenoic acid (DPA) and α-linolenic acid (ALA). In addition, reindeer liver contains high concentrations of vitamins A, B9, B12, Fe and Se. The ratios of ∑PUFA n-6/ ∑PUFA n-3 in meat, tallow and bone marrow are high enough to cover the RDA. The tallow contains a high concentration of vitamin B12 (2.2 µg/100g) compared to bone marrow (1.2 µg/100g) , while the later contains the highest concentrations of vitamin E (2.3 mg/100g) and Ca (340 mg/100g). The vast majority of nutrient concentrations in reindeer liver, tallow and bone marrow were significantly ($p < 0.05$) different from the concentrations in meat (mostly higher than those found in meat). Most vitamin concentrations in liver, tallow and bone marrow were significantly correlated with the concentrations in meat ($p < 0.05$).

Paper II

Level of selected toxic elements in meat, liver, tallow and bone marrow of young semi-domesticated reindeer (*Rangifer tarandus tarandus L.*) from northern Norway

Int J Circumpolar Health 2012, 71: 18187.

Our main purpose was to study the concentration of selected toxic elements - cadmium (Cd), lead (Pb), arsenic (As), nickel (Ni) and vanadium (V) - in the meat, liver, tallow and bone marrow from semi-domesticated reindeer (n= 31), particularly the association between liver and meat concentrations. Additionally, we wanted to relate our results on toxic elements in meat and the rest of the studied tissues to the EC's maximum level (ML) and FAO/WHO – JECFA's provisional tolerable weekly/monthly intake (PTWI/PTMI) limits available for certain toxic elements.

Liver had as expected the highest toxic element concentrations with the exception of Ni, which was highest in bone marrow. Significant correlations among the detected elements between liver and meat were observed only for Cd and As. Therefore, liver is not a good indicator for lead in meat. Based on the measured levels of the present studied elements and their relation to the EC's maximum level (ML) and the provisional tolerable weekly/monthly intake (PTWI/PTMI) limits, we could infer that the consumption of reindeer meat is not associated with any health risk for consumers. The Cd level exceeded the EC's maximum level (ML) for bovine animals in 52% of the liver samples. Nonetheless, the monthly Cd intake of 2.3µg/kg body weight from liver was well below the PTMI of 25 µg/kg body weight set by FAO/WHO – JECFA. This would further indicate a necessity to not use the ML alone when relating toxic element levels in reindeer and games to human intake of such elements. The tolerable intakes set by the JECFA would be more appropriate to use when dietary frequency could be estimated through questionnaire data. Levels of toxic elements in reindeer tissues were much below the ML, except for Cd in liver as stated above. Due to the fact that meat is more frequently consumed than liver and most of the toxic elements were not correlated between liver and meat, future assessments should possibly focus on meat.

Paper III

Concentrations and geographical variations of selected toxic elements in meat from semi-domesticated reindeer (*Rangifer tarandus tarandus L.*) in mid- and northern Norway: Evaluation of Risk Assessment

Int J Environ Res Pub Health 2012, 9 (5), 1699-1714

The main purpose of this paper was to study the concentrations and geographical variations of selected toxic elements – cadmium (Cd), lead (Pb), arsenic (As), copper (Cu), nickel (Ni) and vanadium (V) - in meat from semi-domesticated reindeer (n= 100) in the selected grazing districts (n= 10) in mid- and northern Norway.

The concentrations of the toxic elements detected in this study were low and much below the maximum levels (ML) available for hazardous toxic elements. Geographical variations in toxic element concentrations between some districts were demonstrated, with As and Cd being the elements which had the largest geographical differences. No clear geographical trend was observed except for the east-west gradient for As, with the highest concentrations measured in the east (near the Russian border). The Cd was shown to be at higher concentrations in older animals, while the other toxic elements did not exhibit an age effect. The district Fávrrosorda had the highest Pb concentration (7.4 ng/g ww), while neighbouring Ábborašša with its gold mining facilities had a significantly lower Pb concentration (1.6 ng/g ww, $p < 0.01$). Human exposure to toxic elements through intake of reindeer meat was much lower when compared to provisional tolerable weekly intake (PTWI) and provisional tolerable monthly intake (PTMI) limits as set by the Joint Committee on Food Additives (JECFA). Thus, reindeer meat is not likely to contribute significantly to the human body burden of toxic elements.

Paper IV

Selected vitamins and essential elements in meat from semi-domesticated reindeer (*Rangifer tarandus tarandus L.*) in mid- and northern Norway: Geographical variations and effect of animal population density

Submitted

The main purpose of this work was to increase knowledge about nutrients in reindeer meat by studying geographical variations and effect of animal population density on selected vitamins (A, B3, B7, B12 and E) and essential elements (Ca, Fe, Zn, Se, Cr and Co) in meat from semi-domesticated reindeer (n= 100) originating from mid- and northern Norway.

Reindeer meat contained higher vitamin B12 (4.7 µg/100g), Fe (2.8 mg/100g), Zn (6.4 mg/100g) and Se (19.4 µg/100g) concentrations when compared with Norwegian beef, lamb, mutton, pork and chicken meat. The geographical differences revealed in this study were not large and will most likely have no impact for consumers. Vitamin E and Selenium demonstrated relatively large geographical variations. Calves had a significant lower Zn concentration (4.7 mg/100g) than young and older animals (6.9 mg/100g, $p< 0.01$), whereas young animals had a significant lower Se concentration (16.6 µg/100g) than calves and older animals (25.7 µg/100g, $p< 0.05$). Positive correlations were revealed between iron and calcium ($r= 0.34$, $p< 0.01$), and vitamin B12 and zinc ($r= 0.35$, $p< 0.05$). Animals originating from districts with low animal population density had on average 12.4 µg/ 100g higher selenium than those from districts with medium and high population densities.

5. GENERAL DISCUSSION

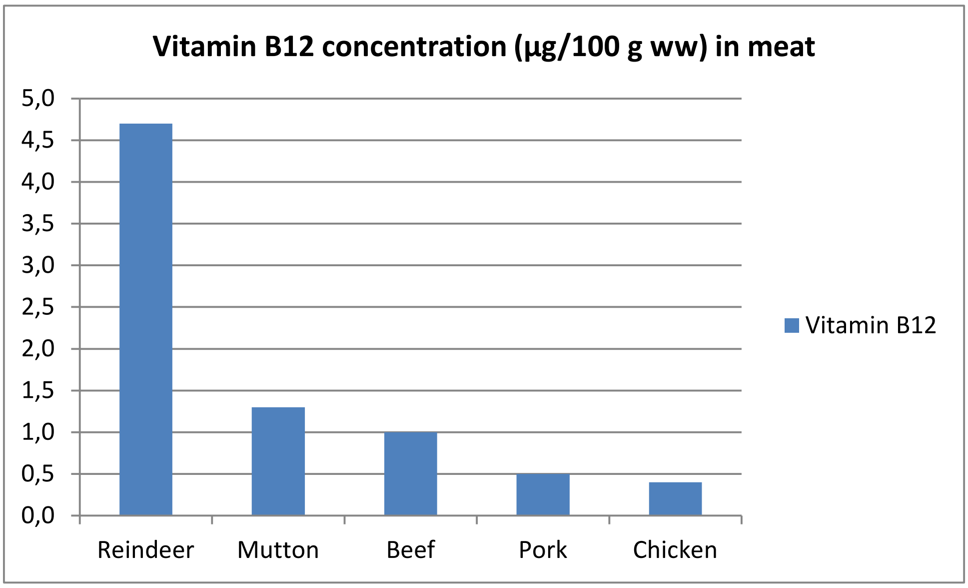
5.1 Arctic dilemma and food safety aspect

Arctic food is healthy and rich in nutrients such as vitamins, essential elements and polyunsaturated fatty acids (n-3). Additionally, it is at the same time prone to environmental pollution. This combination of both positive and negative aspects with some of the local food harvested in the Arctic reflects the issue known as the Arctic dilemma. Aquatic foods have been the main focus. However, high concentrations of toxic elements such as cadmium in liver and kidney from some Arctic terrestrial food animals may point towards inclusion of such animals under this term [49, 53]. Food safety regarding Arctic terrestrial animals has been an issue of concern to the food safety authorities and researchers in the involved countries with most of the focus on cadmium. In Arctic Canada, a recommendation not to eat more than 4-16 caribou livers per person a year was issued [51, 76-78]. The Swedish National Food Administration (SLV) has recommended people not to eat liver from adult reindeer more than 1-2 times a month [53]. In Finland, the Agriculture and Forestry Department (AFD) recommended people not to eat moose liver/kidneys, which is traditionally a common practice among hunters, in addition to the prohibition of selling liver/kidneys from moose older than 1 year [79]. These issued recommendations were based on the maximum level (ML) set by the European Commission (EC) and the provisional tolerable weekly/monthly intakes (PTWI/ PTMI) set by the Joint Expert Committee on Food Additives (JECFA) of the Food and Agricultural Organization (FAO) and Wold Health Organisation (WHO) [68, 69, 80].

5.2 Level of selected nutrients in meat from reindeer and Norwegian domestic animals

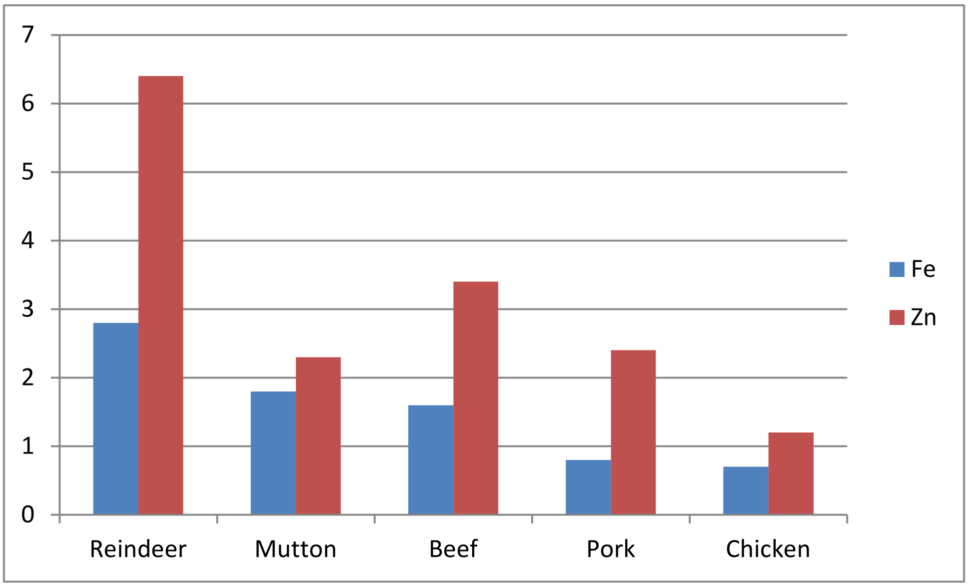
Consumption of reindeer meat in Norway is low compared to that of domestic animals, with the highest consumption among reindeer herders and their families [81]. Despite the low consumption, reindeer meat could contribute significantly to human need for vitamin B12, Fe, Zn and Se when compared to meat from domestic animals (Figures 3, 4 and 5). Iron concentration has previously found to be higher in blood of Sami people compared to ethnic Norwegians and has been related to the consumption of reindeer meat and products [82].

Figure 3. Concentration of vitamin B12 in meat from reindeer and domestic animals



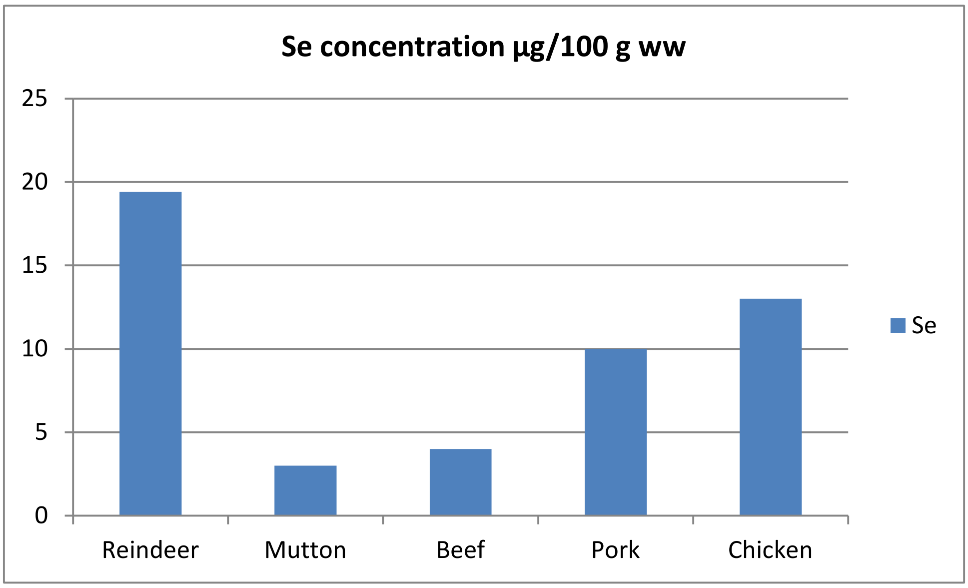
References: Reindeer, mutton, beef, pork and chicken [83, 84].

Figure 4. Iron and zinc concentrations (mg/100g ww) in meat from reindeer and other domestic animals



References: Reindeer, mutton, beef, pork and chicken [83, 84].

Figure 5. Selenium concentration in meat from reindeer and other domestic animals

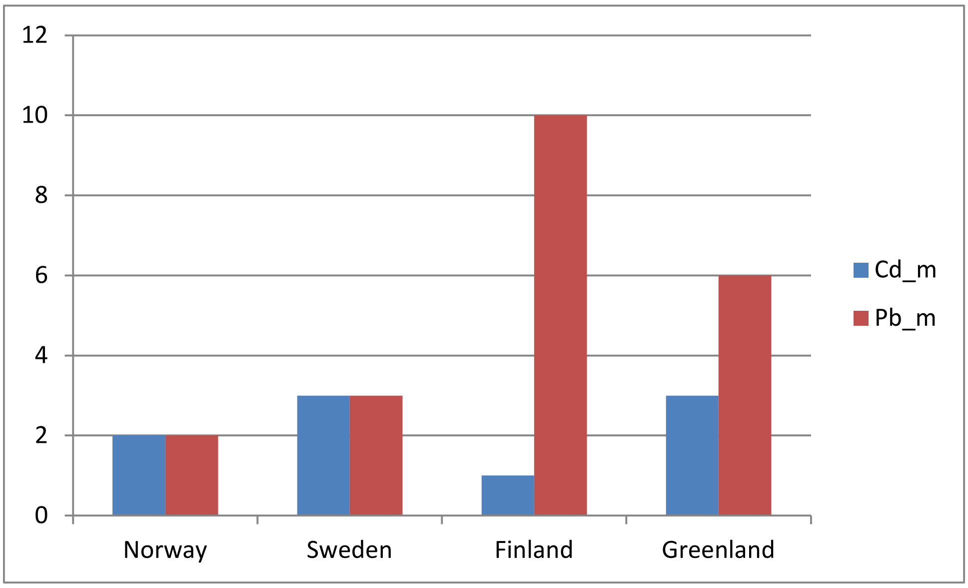


References: Reindeer, mutton, beef, pork and chicken [83, 84].

5.3 Levels of Cd, Pb and As in reindeer

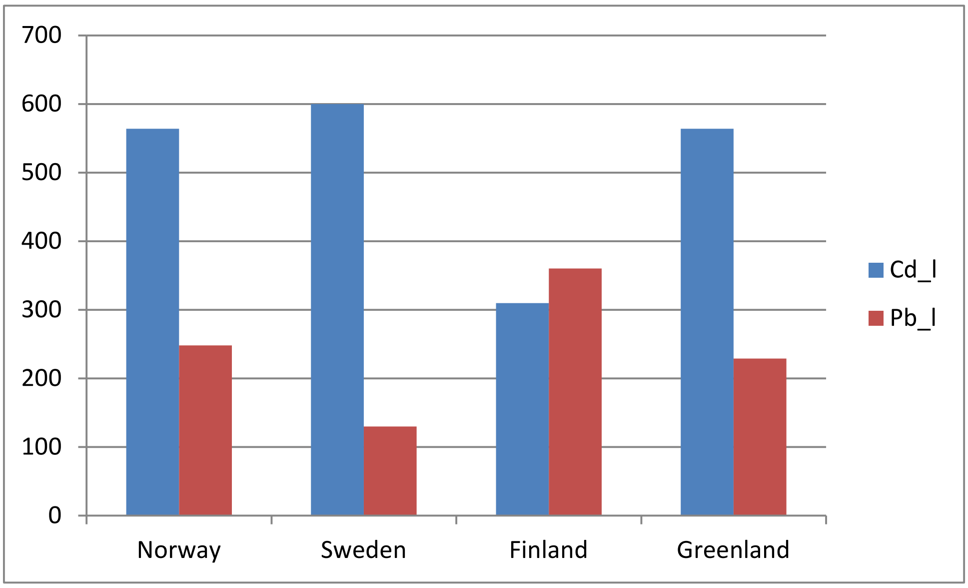
Results referred to as Norway in figures 6-8 are ones from the present study (papers II and III). There is variation in reported level of toxic elements in reindeer between countries as we see in the figures 6, 7 and 8 below. Such variations are expected both within and between countries due to the fact that exposure to toxic elements varies from place to another and over time due to many reasons [45, 85]. Factors such as susceptibility of specific areas to pollution, duration and continuity of exposure are some examples. Furthermore, lichens the main reindeer winter diet have been reported to accumulate such toxic elements [86-89]. Thus, the varying availability of lichens across geography could as well contribute to the explanation of such variations in toxic element concentrations in reindeer.

Figure 6. Levels of cadmium and lead (ng/g ww) in reindeer meat



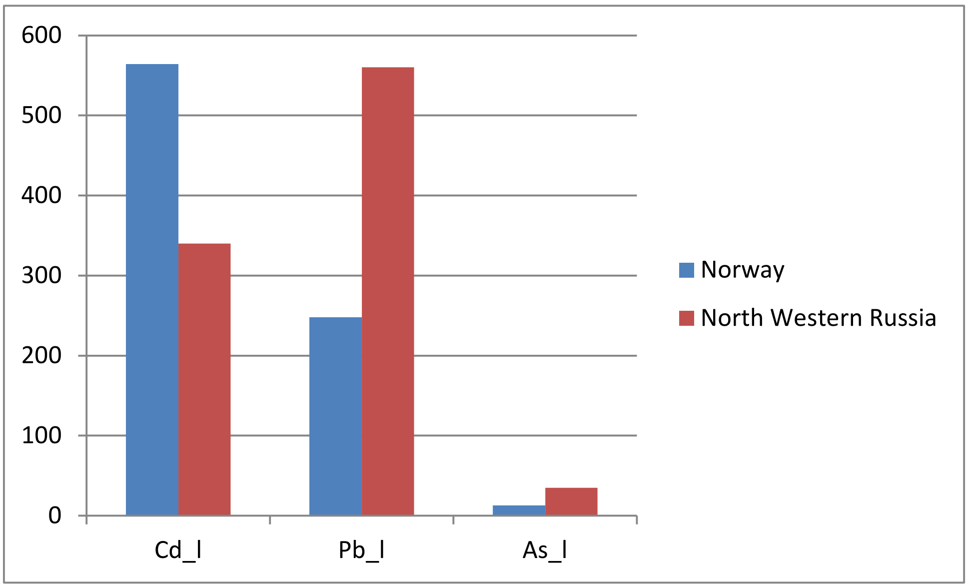
References: Norway, Sweden, Finland and Greenland [50, 52, 54, 90].

Figure 7. Levels of cadmium and lead (ng/g ww) in reindeer liver



References: Norway, Sweden, Finland and Greenland [50, 52, 54, 90].

Figure 8. Levels of cadmium, lead and arsenic (ng/g ww) in liver of Norwegian and Russian reindeer



References: Norway and Russia [90, 91].

5.4 Methodological aspects

Study design

Many methodological problems can be overcome or minimized by proper sample size and study design. The results should always be interpreted in relation to the methodological strengths and weakness of the design. All of the four articles were based on a cross sectional design on population of semi-domesticated reindeer originating from Northern and Mid-Norway.

Challenges of sample collection

Reindeer slaughter season in Norway is once a year and extends from late autumn to early winter the following year. Additionally, small districts have only one delivery (with fewer animals than those delivered from other districts) to slaughterhouse per year and they may reach the slaughterhouse within short time or without prior notice. Besides that, weather conditions that lead to postpone of delivery of reindeer to slaughterhouse (experience of waiting many days above the stated appointment to get samples from animal in the specific districts). These issues are unavoidable, planning-hinder and make fulfilment of sample collection impossible within one year. Due to these reasons, we had to deviate from the protocol regarding age of sampled animal. Samples like bone marrow are difficult to get amount enough for doing the different laboratory analyses included in the project (*e.g.*, fatty acids, vitamins, essential and toxic elements), in spite of the fact that all the four legs of the animal were tried in hope to get the amount needed. This has generated missing values in articles I and II. Missing of laboratory measurements due to inadequate amount of samples is one of the many reasons behind incomplete data [92].

Sample size and chance

Chance is one of the reasons behind the fact that findings are not valid; hence determining whether findings are due to chance or not is an important aspect in statistical analysis. Hypothesis testing is one of the statistical tools used to assess whether findings are real or due to chance and require a clear statement of the hypothesis under testing and formulation of an appropriate null hypothesis [93]. Problems associated with sample size reflect mainly on statistical power and random error [94].

In order to be able to generalize the obtained results, the sample should be representative for the population from which the sample was drawn. The representation could be achieved by statistical calculation for the sample size suitable for the specific study design [95]. In some study designs, the statistical calculation for sample size might not be necessary in case in which results expected to be obtained from the outcome variable are based on metabolic mechanism. An example of this is nutrient levels in healthy humans or animals' body since what has been eaten undergo the same digestion process within the same species. Thus, small sample size could be representative for biological process in the whole species. This is the case in paper I, except for fatty acids in tallow and bone marrow (n=3) in which results may only be indicative and should be interpreted with caution.

Validity

Good procedures for data collection is important in terms of assuring good data quality and is the first step towards drawing a valid conclusion. Validity is divided into internal and external [96]. The internal validity refers to the quality of the methods used in the study and depends on whether chance, bias, measurement errors and confounders are properly controlled for. The external validity, also known as generalizability, refers to whether the obtained results could be generalized to the population/species from which samples were drawn. On the basis of the information that has been provided in the section regarding methodological aspects, we could conclude that the internal validity of this study is satisfying and the results could be generalized to the reindeer populations from which samples were drawn with the limitations being mentioned later on in this section under limitations and weaknesses of the study. However, there might still be point sources we don't know of, although this doesn't seem very likely. Due to the large sample size and broad geographical variation, we consider the external validity of this study as of high quality and we believe the results obtained could be generalized to the semi-domesticated reindeer in the Norwegian reindeer husbandry areas.

Random and systematic errors

Random errors are the ones that would be reduced to zero if a study become infinitely large, while systematic errors remain even if an infinitely large study are involved [97]. The random error is classified into two types; types 1 and 2. The type 1 random error (also known as α-error) is defined as rejecting the null hypothesis when the null hypothesis is true ending up with false positive result, while type 2 (known as β- error) is accepting the null hypothesis when the null hypothesis is not true ending up with false negative result [98].

Bias

Bias is a systematic error in a study and one of the most important problems in epidemiological studies that leads to wrong conclusion and invalid results [96]. Sources of bias can be several. However, we consider selection and measurement bias relevant for our study.

Selection bias

Selection bias results from the procedures used to select subjects and from factors that may influence study participation. It occurs when the study sample is not representative for the total population from which sample is drawn [96]. In all of the four articles, samples from reindeer were randomly selected to avoid selection bias. Animals were not pre marked in the slaughterhouse fence when they were alive, but they were first introduced to the sample collector in the slaughter line as carcasses. The only thing known was that the district from which sample was going to be drawn. Identification of animals were first being made when they reached carcass classification station inside the slaughterhouse, thus animals from different herds within the same district could have same chance of selection and be represented for their district. Moreover, there was no systematic method of carcass selection such as choosing those of 1.5 year with good body scores and leaving out those with poor ones.

Due to limited availability of young animals (1.5 years) in some districts, a number of calves and older animals were chosen; 20% calves and 10% adult animal out of the total of 31 animals (Papers I & II), and 12% calves and 11% adult animal out of the total of 100 animals (Papers III & IV). Thus, percentages of young animals were 70% in paper I & II and 77% in papers III & IV. A Finish study reported that reindeer calves have 7-10% higher vitamin levels than older animals [25, 39]. However, the statistical analyses that were done on vitamin concentrations (Paper IV) from districts with homogenous age group and other with mixed ones did not reveal any significant difference. Thus, presence of calves and older animals is not likely to bias the results on vitamins.

Measurement bias

Measurement bias occurs when measurements of outcomes and/or exposure variables are inaccurate (e.g., defect in the measurement instruments). Effects of measurement error depend mainly on its magnitude and distribution; the bigger and more distributed the error through variables, the more biased result one would get [99].

Measurements error in laboratory analysis may occur due to many factors. Laboratory equipment that is not calibrated gives biased results which may lead to over or under estimation of the outcome variable. Contamination of samples and laboratory tools and equipment during sample collection and/or analysis generates as well measurement error. Microbiological contamination alter contents of nutrient elements in the biological samples due to bacterial activities result in depletion of these nutrients, besides adding substances that may affect the analysis result. Environmental contaminants is also a problem in samples intended to be analysed for essential and toxic elements as some of these contaminants are widely spread in the environment, thus they may add positive false contribution to levels of the studied elements . Samples intended of analyses for essential and toxic elements may also be contaminated by metals in the laboratory equipment and from the dust in the ambient environment. For concentrations close to the limit of detections (LOD), the measurement uncertainty is higher than those with greater distance to the LOD values.

A number of measures have been taken in this study (Papers I-IV) to avoid or minimize measurements error. Samples intended for essential and toxic elements were collected in acid rinsed plastic containers and analysed using non-metallic laboratory tools (crystal glass and plastic). Dust was avoided by conducting the analysis in a closed glass cabinet. Samples intended for vitamin analyses were covered with aluminium foils to avoid light exposure which may lead to oxidation of some vitamins altering their real concentrations in the samples and kept at – 20° C (- 70° C in cases of samples in which vitamin C was analysed) to ensure their stabilities. Blank samples have been analysed in order to check for possible contamination in the laboratory under samples processing.

In papers I-IV, concentrations of toxic elements were given numeric values at halve the limits of detection (LOD), whereas nutrients concentrations were replaced by zero prior to the statistical analyses, in cases in which concentrations were below the LOD. The background of using such practice in toxic elements (LOD/2) was based on the fact that these elements have the ability to accumulate in body tissues and can be found in concentrations lower than those detectable by the available laboratory facilities. There are several methods discussed in literature deal with concentration of environmental contaminants below the LOD prior to application of data analysis [74, 100]. The replacement of nutrient concentrations below the limit of detection by zero was based on the fact that some nutrients are likely to be absent in some food items. Thus, it would be unreasonable giving such nutrients halve value of the detection limits.

Confounding

Confounding is similar to bias and is often confused. Bias involves error in the measurement of a variable whereas confounding involves error in the interpretation of what may be an accurate measurement. In epidemiologic study design, confounding is a central term that refers to the fact that the effect of the exposure is mixed together with the effect of another variable. The confounding variable reported to be causally associated with the outcome and none causally or causally associated with the exposure, but it is not an intermediate variable in the causal pathway between exposure and outcome[96]. This will lead to bias of the results and hence a deceptive conclusion. Stratification of data and adjustment are key issues in confounding problems' solutions. Furthermore, confounding can be controlled by stratified analysis, standardization methods and multivariable analysis in which we enter confounding factors as covariates [101, 102]. Furthermore, randomization was reported to be the best defence against unknown confounders. This is obviously because unknown confounders are much trickier than known ones due to the fact that an apparent association between a risk factor or an intervention and an outcome is always under the risk of being mediated by an unknown confounder.

Numerous confounding factors were considered and adjusted for in this study (Papers III & IV). Age of animals was stratified into three groups; calves (n= 12), young (n= 77) and older animals (n= 11) and sex into; male (n= 52) and female (n= 48). Animal population density was stratified into three groups; low (0.8-1.9), medium (3-5.3) and high (6-13.7) animals/ km^2. Stratified data on concentrations of Cd, Zn and Se were given due to the significant age effect on Cd and Zn, and animal population density effect on Se.

Due to presence of pooled vitamins samples from some districts (n= 4 districts) with mixed age groups (Paper IV), it was not possible to adjust for age directly. Additional statistical analyses were done on districts with homogenous age group and districts with mixed ones in order to investigate the effect of age on vitamin concentrations. No significant effect for age was observed on vitamin concentrations.

5.5 Strengths of the study

This study has several strengths related to both the magnitude of the data, samples and novelty in analyses done. Sample size for papers I and II were comparable to those from most of the previous reindeer studies on nutrients and toxic elements. For papers III and IV, sample size was larger than those previous done on Norwegian reindeer in the same topic.

Inclusion of four biological samples (meat, liver, tallow and bone marrow) and large number of nutrients and toxic elements makes this study unique compared to previous studies. This is besides the inclusion of broad geographical area representing 13 different grazing districts distributed over four different Norwegian counties extended from the middle to the northernmost part of the country (Sør-Trøndelag, Nordland, Troms and Finnmark Counties).

Concentrations of numerous nutrients and toxic elements in the present study (Papers I - IV) were measured for the first time in meat, liver, tallow and bone marrow from reindeer and caribou, in particular those in tallow and bone marrow.

Effect of animal population density on vitamin and essential element concentrations in meat (Paper IV) was studied for the first time not only in semi-domesticated reindeer, but also caribou.

The correlation studies of toxic elements between meat and liver (Paper II) provide important information on how good is liver as indicator for toxic elements in meat.

5.6 Limitations and weaknesses of the study

Sampling is often associated with some challenges regarding edible tissues from semi-domesticated reindeer as stated earlier in this section under challenges of sample collection, which could further result in some limitations and weaknesses of such studies. Laboratory analyses for fatty acids and lipids in tallow and bone marrow (paper I) were based on samples from few animals (n= 3) due to inadequate amount of samples. Therefore, the results on fatty acids and lipids in tallow and bone marrow may only be indicative.

Due to limited availability of young animals (1.5 years) in some districts, a number of calves and older animals were chosen; 20% calves and 10% adult animal out of the total of 31 animals (papers I & II), and 12% calves and 11% adult animal out of the total of 100 animals (Papers III & IV). Thus, percentages of young animals were 70% in paper I & II and 77% in papers III & IV. A Finish study reported that reindeer calves have 7-10% higher vitamin levels than older animals [25, 39]. However, the statistical analyses that were done on vitamin concentrations (Paper IV) from districts with homogenous age group and other with mixed ones did not reveal any significant difference. Thus, presence of calves and older animals is not likely to bias the results on vitamins, but worth mentioning as deviation from the target group (young animals).

Vitamins samples were pooled in the laboratory analysis (Papers I & IV). Pooled samples from some districts had individual samples from mixed age group. This doesn’t allow for direct control of age effect (see also the above mentioned point; age of sampled animals).

Samples for papers I and II were collected in 2004-2005, while those for papers III and IV were collected in 2008-2009. The laboratory analyses for all samples were done in the same year of sample collection.

Data from grazing districts such as quantity/ quality of pasture, distribution and intensity of summer flies are lacking by today. Presence of such data could positively contribute to better explanation of the variations that have been revealed.

6. CONCLUDING REMARKS

Reindeer meat is lean, but a good source of docosapentaenoic acid (DPA), α-linolenic acid (ALA), vitamin B12, Fe, Zn and Se. Moreover, meat contained higher vitamin B12, Fe, Zn and Se concentrations when compared to Norwegian beef, lamb, mutton, pork and chicken meat. Thus, concentrations of the studied nutrients in reindeer meat, liver, tallow and bone marrow contribute significantly to the recommended dietary allowances (RDA) for people who regularly consume reindeer edible tissues even though the amount consumed in each occasion is low.

Liver was the organ that had in most cases the highest nutrient and toxic element concentrations. Significant differences in nutrients and toxic element concentrations between meat and the rest of the studied tissues were revealed. Levels of toxic elements in reindeer were generally low, except for Cd in liver in which 52% of samples had concentrations above the maximum level (ML).

Concentrations of most of the nutrients in meat correlate between meat and the rest of the studied tissues. In concern to toxic elements correlation between liver and meat, the only significant correlations were found for Cd and As. Arsenic concentration in meat was comparable to that of liver. Although Cd concentration was significantly correlated between meat and liver, the concentration in liver was nearly 400 times higher than that of meat. This raises a question about liver as an indicator for toxic elements in meat when only concentration of As is correlated and comparable between liver and meat.

Little geographical differences were observed for nutrients compared to toxic elements, with vitamin E, Se, Cd and As being the ones that demonstrated the largest geographical variations. The observed geographical differences in nutrients and toxic element concentrations will most likely have no impact on consumer.

Animal population density had only significant effect on Se in which animals originating from grazing districts with low population density had on average higher Se concentrations compared to that found in animals from districts with high density.

Concentrations of toxic elements in meat and the rest of the studied tissues were generally low. Despite the high Cd concentration in liver, human exposure to Cd from reindeer liver is considerably low and constitutes 9.2% of the provisional tolerable monthly intake (PTMI) due to the low liver consumption. Therefore, toxic elements in reindeer are not a major contributor to human toxic element body burden.

7. FUTURE PERSPECTIVES

Based on the results obtained from the present study, the following perspectives are recommended in future research:

- Due to the fact that meat is more frequently consumed than liver and most of the toxic elements were not correlated between liver and meat, future assessments for monitoring purposes regarding food safety should possibly be done on meat.
- There is a need of study on predictors of high consumers of reindeer edible tissues in order to broaden the knowledge around reindeer as foodstuff.
- There is a need of study on persistent organic pollutants (POPs), particularly dioxin, in order to get a larger food safety aspect.
- There is need of knowledge regarding the data from grazing districts such as quality/quantity of reindeer summer/ winter pasture and the spread/ intensity of summer insects (*e.g.*, warble flies).
- We suggest that the link between lichens availability and selenium concentration in relation to animal population density is investigated further.

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ERRATA

Thesis: Page 30, subtitle 3.3. Vitamins analyses, lines 6 - 8.

Paper IV: Page 4, subtitle 2.3.1.Vitamins, lines 4 - 6.

The sentence

‘’The vitamin E concentration is composed of all tocopherols (α, β, γ and Δ tocopherols), whereas vitamins A and B3 concentrations refer to retinol and niacin, respectively.’’

Should be changed to

‘’The vitamins A, B3 and E concentrations refer to retinol, niacin and α-tocopherol, respectively.’’

Paper IV: Page 12, second paragraph, lines 1 - 2.

The sentence

‘’Concentration of vitamin E (α, β, γ and Δ tocopherols) detected in the present study (0.5 mg/100 g) was comparable to those previously reported by same authors.’’

Should be changed to

‘’Concentration of vitamin E (α- tocopherol) detected in the present study (0.5 mg/100 g) was comparable to those previously reported by same authors.’’

Paper I

ORIGINAL RESEARCH ARTICLE

Level of selected nutrients in meat, liver, tallow and bone marrow from semi-domesticated reindeer *(Rangifer t. tarandus L.)*

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Objectives: To acquire new knowledge on the nutritional composition of semi-domesticated reindeer *(Rangifer t. tarandus L.)* and their nutritional value for humans. The results could be useful in updating the Norwegian Food Composition Database, whose current data on reindeer is limited.

Study design: Cross-sectional study on population of semi-domesticated reindeer from 2 northern Norwegian counties (Finnmark and Nordland).

Methods: Semi-domesticated reindeer carcasses ($n = 31$) were randomly selected, from which meat, liver, tallow and bone marrow samples were collected. Selected vitamins, minerals, fatty acids and total lipids were studied.

Results: As expected, reindeer meat was found to be lean (2% total lipid), thus it is a good source of low-fat meat. The meat was also found to be a good source of vitamin B12, docosapentaenoic acid (C22:5 n-3) and α-linolenic acid (C18:3 n-3). Statistically significant differences ($p < 0.05$) in most of the nutrient levels between meat and the rest of the studied reindeer tissues were observed. In most cases, the liver, tallow and bone marrow had higher nutritional values when compared to meat. Liver had the highest concentrations of vitamin A, all vitamin B types, vitamin C, iron, selenium and the total amount of polyunsaturated fatty acids (n-3). Additionally, liver was the only edible tissue that contained vita-mins B9 and C. The vast majority of the vitamin concentrations in liver, tallow and bone marrow were significantly correlated with the concentrations in meat ($p < 0.05$).

Conclusions: The studied tissues from reindeer demonstrated that reindeer is a valuable food source that could meet or contribute to the consumers' nutritional recommended daily allowance (RDA).

Keywords: *Nutrients*; *edible tissues*; *reindeer*; *Sami*; *Norway.*

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Data on the meat composition of reindeer and caribou are very limited in comparison to other meat types and are mostly restricted to unpublished data such as local reports. Consequently, the Norwegian Food Composition Database lacks information on some levels for the nutri-tional elements of reindeer meat and meat products (1,2). Semi-domesticated reindeer (*Rangifer t. tarandus L.*) liver, tallow and bone marrow are important nutritional food substances in the traditional Sami diet.

The Sami are an Indigenous people and an ethnic minority in northern Fenno-Scandinavia and the Kola Peninsula in Russia. The greatest proportion of the total Sami population lives in Norway. Semi-domesticated reindeer are a fundamental component in sustaining the Sami culture, as well as an important food substance. The total number of reindeer in Norway is reported to be approximately 243,200, with the highest concentration in Finnmark County, the northernmost county in Norway, which has over 50% of the total amount (3). However, the

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numbers of reindeer and caribou herds are declining across the circumpolar region due to global climate change and modernization (4). The flock structure varies by district, and appears to be dominated by female reindeer at 69–78%, followed by calves at 17–21% and males at 4–11% (3).

Unlike cattle, the semi-domesticated reindeer in Norway primarily graze on a natural pasture throughout the year, of which a large number of species of vascular plants and lichens are the main component (5). However, some supplement feed such as dried hay and pellet concentrates may also sometimes be provided by owners during harsh winters when the natural pasture is inaccessible. Generally speaking, summer grazing is necessary for reindeer, as the green pasture is rich in proteins, minerals and cellulose and provides a reserve that can be used in winters during which carbohydrate-rich lichens are the main type of feed. This also allows reindeer to be in better physical condition in autumn than in spring when most of their reserves are depleted. Seasonal variations and animal feed types were reported to influence the varying nutritional composition of the reindeer meat and other edible tissues (6,7). The movement of reindeer herds through mountains, forests and plains differentiates their grazing habits from other Norwegian ruminants that are fed while indoors in the winter and outdoors on farmyards in the spring and autumn. Norwegian sheep are an exception since their free grazing in summer very much resembles that of the reindeer.

Semi-domesticated reindeer slaughterhouses are small in size and limited in capacity with line speeds varying between 20 and 60 carcasses per hour, depending on the structure of the slaugh-terhouse in comparison to other red meat slaugh-terhouses in Norway. In addition to the stationary slaughterhouses, there are a few mobile ones that contribute to the slaughter of a limited number of reindeer. The majority (69%) of slaughtered reindeer are calves, which are approximately 6–10 months old (3). The reindeer slaughtering season starts in early September and ends in late January of the following year.

Traditional foods for Indigenous people were reported to be the main sources of protein, fat, most minerals, vitamin D and long-chain n-3 fatty acids (8). In general, the nutritional values of meat vary in relation to many factors such as feed types, anatomy, species and animal physiology. The level of minerals, trace elements and vitamin B were reported to be much higher in reindeer meat than in cattle and pig meat (7). Studies on minerals in reindeer and other rumi-nants revealed a higher selenium concentration in the internal organs of reindeer 9–11, and the total lipid content in reindeer meat was reported to be low in comparison to other meat types (7,12,13).

The main purpose of this study was to obtain new knowledge on the nutrient value of semi-domesticated reindeer through the measurement of levels of selected vitamins, minerals, fatty acids and total lipids in the meat, liver, tallow and bone marrow. Our intent was to compare the nutrient value of reindeer meat, liver, tallow and bone marrow with the same from other species. Additionally, we wanted to also compare our results on reindeer meat, liver, tallow and bone marrow to consumers' nutritional recommended daily allowance (RDA). Hopefully, our results will be useful in updating the Norwegian Food Compo-sition Database, whose current data on reindeer is limited.

Materials and methods

Sample collection

Muscle, liver, tallow and bone marrow samples were randomly collected from semi-domesticated reindeer carcasses (n = 31 animals) from 7 districts in Finnmark (n = 6) and Nordland (n = 1) Counties in northern Norway. The selec-tion of the 2 counties was based on the fact that Finnmark has the largest number of semi-domes-ticated reindeer, while Nordland was chosen to provide a sample of the possible geographical variation in the concentration of nutrients. The average age of the reindeer from which the samples were collected from September 2004 to January 2005 was 1.5 years old, according to internal procedures (not published). However, a limited number of calves (approximately 6–10 months old, n = 6) and adult animals (over 2 years old, n = 3) had to be chosen because of a scarcity of slaughtered animals with the age of 1.5 years. Meat samples were collected from the muscles in the dorsal neck region. Liver samples were collected from the main loop, tallow was collected from the fat tissue surrounding kidneys, and bone marrow was collected from the hind and front legs. All of the samples were collected in pre-marked plastic bags immediately after the slaughter/ dressing/carcass weighing process and then divided into different sample glasses/ plastic boxes. Each glass/plastic box was then labelled with the sample type, carcass number, district name/number and date of sample collection. Samples were put on ice (approximately 4°C) immediately after collection and distribu-tion into dedicated containers and kept frozen at −20°C (within 12 hours from the sample collec-tion) until analysis. Samples for vitamin analysis were stored in a −70°C freezer until they were shipped frozen to the laboratory for analysis. Glasses with samples for vitamin analysis were covered with aluminium foil to prevent them from being exposed to light. Hygienic measures were taken during the sample collection to avoid the possible microbiological contamination of samples.

Due to the high cost of vitamin analysis, pooled samples of meat, liver, tallow and bone marrow from the same district (a maximum of 5 animals of mixed age in a pooled sample) were prepared.

Laboratory analyses

The analyses of vitamins were conducted by GBA-Food (Hamburg, Germany) according to methods approved by the German Food Act LMBG § 35, LFGB § 64 and the standard methods of the Association of Official Analytical Chemists (14,15). The laboratory is accredited with the methods used in the analyses according to Staatliche Akktreditierungsstelle Hanover, AKS-P-20213-EU.

For mineral analyses, the meat and internal organs of reindeer were separately digested using a microwave oven technique. In short, concentrated supra-pure HNO3 (5 ml) and H2O2 (3 ml) were added to the sample (0.6–0.7 g) before undergoing the microwave oven treatment. Hence, the following temperature regimes were used in the microwave: 20–50°C (5 min.), 50–100°C (10 min.), 100–180°C (5 min.) and 180°C (15 min.). After cooling down the heated decomposed sample, the solution was diluted to 50 ml. The sample solution was analysed using an inductively coupled plasma high-resolution mass spectrometer (ICP-HRMS), Thermo Scientific Finnigan Element-2, Germany. All standards and calibration solutions contained 1 ppb Rhenium (Re) as an internal standard and 1% nitric acid (HNO3). The calibration curve was verified by use of a standard quality control (QC) sample, National Institute of Standards and Technology (NIST), USA. The resolutions used for minerals were low (at 10) for (Zn), middle (at 20) for (Ca, Fe), and high (at 30) for (Se). The lens adjustment was optimized daily to ensure maximum intensity and top separation.

Precautionary measures, such as the use of closed cabinet, non-metal sampling devices, tools and containers, were taken when preparing the decomposed samples to avoid contamination by dust or from mineral alloys in laboratory tools.

The analyses of fatty acids and total lipids were undertaken by Unilab Analyse A/S in the Fram Centre, Tromsø, Norway, according to a method for the isolation and purification of total lipids from animal tissues by Folch et al. (16). The laboratory is accredited for the methods used in the analyses according to the European standard NS-EN ISO/IEC 17025. Fatty acids are described by a shorthand nomenclature of chain length (number of carbon atoms): the number of double bonds and n-x which indicate the position of the last double bond related to the terminal methyl end. Additionally, common fatty acids names are used in polyunsaturated fatty acids.

Statistical analyses

Results were presented as mean values ± standard deviation (SD). The data set was entered in Excel® 2003 for Windows. Analytical results for vita-mins, minerals and fatty acids below the limits of detection (LOD) were replaced by zero, with the data then transferred to Stata/SE 11.0 for Windows (Stata Corp. College Station, TX) for further statistical analyses. Summary statistics was used to determine the mean, SD, minimum and maximum values. A dependent-sample t-test was used to test differences in the concen-trations of nutrient concentrations between meat and the other studied tissues. The differ-ences in fatty acid concentrations were only tested between meat and liver due to a small number of observations ($n = 3$) for tallow and bone marrow. A Pearson's correlation test was used to test for possible statistically significant correlations of nutrient concentrations between meat and the other studied tissues. The level of statistical significance was set at $p < 0.05$ for all performed analyses.

Ethics

The study did not include any living animals, did not have any adverse environmental health effects, with samples collected from reindeers that had been slaughtered for human consump-tion. Animals were fixed prior to slaughter, made unconscious using a bolt pistol and put down under the inspection of an official veterinarian according to Norwegian regulations on animal welfare in slaughterhouses (17).

RESULTS

Vitamins

Mean vitamin concentrations (per 100 g edible raw tissue) in meat, liver, tallow and bone marrow are shown in Table I.

Vitamins D2 and D3 were neither detected in meat nor in any of the studied tissues. Vitamins B9 and C were only detected in the liver, but as expected the liver had the highest concentration of vitamin B9 in relation to the meat, tallow and bone marrow. Vitamin A was the only vitamin found to reveal a large variation in concentrations among the 4 studied edible raw tissues (Table I), although the variation was harmonic in the districts from where the samples were collected (data not shown). The meat, liver and tallow samples had the same mean concentration of 0.48 μg/100g for vitamin E, while bone marrow had a 4 times higher ($p < 0.01$) concentration for this vitamin.

The majority of vitamin concentrations in liver, tallow and bone marrow were signifi-cantly correlated with concentrations in meat as shown: Vitamin A: meat-liver ($r = -0.95$, $p < 0.01$) and meat-tallow ($r = -0.90$, $p < 0.01$). Vitamin B1: meat-liver ($r = 0.99$, $p < 0.01$), meat-tallow ($r = 0.55$, $p < 0.05$) and meat-bone marrow ($r = 1.0$, $p < 0.01$). Vitamin B2: meat-bone marrow ($r = 1.0$, $p < 0.01$). Vitamin B3: meat-tallow ($r = 0.99$, $p < 0.01$). Vitamin B5: meat-tallow ($r = 0.90$, $p < 0.01$)

Table I. Mean vitamin concentrations in meat, liver, tallow and bone marrow of reindeer per 100 g of edible raw tissue

Vitamin	Meat (n = 20) Mean ± SD	Liver (n = 20) Mean ± SD	Tallow (n = 15) Mean ± SD	Bone marrow (n = 10) Mean ± SD	RDA[c] Female	Male	
Vitamin A (RAE)	19.93 ± 22.55	20915.5 ± 5310.22**	117 ± 22.98**	45.25 ± 47.70	700	900	RAE[b]
Vitamin B1 (mg)	0.09 ± 0.07	0.33 ± 0.30**	0.03 ± 0.02**	0.02 ± 0.02**	1.1	1.4	mg
Vitamin B2 (mg)	0.26 ± 0.07	2.63 ± 0.15**	0.15 ± 0.04**	0.51 ± 0.46*	1.3	1.7	mg
Vitamin B3 (mg)	4.28 ± 0.51	14.9 ± 4.77**	1.67 ± 1.22 **	n.d.[a] (< 0.2 mg)	15	19	mg
Vitamin B5 (mg)	1.14 ± 1.23	5.85 ± 1.25**	0.41 ± 0.36**	0.18 ± 0.03**	–	–	
Vitamin B6 (mg)	0.19 ± 0.04	0.53 ± 0.08**	0.02 ± 0.03**	n.d.[a] (< 0.05 µg)	1.2	1.6	mg
Vitamin B7 (µg)	1.23 ± 1.03	19.45 ± 7.88**	4.3 ± 3.48**	0.29 ± 0.31*	–	–	
Vitamin B9 (µg)	n.d.[a] (< 2 µg)	302.78 ± 184.52	n.d.[a] (< 2 µg)	n.d.[a] (< 2 µg)	300	300	µg
Vitamin B12 (µg)	3.34 ± 1.83	161.73 ± 48.48**	2.23 ± 1.64**	1.24 ± 0.59*	2	2	µg
Vitamin C (mg)	n.d.[a] (< 0.1 µg)	11.88 ± 12.93	n.d.[a] (< 0.1 µg)	n.d.[a] (< 0.1 µg)	75	75	mg
Vitamin D2 (µg)	n.d.[a] (< 0.5 µg)	n.d.[a] (< 0.5 µg)	n.d.[a] (< 0.1 µg)	n.d.[a] (< 0.1 µg)	7.5	7.5	µg
Vitamin D3 (µg)	n.d.[a] (< 0.5 µg)	n.d.[a] (< 0.5 µg)	n.d.[a] (< 0.5 µg)	n.d.[a] (< 0.5 µg)			
Vitamin E (mg)	0.48 ± 0.30	0.48 ± 0.20	0.46 ± 0.07	2.25 ± 0.47**	8	10	α-TE

[a]n.d. = Not detected (below the limit of detection).
[b]RAE = Retinol activity equivalent (µg).
[c]Recommended daily allowance (RDA) for adult males and females based on Nordic nutritional recommendations (28).
α-TE = α-Tocopherol equivalent (mg).
*Concentrations were significantly different from those in meat ($p < 0.05$).
**Concentrations were significantly different from those in meat ($p < 0.01$).

and meat-bone marrow ($r = 1.0$, $p < 0.01$). Vitamin B6: meat-liver ($r = -0.57$, $p < 0.01$) and meat tallow ($r = -0.60$, $p < 0.05$). Vitamin B7: meat-liver ($r = -0.52$, $p < 0.05$) and meat-bone marrow ($r = 1.0$, $p < 0.01$). Vitamin B12: meat-liver ($r = 0.84$, $p < 0.01$), meat-tallow ($r = 0.93$, $p < 0.01$) and meat-bone marrow.

Minerals

Table II shows the mean mineral concentrations of µg/100 g for Se and mg/100 g of edible raw tissue for Ca, Fe and Zn.

The highest Ca concentration of 339.70 ± 277.30 mg/100 g was found in bone marrow ($p < 0.01$). The Ca concentrations in meat and tallow were comparable (5.40 ± 1.0 and 5.10 ± 2.10 mg/100 g, respectively). Liver appeared to be the best source of Fe in terms of mean concentration at 41.10 ± 21.70 mg/100 g ($p < 0.01$). There were no differences in the Fe concentrations of meat, tallow and bone marrow ($p = 0.25$), with the following concentrations: 3.30 ± 0.70, 4.10 ± 1.50 and 3.1 ± 1.30 mg/100 g. Liver also had the highest Se content of 48.70 ± 48.90 µg/100 g ($p < 0.01$). The Se concentrations in meat, liver, tallow and bone marrow (Table II) were characterized by varia-tions among districts and from animals within the same district (results not shown), with one animal demonstrating a remarkably high Se concen-tration (data not shown). These variations have resulted in standard deviations similar or higher than the mean concentration. The Zn in liver was the only mineral that was significantly correlated with meat ($r = 0.77$, $p < 0.01$).

Fatty acids and total lipids

Fatty acids and total lipids concentrations were given in g/100 g edible tissue. The concentra-tions of saturated fatty acids (SFA), monounsat-urated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are shown in

Table II. Mean mineral concentrations per 100 g of edible raw tissue

Element	Mean ± SD Meat (n = 29)	Liver (n = 29)	Tallow (n = 15)	Bone marrow (n = 9)	RDA[a] Female	Male
Zn (mg)	6.8 ± 0.2	3.5 ± 1.7*	0.2 ± 0.1*	0.1 ± 0.1*	7 mg	9 mg
Ca (mg)	5.4 ± 1.0	6.4 ± 1.1*	5.1 ± 2.1	339.7 ± 277.3*	1 g	1 g
Fe (mg)	3.3 ± 0.7	41.1 ± 21.7*	4.1 ± 1.5	3.1 ± 1.3	15 mg	9 mg
Se (µg)	3.0 ± 3.2	48.7 ± 48.9*	0.8 ± 1.6	0.2 ± 0.5	40 µg	50 µg

[a]Recommended daily allowance (RDA) for adult males and females based on Nordic nutritional recommendations (28), the RDA for Ca is based on data from the USDA (29).
*Concentrations were significantly different from those in meat ($p < 0.01$).

Table III. Mean fatty acids concentration of reindeer meat, liver, tallow and bone marrow in 100g of edible raw tissue

Fatty acids	Mean ± SD Meat (n = 28)	Liver (n = 30)	Tallow (n = 3)	Bone marrow (n = 3)
Saturated (SFA)				
C14:0	0.02 ± 0.01	0.01 ± 0.001**	0.78 ± 0.38	0.68 ± 0.28
C15:0	0.01 ± 0.001	0.01 ± 0.002	0.07 ± 0.02	0.07 ± 0.02
C16:0	0.26 ± 0.10	0.80 ± 0.21**	15.57 ± 1.99	14.84 ± 1.65
C17:0	0.01 ± 0.01	0.05 ± 0.02**	0.89 ± 0.11	0.35 ± 0.06
C18:0	0.23 ± 0.05	0.81 ± 0.08**	19.26 ± 1.61	3.22 ± 0.46
C20:0	1.31 ± 0.003	0.01 ± 0.003**	0.22 ± 0.06	0.06 ± 0.01
C22:0	0.002 ± 0.001	0.01 ± 0.001**	0.02 ± 0.01	0.01 ± 0.001
C24:0	0.001 ± 0.001	0.01 ± 0.001**	0.02 ± 0.02	0.01 ± 0.001
ΣSFA	1.84 ± 0.16	1.62 ± 0.23	36.83 ± 3.66	19.24 ± 2.42
Monounsaturated (MUFA)				
C14:1	0.002 ± 0.001[a]	0.003 ± 0.002[a]	0.06 ± 0.03	0.94 ± 0.20
C14:1 n-5	0.002 ± 0.001[a]	0.001 ± 0.002[a]	–	–
C16:1 n-5	0.002 ± 0.001	0.003 ± 0.001	0.04 ± 0.01	0.31 ± 0.08
C16:1 n-7	0.02 ± 0.01	0.03 ± 0.02**	0.65 ± 0.12	7.54 ± 2.18
C16:1 n-9	0.01 ± 0.002	0.02 ± 0.01**	0.33 ± 0.04	0.19 ± 0.01
C17:1	0.003 ± 0.002	0.01 ± 0.004*	0.20 ± 0.02	0.52 ± 0.03
C18:1 n-7	0.01 ± 0.003	0.03 ± 0.01**	0.46 ± 0.06	1.99 ± 0.80
C18:1 n-9	0.37 ± 0.12	0.91 ± 0.30**	31.70 ± 3.48	35.44 ± 1.15
C20:1 n-7	0.004 ± 0.001	0.001 ± 0.001*	0.02 ± 0.01	0.02 ± 0.01
C20:1 n-9	0.002 ± 0.001	0.01 ± 0.002**	0.15 ± 0.05	0.13 ± 0.02
C21:1 n-11	0.003 ± 0.001[a]	n.d.	0.003 ± 0.001	–
C22:1 n-7	0.001 ± 0.0002	0.001 ± 0.001	n.d.	n.d.
C22:1 n-9	0.001 ± 0.0001[a]	0.0002 ± 0.0002*	n.d.	n.d.
C22:1 n-11	0.003 ± 0.001	0.002 ± 0.001**	0.003 ± 0.001	0.01 ± 0.01
C24:1 n-9	0.001 ± 0.0003	0.002 ± 0.001**	n.d.	n.d.
ΣMUFA	0.43 ± 1.14	1.02 ± 0.35	33.62 ± 3.83	47.10 ± 4.48
Polyunsaturated (PUFA)				
C16:2 n-7	0.0002 ± 0.0002	0.0002 ± 0.0004	0.01 ± 0.01	0.01 ± 0.004
C16:3 n-4	0.001 ± 0.001	0.003 ± 0.001**	n.d.	n.d.
C16:4 n-1	0.01 ± 0.004	0.02 ± 0.01	0.36 ± 0.07	0.33 ± 0.06
C18:3 n-3	0.01 ± 0.004	0.03 ± 0.01	0.04 ± 0.03	0.04 ± 0.01
C18:4 n-3	0.002 ± 0.001	0.003 ± 0.003**	0.02 ± 0.001	0.03 ± 0.02
C18:5 n-3	0.001 ± 0.001	0.01 ± 0.01*	0.03 ± 0.01	0.04 ± 0.02
C20:3 n-3	0.001 ± 0.0004	0.01 ± 0.003**	0.002 ± 0.002	0.01 ± 0.01
C20:4 n-3	0.001 ± 0.0002	0.002 ± 0.001**	0.01 ± 0.002	0.01 ± 0.003
C20:5 n-3	0.01 ± 0.004	0.03 ± 0.01	0.01 ± 0.002	0.01 ± 0.01

Table III (Continued)

Fatty acids	Mean ± SD Meat (n = 28)	Liver (n = 30)	Tallow (n = 3)	Bone marrow (n = 3)
C22:5 n-3	0.04 ± 0.01	0.26 ± 0.09**	0.02 ± 0.01	0.03 ± 0.01
C22:6 n-3	0.003 ± 0.001	0.05 ± 0.02**	0.003 ± 0.001	n.d.
C18:2 n-6	0.15 ± 0.05	0.36 ± 0.06**	0.82 ± 0.19	0.67 ± 0.18
C18:3 n-6	0.0002 ± 0.0001	0.0002 ± 0.001	0.004 ± 0.001	0.01 ± 0.004
C20:2 n-6	0.002 ± 0.001	0.01 ± 0.002**	n.d.	n.d.
C20:3 n-6	0.01 ± 0.003	0.02 ± 0.01	0.01 ± 0.001	0.01 ± 0.002
C20:4 n-6	0.09 ± 0.04	0.42 ± 0.07**	0.11 ± 0.03	0.12 ± 0.04
C22:2 n-6	n.d.	n.d.	n.d.	n.d.
C22:4 n-6	0.01 ± 0.004	0.07 ± 0.02**	0.01 ± 0.002	0.004 ± 0.002
C22:5 n-6	0.0001 ± 0.0003	0.001 ± 0.001	0.01 ± 0.01	0.02 ± 0.01
ΣPUFA	0.34 ± 0.11	1.30 ± 0.17	1.47 ± 0.33	1.34 ± 0.14
ΣPUFA n-3	0.07 ± 0.02	0.40 ± 0.10	0.14 ± 0.05	0.17 ± 0.05
ΣPUFA n-6	0.26 ± 0.09	0.88 ± 0.12	0.96 ± 0.21	0.84 ± 0.14
ΣPUFA n-6/ ΣPUFA n-3	3.71 ± 0.003	2.20 ± 0.004	6.86 ± 0.10	4.94 ± 0.15

[a]Observations less than those given in the main column: Meat (n = 14) and liver (n = 17) for C14:1. Meat (n = 14) and liver (n = 13) for C14:1 n-5. Meat (n = 14) and liver (n = 13) for C14:1 n-5. Meat (n = 27) for C21:1 n-11. Meat (n = 27) for C22:1 n-9.
n.d = Not detected.
*Concentrations were significantly different from those in meat ($p < 0.05$).
**Concentrations were significantly different from those in meat ($p < 0.01$).

Table III. Eight polyunsaturated fatty acids n-3 (PUFA n-3) were detected in the meat, liver and tallow, while 7 were detected in bone marrow.

Docosapentaenoic acid, DPA (C22:5 n-3) and α-linolenic acid, as well as ALA (C18:3 n-3), were detected in all studied reindeer tissues and had the highest concentrations among PUFA n-3 (Table III). Meat had the lowest concentration for PUFA n-3 among all the sample types.

Seven types of PUFA n-6 were found in the meat and liver, while 6 were found in the tallow and bone marrow. The highest concentration of PUFA n-6 was 0.15 ± 0.05 g/100 g in meat for linoleic acid (C18:2 n-6), followed by 0.42 ± 0.07 g/100 g in liver for arachidonic acid, AA (C20:4 n-6). Tallow and bone marrow had the lowest PUFA n-6 concentrations. The eicosapentaenoic acid, EPA (C20:5 n-3) concentrations in meat, liver, tallow and bone marrow were 0.01 ± 0.004, 0.03 ± 0.01, 0.01 ± 0.002 and 0.01 ± 0.01g/100 g, respectively. However, the docosahexaenoic acid, DHA (C22:6 n-3) concentrations in meat, liver and tallow were 0.003 ± 0.001, 0.05 ± 0.02 and 0.003 ± 0.001 g/100 g, respectively. The DHA was not detected in bone marrow samples. The ratios of n-6 to n-3 PUFA in meat, liver, tallow and bone marrow were 3.71 ± 0.003, 2.20 ± 0.004, 6.86 ± 0.10 and 4.94 ± 0.15 g/100 g, respectively.

The mean percentages (g/100 g) of the total lipid content in meat, liver, tallow and bone marrow were 2 ± 0.84 ($n = 27$), 5.64 ± 0.88 ($n = 31$), 78.89 ± 6.84 ($n = 3$) and 71.79 ± 15.99 ($n = 3$), respectively.

As expected, the results from Tables I, II and III revealed considerable differences in nutrient levels between the meat and the rest of the studied tissues. These differences were statistically signifi-cant ($p < 0.05$), except for the Se and Fe in tallow and bone marrow, the total lipid in tallow and bone marrow and the fatty acids C14:1, C14:1 n-5, C16:1 n-5, C16:1 n-7, C16:2 n-7, C16:4 n-1, C18:3 n-3, C18:3 n-6, C20:3 n-6, C20:5 n-3, C22:1 n-7, C22:5 n-6 in the meat and liver. In most cases, the liver, tallow and bone marrow had higher nutrient levels in comparison to meat ($p < 0.05$).

Significant correlations in fatty acid concentrations between meat and the other studied tissues were only detected between the meat and liver. The fatty acids that were significantly corre-lated were C16:3 n-4 ($r = -0.43$, $p < 0.05$), C18:1 n-7 ($r = 0.78$, $p < 0.01$), C18:2 n-6 ($r = 0.45$, $p < 0.05$), C22:4 n-6 ($r = 0.44$, $p < 0.05$) and C22:5 n-3 ($r = 0.48$, $p < 0.01$). No significant correlation was detected for total lipid content between the meat and liver. Moreover, the correlation was not tested between meat-tallow and meat-bone marrow due to the small number of animals ($n = 3$) included in the total lipid analysis of tallow and bone marrow.

Discussion

Meat from the semi-domesticated reindeer is lean and a good source of vitamin B12, docosapentaenoic acid (C22:5 n-3) and α-linolenic acid (C18:3 n-3). Statistically significant differences ($p < 0.05$) were observed in most of the nutrient levels between meat and the rest of the studied tissues. Liver had the highest concentrations of vitamin A, all vitamin B types, vitamin C, iron, selenium and the total amount of polyunsaturated fatty acids (n-3).

Additionally, liver was the only edible tissue that contained vitamins B9 and C. The vast majority of the vitamin concentrations in liver, tallow and bone marrow were significantly corre-lated with the concentrations in meat ($p < 0.05$). To the best of the authors' knowledge, this study is unique and the first of its kind to include a relatively large number of animals (31 reindeer) and to study nutrient concentrations from the meat, liver, tallow and bone marrow of semi-domesti-cated reindeer in Norway.

The mean concentration in the meat for vitamin B3 in this study is in agreement with Rastas (18). Still, our concentrations of vitamins B1, B2 and E were half the values in the referred study, whereas the vitamin A concentration was 3 times higher. Vitamins B9 and C in meat were not detected in our study, though their respective mean concentration ($n = 24$) has been formerly reported to be 2.6 and 3.3 μg/100 g (7). In agree-ment with a study by Nieminen (7), the meat and liver samples exhibited a similar mean value for vitamin E. However, concentrations reported in the same study for vitamins B1, B2 and B3 in meat were slightly higher with an exception for vitamin A, which was 3 times higher in our study. The liver contained higher vitamin concentrations than the meat, with the exception of vitamin E, which had a similar concentration in both sample types. The liver of reindeer, pork and cattle was also reported to be much richer in vitamins compared to meat (7). Although liver is rich in vitamin B9 (folic acid), it is difficult to find the entire amount in raw tissue when heated since cooking food at a high heat will reduce or demolish the amount of vitamin B9. Concentrations of vitamins B2, B3, B6 and B9 in caribou meat were reported to be 1.14 mg, 10.91 mg, 0.47 mg and 11.80 μg/100 g, respectively, while the concentrations of these vitamins in liver were found to be 1.58 mg, 15.64 mg, 1.97 mg and 374.30 μg/100 g, respectively. Furthermore, vitamin B2 and B6 concentrations in bone marrow have been reported to be 0.30 mg and 0.09 mg/100 g, respectively (19).

In one of the districts from which the samples were collected, vitamin A concentrations in meat and internal organs were much higher than in the other districts. The large variation in vitamin A in one district resulted in standard deviation that was higher than the mean concentrations in meat and bone marrow. Nonetheless, the variation in the vitamin A concentration in meat, liver, tallow and bone marrow in this district was harmonic, that is, it followed the same pattern for vitamin A in other districts in which the concentrations were the highest in liver, followed by tallow, bone marrow and meat.

The vitamin A mean concentrations in meat and liver were 19.93±22.55 and 20915.50±5310.22 RAE/100 g, respectively, in our study, whereas Nieminen's respective concentrations were 6.60 and 26000 RAE/100 g (7). The vitamin E concen-trations for meat, liver and tallow of caribou were found to be 0.15, 12.64 and 0.68 mg/100 g, respectively, while the respective vitamin A concentrations were 0.15, 12.64 and 0.68 mg/100 g (20). The concentration of vitamin D in our study was below the detection limits of all sample types. However, its concentration of 1.40 μg/100 g was only detected in the liver of caribou (20). The vitamin C concentration in the meat and liver of caribou were reported to be 0.86 and 23.76 mg/100 g, respectively (21).

The Se concentrations in meat, liver, tallow and bone marrow (Table II) were characterized by variations among both districts and in animals within the same district (results not shown). One animal had a remarkably high Se concentra-tion (data not presented). These variations have resulted in standard deviations which are similar or higher than the mean concentrations. The meat Se concentration in this study was 12.5% lower than the value of 24.0 μg/100 g reported elsewhere (7,18), whereas the liver Se concen-tration in our study was 5 times higher than the value of 10.0 μg/100 g reported by Fediuk et al. (21). The concentrations of meat and liver Ca, Zn and Fe in this study are comparable with those reported in previous studies on reindeer and caribou (7,18,21,22).

Even though most of our results on fatty acids in reindeer meat were 34% to 68% lower than those reported by Sampels et al. (12), the concentrations of docosapentaenoic acid, DPA (C22:5 n-3) and adrenic acid (C22:4 n-6) were in accordance with the same reference. Compared to Sampels et al. (12), the low results on fatty acids in the present study may be explained in part by the fact that the reindeer used by Sampels et al. were calves (about 10 months) that had been fed a pelleted feed mixture for two months prior to slaughter. The concentrations of fatty acids C18:2 n-6, C18:3 n-6, C18:3 n-3 and C18:4 n-3 in reindeer bone marrow reported by Soppela and Nieminen (23) were 2 to 19 times higher than those reported in our study, while the fatty acid C20:4 n-6 was twice as high in our study. Moreover, ΣPUFA n-3 and n-6 in bone marrow reported by Soppela and Nieminen (23) were twice as high than those found in our study.

It is a well-established fact that aquatic animals have higher concentrations of PUFA n-3 compared to terrestrial ones. Nevertheless, the PUFA n-3 concentrations of DPA and ALA in reindeer meat from this study are comparable to those formerly reported for DPA and ALA in crab, scampi, mussels and oysters and DPA in code (24). A study on turkey meat reported a DHA concentration comparable with our results for reindeer meat, whereas the concentrations of DPA and ALA were lower in turkey meat (25). Species, animal diet, environmental and genetic factors were reported to affect the fatty acid composition of meats (26).

The results for the low total lipid percentage in the present study from reindeer meat is in accordance with previous reindeer and caribou studies (7,12,13,21,22). With its lower fat content compared to domestic ruminants, this seems to indicate that reindeer meat can be considered an excellent source in meeting the consumer demand for low-fat meat. The total lipid percentage of rein-deer liver measured in this study is 5.64%, though the total lipid percentages in the liver of reindeer and caribou have formerly been reported to be 4% and 3%, respectively (7,21). The total meat lipid of wild ruminants was reported to be lower than that for domestic ruminants (27). Moreover, the total meat lipids in chickens and calves are comparable to those of reindeer, while turkey meat was reported to have a lower total meat lipid percentage (25).

Based on Nordic nutritional recommenda-tions (28), the concentration of nutrients in semi-domesticated reindeer meat, liver, tallow and bone marrow could meet or contribute to the recommended daily allowance (RDA), see Tables I, II, III. In addition, a ratio of n-6 to n-3 polyunsaturated fatty acids between 3 and 9 in the diet is considered to be sufficient in meeting the RDA (28). The ratios of ΣPUFA n-6/ ΣPUFA n-3 obtained from our study on meat, tallow and bone marrow fell between 3.71 and 6.87. The calculations for the contribution to the RDA done in this study were based on raw tissues; therefore, it is important to consider the impact of cooking since cooking will have an effect on the nutrient content.

The overview for the concentrations of some of the nutrients on reindeer meat and liver from this study, as well as the nutrient concentrations for the other meat and liver types presented in the Norwegian Food Composition Database (1), is shown in Table IV and V. In a similar manner, an overview of the nutrient concentrations on rein-deer meat from our study and the meat of other cervides (e.g. moose and roe deer) presented in the United States National Nutrient Database for Standard Reference (29) are listed in Table VI. Such overviews should be put in a nutritional context in terms of which meat type may be the best source for the specific nutrient, rather than in a comparative one. Species, animal diet, physi-ological and methodological variations need to be taken into consideration. The

Table IV. Mean vitamins, minerals and total lipid concentrations of reindeer meat and other meat types.

			Data from the Norwegian Foods Database[b]				
Nutrient*	Reindeer[a]	95%CI[a]	Lamb	Beef	Calves	Pork	Chicken
Vitamin A (RAE)	19.93	9.37–30.47	7.00	7.00	–	4.00	**11.00**
Vitamin B1 (mg)	0.09	0.05–0.12	**0.09**	0.03	**0.09**	0.42	0.14
Vitamin B2 (mg)	0.26	0.22–0.29	0.15	0.11	**0.22**	0.15	0.18
Vitamin B3 (mg)	4.28	4.04–4.51	6.00	4.10	5.10	5.10	7.80
Vitamin B5 (mg)	1.14	0.56–1.72	–	–	–	–	–
Vitamin B6 (mg)	0.19	0.17–0.21	0.16	0.15	0.40	0.23	0.38
Vitamin B7 (μg)	1.23	0.75–1.71	–	–	–	–	–
Vitamin B9 (μg)	<2.00	–	1.00	3.00	5.00	5.00	11.00
Vitamin B12 (μg)	3.34	2.48–4.20	1.30	1.00	1.30	0.50	0.40
Vitamin E (mg)	0.48	0.34–0.62	0.20	0.20	0.20	**0.60**	0.20
Zinc (mg)	6.80	5.90–7.71	2.30	3.40	4.30	2.40	1.20
Calcium (mg)	5.40	5.02–5.75	10.00	7.00	12.00	6.00	6.00
Iron (mg)	3.30	2.99–3.52	1.80	1.60	1.50	0.80	0.70
Selenium (μg)	3.00	1.79–4.28	**3.00**	**4.00**	6.00	10.00	13.00
Total lipid %	2.00	1.67–2.33	17.10	9.00	3.00	15.50	**2.10**

*Vitamins C, D2 and D3 in reindeer were below the limit of detections (LOD); their values in other animals were not given by the referred database (1).
[a]Results on reindeer meat from this study.
[b]Results based on data from the Norwegian Food Composition Database (1).
– = Not given.
Note: Values in **bold** are the ones that fall within a 95% CI for mean nutrients values of reindeer meat in this study.

Table V. Mean vitamins, minerals and total lipid concentrations per 100 g of raw reindeer liver and other raw liver types

			Data from the NFC Database[b]			
Nutrient	Reindeer[a]	95% CI[a]	Lamb	Cattle	Pig	Chicken
Vitamin A (RAE)	20915.50	18430.24–23400.76	32760	**23220**	23580	9702
Vitamin B1 (mg)	0.33	0.18–0.47	**0.39**	**0.30**	**0.43**	0.63
Vitamin B2 (mg)	2.63	2.55–2.70	3.49	2.79	2.86	3.40
Vitamin B3 (mg)	14.90	12.67–17.13	**14.00**	10.30	11.00	9.20
Vitamin B5 (mg)	5.85	5.26–6.44	–	–	–	–
Vitamin B6 (mg)	0.53	0.49–0.57	**0.53**	0.74	0.64	0.80
Vitamin B7 (μg)	19.45	15.76–23.14	–	–	–	–
Vitamin B9 (μg)	302.78	216.42–389.13	**281**	529	813	740
Vitamin B12 (μg)	161.73	139.03–184.42	114.00	200	30.00	21.00
Vitamin C (mg)	11.88	5.83–17.92	20.00	25.00	**15.00**	34.00
Vitamin D2 (μg)	<0.50		0.50	1.70	1.10	0.20
Vitamin D3 (μg)	<0.50		0.50	1.70	1.10	0.20
Vitamin E (mg)	0.48	0.39–0.57	1.50	1.30	1.50	0.20
Zinc (mg)	3.50	2.80–4.11	**3.90**	**3.80**	8.70	2.40
Calcium (mg)	6.40	6.02–6.85	5.00	4.00	5.00	7.00
Iron (mg)	41.10	32.84–49.34	9.60	7.40	18.70	7.30
Selenium (μg)	48.70	30.07–67.24	24.00	15.00	**46.00**	**44.00**
Total lipid %	5.64	5.32–5.97	4.10	3.20	3.40	3.80

[a]Results on reindeer liver from this study.
[b]Results based on data from the Norwegian Food Composition (NFC) Database (1).
– = Not given.
Note: Values in **bold** are the ones that fall within a 95% CI for the mean nutrient values of reindeer liver in this study.

Table VI. Mean vitamins, minerals and total lipid concentrations of reindeer meat and other related species meat

		Data from the USDA National Nutrient Database[b]			
Nutrient*	Reindeer[a]	Caribou	Deer	Moose	Antelope
Vitamin A (RAE)	19.93	0.0	0.0	0.0	0.0
Vitamin B1 (mg)	0.09	0.32	0.22	**0.07**	0.30
Vitamin B2 (mg)	0.26	0.72	0.48	**0.24**	0.60
Vitamin B3 (mg)	4.28	5.50	6.37	5.00	–
Vitamin B5 (mg)	1.14	2.50	–	–	–
Vitamin B6 (mg)	0.19	0.37	0.37	–	–
Vitamin B7 (μg)		1.23	–	–	–
Vitamin B9 (μg)	<2.00	4.00	4.00	–	–
Vitamin B12 (μg)	3.34	6.31	6.31	–	–
Vitamin C (mg)	<0.10	0.0	0.0	4.00	0.0
Vitamin E (mg)	0.48	–	0.20	–	–
Zinc (mg)	6.80	4.00	2.09	2.80	1.30
Calcium (mg)	5.40	17.00	5.00	5.00	3.00
Iron (mg)	3.30	4.69	**3.40**	**3.28**	**3.20**
Selenium (μg)	3.00	10.20	9.70	–	9.70
Total lipid %	2.00	3.36	2.42	1.50	**2.00**

*Vitamins D2 and D3 in reindeer were below the limit of detections (LOD); their values in other related species were not given by the referred database (29).
[a]Results on reindeer meat from this study, a 95% CI were given in Table V.
[b]Results based on data from the USDA National Nutrient Database (29).
– = Missing or incomplete value.
Note: Values in **bold** are the ones that fall within a 95% CI for mean nutrient values of reindeer meat in this study.

nutrient values, which are written in bold for other meat and liver types (Table IV, Table V and Table VI), fell within the 95% confidence interval for the mean nutrient values of reindeer meat and liver in this study. As a result, the nutrient values in bold did not differ from those values obtained on reindeer meat and liver. The opposite was true for the data on other meat and liver types not written in bold, as they differ from the values obtained from our study. Furthermore, it was difficult to say something about whether these differences were statistically significant or not since neither confidence intervals nor standard deviations were available on the referred data used in the overview.

There was a scarcity of slaughtered animals aged 1.5 years; thus we were compelled to choose animals (30%, n = 9) out of the protocol scope (20%, n = 6 claves and 10%, n = 3 adult animals). The deviation made by choosing animals (30%) out of the protocol age scope may influence the mean nutrient value if nutrient levels tend to be age dependent. Nevertheless, our raw data revealed little or no varia-tion in nutrient concentrations among the 31 animals. The vitamin levels of reindeer calves were reported to be higher (7–10%) than those of adult animals (7,30).

The vitamin analysis in meat, liver, tallow and bone marrow was based on the pooled sample. The advantage of the pooled sample is that fewer samples have to be analysed, thereby being a time-saving and cost-beneficial method. The disadvantage is that the individual nutrient concentration is based on the combined concentration of all animals in the pooled sample, and cannot be obtained for each animal on an individual basis. Hence, all the animals in the specific pooled sample received the same nutrient concentration.

The bone marrow samples were very limited, and varied in relation to the number of different laboratory analyses types conducted in this study (see Tables I, II and III). This was due to practical reasons around the sample collection and an insufficient sample quantity. Consequently, concentrations of fatty acids on tallow and bone marrow (n = 3 animals) may only be indicative.

In conclusion, the study provided information about the nutrient quality of reindeer meat, liver, tallow and bone marrow. The judge-ment as to whether nutrient concentrations are high or good sources for humans was based on how much these nutrients may contribute to the recommended daily allowance (RDA). Semi-domesticated reindeer meat is lean, thus it suit-ably meets consumers' need for low-fat meat, and meat is also a good source of vitamin B12, docos-apentaenoic acid (DPA) and α-linolenic acid (ALA). In addition, reindeer liver contains high concentrations of vitamins A, B9, B12, Fe and Se. The ratios of ΣPUFA n-6/ ΣPUFA n-3 in meat, tallow and bone marrow are high enough to cover

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the RDA. The tallow contains a high concentration of vitamin B12, while bone marrow contains the highest concentrations of vitamin E and Ca. The presence of reindeer meat, liver, tallow and bone marrow in a meal is a good approach for meeting or contributing to consumers' nutrient RDA needs. The vast majority of nutrient concen-trations in reindeer liver, tallow and bone marrow were significantly ($p < 0.05$) different from the concentrations in meat (mostly higher than those found in meat). Most vitamin concentrations in liver, tallow and bone marrow were signifi-cantly correlated with the concentrations in meat ($p < 0.05$).

Further research, which includes more animals from many different grazing districts, is needed to take into account factors that we could not investigate in this study, such as geographical variations, in order to look into their association to the nutrient composition of reindeer.

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Paper II

ORIGINAL RESEARCH ARTICLE

CO-ACTION PUBLISHING

Level of selected toxic elements in meat, liver, tallow and bone marrow of young semi-domesticated reindeer (*Rangifer tarandus tarandus L.*) from Northern Norway

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Objectives. To gain knowledge on toxic elements in semi-domesticated reindeer and their distribution in meat, liver, tallow and bone marrow. The correlations between concentrations in meat and liver, as well as the use of the latter as an indicator for toxic elements in meat, were also investigated.

Study design. Cross-sectional study on population of semi-domesticated reindeer from 2 northern Norwegian counties (Finnmark and Nordland).

Methods. Semi-domesticated reindeer carcasses ($n=31$) were randomly selected, from which meat, liver, tallow and bone marrow samples were collected. Selected toxic elements (cadmium, lead, arsenic, nickel and vanadium) were studied.

Results. Liver was the organ with the highest level of all elements except for nickel, which was highest in bone marrow. Meat had the lowest levels, whereas levels in tallow and bone marrow were between those of meat and liver. Concentrations of cadmium, lead and arsenic were significantly different ($p<0.05$) between meat and liver, while only arsenic and cadmium were significantly correlated in meat ($r_s=0.71$, $p<0.01$) and liver ($r_s=0.72$, $p<0.01$). The cadmium level exceeded the European Commission's (EC) maximum level set for bovine meat and liver in 52% of the liver samples ($n=29$). Nevertheless, the estimated monthly cadmium intake from liver of 2.29 μg/kg body weight was well below the provisional tolerable monthly intake of 25 μg/kg body weight set by the FAO/WHO Joint Expert Committee on Food Additives.

Conclusions. Based on the measured levels and their relation to the maximum level and to the provisional tolerable weekly/monthly intake limits, it could be inferred that consumption of reindeer meat is not associated with any health risk related to the studied toxic elements for consumers.

Keywords: *reindeer*; *edible tissues*; *cadmium*; *lead*; *arsenic*; *nickel*; *vanadium*

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Knowledge on toxic element concentrations in reindeer as terrestrial animals is necessary for assessing the potential effect in humans from the consumption of contaminants in these animals. Moreover, there has been an increasing awareness among consumers with concern to food safety and environmental toxicant issues over the past 20 years. Toxic elements (e.g. mercury, cadmium, lead and arsenic) have been an issue in Arctic terrestrial animals. The levels of persistent organic pollutants (POPs) in the Arctic terrestrial animals are low (1). Even though contaminant levels are low in the meat of Arctic terrestrial animals, it is important to monitor their levels regularly because of high concentrations reported in their liver and kidneys. Liver has been reported to have higher concentrations of toxic elements than meat and is considered to be a good indicator of such elements in animal's body (2,3). Therefore, liver is used in food safety control routines as an indicator of toxic elements in meat.

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Toxic elements are chemicals that are persistent and not metabolized, although their chemical forms may change as they pass through the intestinal tract or during storage in animal tissues (4). They are regarded as toxic to living organisms since they have tendency to accumulate in selected human and animal target tissues with the potential of causing nephrological, carcinogenic, teratogenic and immunological disorders (5,6). Toxic elements are capable of being transported over long distances (thousands of kilometres) or may be deposited near their source of origin, thereby having a local impact (1). The long range transportation of toxic elements through the atmosphere mainly depends on the size and composition of particles with which toxic elements are associated as well as their solubility.

Reindeer feed on vascular plants and lichens throughout the year with largest amount of lichens, which account for more than 50% of the winter diet for reindeer (7–9). Lichens naturally contain low amounts of essential elements (e.g. zinc and manganese) when compared with grass and are highly dependent on atmospheric supplementation in covering the reindeer's need for essential elements. Thus, high levels of such elements in lichen tissues are due to atmospheric contamination (10). The long survival, tolerability of high element concentrations and fairly uniform morphology of lichens allow for the absorption and accumulation of contaminants over the entire surface of the lichens make it suitable as a biomarker for atmospheric toxic element pollution (11,12). The availability of lichens as the reindeer's main winter feed has been described as a key factor for determining the level of heavy metals (13).

Toxicological terms such as the provisional tolerable weekly/monthly intake (PTWI/PTMI) limits set by the Joint Food and Agriculture Organization (FAO)/WHO Expert Committee on Food Additives (JECFA), as well as the maximum levels (ML) for certain contaminants set by the European Commission (EC), are relevant in relation to toxic element concentrations in both the human body and foodstuffs, respectively (14–17). The PTWI/PTMI limits are end points used for contaminants such as toxic elements with cumulative properties, representing permissible human weekly/monthly exposure to those contaminants that are unavoidably associated with the consumption of contaminated foodstuffs. The ML represents the maximum concentration of contaminants in foodstuffs in which no human health risk is associated, i.e. the concentrations that are acceptable in or on foodstuffs (17).

Our main purpose was to study the level of toxic elements in the meat, liver, tallow and bone marrow of reindeer, particularly the association between liver and meat concentrations. Additionally, we wanted to relate our results on toxic elements in meat and the rest of the studied tissues to the EC's maximum level (ML) and FAO/WHO–JECFA's PTWI/PTMI limits available for certain toxic elements.

Materials and methods

Sample collection

Muscle, liver, tallow and bone marrow samples were randomly collected from semi-domesticated reindeer carcasses ($n = 31$ animals) from Finmark and Nordland Counties in North Norway. The average age of the reindeer from which the samples were collected from September 2004 to January 2005 was young (1.5 years old) according to internal procedures (not published). However, a limited number of calves (approximately 6–10 months old, $n = 6$) and adult animals (over 2 years old, $n = 3$) had to be chosen because of a scarcity of slaughtered animals in the age of 1.5 years. Age of the reindeer was obtained directly from the tags attached to animals' carcasses when they passed the weighing post in the slaughterhouses.

Meat samples were collected from the muscles in the dorsal neck region. Liver samples were collected from the main loop, tallow from the fat tissue surrounding kidneys and bone marrow from the hind and front legs. All of the samples were collected immediately after the slaughter/dressing/carcass weighing process in plastic bags, though prior to being further divided into different acid-rinsed glasses according to the sample type (meat, liver, tallow or bone marrow). Samples were put on ice (approximately 4°C) immediately after collection and distribution into dedicated containers and kept frozen at −20°C (within 12 hours from the sample collection) until analysis.

Chemical analysis

The samples were separately decomposed using a microwave oven technique and concentrated supra-pure HNO_3 (5 ml) and H_2O_2 (3 ml) were added to the sample (0.6–0.7 g) before microwave oven treatment. The following temperature regimes were subsequently used in the microwave oven: 20–50°C (5 min), 50–100°C (10 min), 100–180°C (5 min) and 180°C (15 min). After cooling down the heated decomposed sample, the solution was diluted to 50 ml. The sample solution was analysed using an inductively coupled plasma high resolution mass spectrometer (ICP-HRMS), Thermo Scientific Finnigan Element-2, Germany.

All standards and calibration solutions contained 1 ppb rhenium (Re) as the internal standard, together with 1% nitric acid (HNO_3). The calibration curve was verified by a standard quality control (QC) sample, National Institute of Standards and Technology (NIST), USA. The resolutions used for elements were low [at 10] for (cadmium and lead), middle [at 20] for (vanadium and nickel) and high [at 30] for arsenic. The lens adjustment

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was optimized daily to ensure maximum intensity and top separation. The analyses were done by the NILU (Norwegian Institute for Air Research) Laboratory, Kjeller, Norway. The laboratory is accredited for the methods used in the analyses according to NS-EN ISO/IEC 17025, No. TEST 008. The limits of detections (LODs) for the studied toxic elements were 3 times the standard deviation (SD) of the laboratory blanks, whereas the limits of quantifications (LOQs) were 10 times the SD of the blanks.

Statistical analysis

STATA/SE 11.0 for Windows (STATA Corp. College Station, TX, USA) was used for statistical analyses. The results were presented as a percentage of the detected toxic elements, mean, median and range. Laboratory results for metals below the LOD were given a numeric value at half the detection limit (LOD/2) according to accepted statistical practice (18). Elements concentrations were positively skewed (skewed to the right); therefore, the nonparametric Wilcoxon matched-pairs signed-rank test was used to test for significance differences in element concentrations between meat–liver and tallow–bone marrow. A Spearman's rank correlation test (Spearman's r_s) was used for determining significant correlation coefficients of elements among the different tissues and inter-element correlations within the same tissue. The significance levels of correlations were Sidak-adjusted for pair-wise comparisons. Element ratios were created by dividing element concentrations in the meat by the same element concentrations in the rest of the studied tissues and mean values of the element ratios were obtained. Mean, median, p-value, correlation coefficients and element ratios were not calculated in cases in which the detection percentage was below 50%. The level of statistical significance was set at $p < 0.05$ in all performed analyses.

Calculation of estimated human weekly/monthly toxic elements intake from reindeer

A scenario based on data from a questionnaire on the Population-based Health and Living Conditions in areas with Sami and Norwegian populations – The SAMINOR Study – was used to estimate reindeer meat and liver intake (19). This estimation was used to determine toxic elements exposure on the basis of weekly human reindeer meat and liver intake within this population. This was then compared with the PTWI/PTMI limits in order to assess the different toxic elements exposure for a standard 60 kg human body weight, as set by FAO/WHO–JECFA (17). The percentiles 25, 50 and 75% were used to determine the distribution of meat intake, in which 25% was given to low (23 g/week), 50% to medium (38 g/week) and 75% to high consumption (70 g/week). Due to very low liver consumption, the mean consumption frequency of once in a month or more was used to determine high liver intake (61 g/week). The proportion of the population with high liver intake was about 2% (total n = 12,899). The estimated toxic elements concentrations on the basis of human weekly/monthly reindeer meat and liver intake (EHTI) were calculated according to the following formula:

$$\text{EHTI } (\mu\text{g/kg human body weight}) = I \times T/S.$$

where:

(I) is the mount of estimated weekly edible tissue intake in grams, (T) is toxic element concentration in the meant tissue per gram and (S) is the standard human body weight equal to 60 kg. In addition to the PTWI/PTMI, the ML of toxic elements in a reindeer body was directly calculated per kilogram of meat/liver. Since there are no official ML for toxic elements in the meat and liver of cervine species, the available ML for bovine animals' meat and liver were used (16).

Results

The percentage of samples within the detection limits (% detected), mean, median ng/g wet weight (ww) and range are presented in Table I. The ratios of element concentrations between liver and the rest of the studied tissues are presented in Table II. The toxic element correlations between liver and the rest of the studied tissues are presented in Table III.

Cadmium (Cd)

Cd was detected in 100% of tissues studied, with the highest concentration in liver (mean 653.7, median 563.9 ng/g ww) and the lowest in meat (mean 1.9, median 1.2 ng/g ww, $p < 0.01$). Cd was significantly higher in tallow than in bone marrow ($p < 0.05$). In addition, Cd concentrations in meat and liver were significantly positive correlated (Spearman's $r_s = 0.72$, $p < 0.01$).

Lead (Pb)

Pb was detected in 100% of liver, tallow and bone marrow samples and 83% of meat samples. The highest Pb concentration was measured in liver (mean 272, median 247.8 ng/g ww) and the lowest in meat (mean 7.9, median 7.6 ng/g ww), $p < 0.01$. No significant correlation was found between Pb concentrations in meat, liver or any of the other tissues.

Arsenic (As)

As was detected in all tissue samples with the exception of bone marrow in which only 44% of the concentrations were above the LOD. Liver had the highest As concentration (mean 24.1, median 13.1 ng/g ww). A significant positive correlation coefficient (Spearman's $r_s = 0.71$, $p < 0.01$) was found for As in meat and liver. The As was not significantly inter-correlated with any other element in the 4 tissues studied.

Table I. Levels of toxic elements (ng/g ww) in meat, liver, tallow and bone marrow of semi-domesticated reindeer

	Meat (n = 29)					Liver (n = 29)						
Element	% Detected[a]	Mean	Median	Min	Max	% Detected	Mean	Median	Min	Max	p[b]	LOD
Cd	100	1.9	1.2	0.6	7.1	100	653.7	563.9	174.7	2,186.2	*	0.37
Pb	83	7.9	7.6	1.8	18.1	100	272.0	247.8	144.5	522.7	**	3.61
As	100	19.7	11.6	1.3	82.6	96	24.1	13.1	0.6	156.6	*	1.13
Ni	48	–[c]	–[c]	19.7	101.7	72	50.5	42.5	19.7	185.9	–[c]	39.46
V	48	–[c]	–[c]	0.1	4.9	100	13.1	9.6	4.6	43.7	–[c]	0.24
	Tallow (n = 15)					Bone marrow (n = 9)						
Cd	100	5.7	5.7	2.5	11.9	100	3.8	3.8	2.5	5.5	*	0.37
Pb	100	28.6	26.6	11.3	64.4	100	21.8	22.6	7.0	36.3	n.s	3.61
As	87	2.1	1.9	0.6	4.0	44	–[c]	–[c]	< 1.1	78.1	–[c]	1.13
Ni	13	–[c]	–[c]	19.7	326.1	100	285.6	253.5	46.5	653.0	–[c]	39.46
V	93	1.3	0.9	0.1	6.0	100	2.6	1.0	0.4	7.8	n.s	0.24

[a]Percentage of samples within the detection limits.
[b]Level of significance: * < 0.05, ** < (0.01), n.s (not significant).
[c]Not calculated because more than 50% of the values were below the limit of detection (LOD).
Note: To detect significant differences, meat was tested against liver, and tallow was tested against bone marrow.

Nickel (Ni)

Forty-eight percent of Ni concentrations in meat and 13% in tallow were above the LOD. All Ni concentrations in bone marrow were above the LOD, whereas 72% of concentrations in liver were above the LOD. The concentration in bone marrow was 6 times higher than that in liver, with mean 285.6, median 253.5 and mean 50.5, median 42.5 ng/g ww, respectively. Due to a low detection percentage, the nickel mean ratio was only calculated between liver and bone marrow (0.38).

Vanadium (V)

Forty-eight percent of V concentrations were above the LOD in meat, none were under the LOD in bone marrow, while 93% were above the LOD in tallow. V was the least abundant element in the different tissue samples, with a range between 0.93 and 9.57 ng/g ww. The V concentration was highest in liver (mean 13.11, median 9.57 ng/g ww) and lowest in tallow (mean 1.32, median 0.93 ng/g ww).

Scenarios for estimated human weekly and monthly toxic elements intake from reindeer

Scenarios for estimated human weekly and monthly toxic elements intake from reindeer meat and liver were calculated according to the formula given previously in Materials and methods section on the basis of the following meat and liver consumptions (weekly for As and Pb, and monthly for Cd): (a) Weekly/monthly meat consumption: low (23/92 g), medium (38/152 g) and high (70/280 g), respectively. (b) Weekly/monthly liver consumption: 61 and 244 g, respectively. The estimated human toxic element intake values are presented in Table IV, together with the PTWI and PTMI limits (see calculation of estimated human toxic elements intake from reindeer under Materials and methods section).

Discussion

Levels of the studied selected toxic elements in reindeer were low compared to maximum levels for bovine animals set by the EC, except for Cd in liver. Cadmium

Table II. Ratio of toxic element concentrations between liver and the rest of the studied tissues

	Mean toxic element ratio[a]				
	Cd	Pb	As	Ni	V
Liver/meat	410.22	51.48	1.37	–[b]	–[b]
Liver/tallow	91.07	12.13	7.97	–[b]	31.21
Liver/bone marrow	131.82	18.46	–[b]	0.38	12.34

[a]Toxic element ratio was calculated by dividing the individual concentration of the specific element in liver by that of the same element in the other tissue for the same individual. Then, the mean ratio of all these ratios for each toxic element was obtained and reported, aiming to give an idea about toxic element concentrations in liver when directly compared to the other tissue.
[b]The toxic element ratio between liver and the rest of the studied tissues was not calculated in cases in which at least one of the ratio components had a percentage of samples within the detection limits that was below 50%.

Table III. Toxic element correlations between liver and different tissues from reindeer

	Correlation coefficient					
Element	Meat–liver	p-value	Tallow–liver	p-value	Bone marrow–liver	p-value
Cadmium	0.72	< 0.01	0.38	n.s[a]	0.61	n.s[a]
Lead	0.09	n.s[a]	0.18	n.s[a]	−0.26	n.s[a]
Arsenic	0.71	< 0.01	0.61	n.s[a]	−0.79	n.s[a]
Nickel	–[b]	–[b]	–[b]	–[b]	0.07	n.s[a]
Vanadium	–[b]	–[b]	−0.15	n.s[a]	−0.02	n.s[a]

[a]Not significant.
[b]The correlation was not calculated in cases in which at least one of the correlation components had less than 50% of samples within the detection limits.

exceeded the EC's ML for bovine animals in 52% of the liver samples. This level would not represent a potential health risk to the population as the estimated monthly Cd intake from liver was well below the PTMI limit set by JECFA. The low Cd intake from liver was certainly because of the very low reindeer liver consumption. This would further indicate a necessity to not use the ML alone when relating toxic element levels in reindeer and games to human intake of such elements. The tolerable intakes set by FAO/WHO–JECFA would be more appropriate to use when dietary frequency could be estimated through questionnaire data.

Cadmium

The Cd concentration in reindeer liver from this study was twice the amount detected by Bernhoft et al. (20), nearly half the amount reported by Frank (21) and comparable with those detected elsewhere (22,23), in which levels were 2–3 times of those reported in moose and sheep from same area. Our median Cd concentration of 1.2 ng/g ww (equivalent to 0.0012 mg/kg ww) in reindeer meat was much lower than the EC's ML for Cd in the meat of bovine animals, sheep, pig and poultry of 0.05 mg/kg ww. However, the median Cd level of 563.9 ng/g ww in reindeer livers was 60 ng/g higher than the ML of 0.50 mg/kg ww set for liver (16). Moreover, the Cd level exceeded the EC's ML for bovine animals in 52% of the liver samples (n = 29) collected. High Cd concentrations from reindeer and caribou livers have also been shown previously (13,23–27).

The FAO/WHO–JECFA has recently set the PTMI limit at 25 μg/kg body weight (standard human = 60 kg) (14). The estimated monthly human Cd intake from reindeer meat for low, medium and high consumption, as well as from reindeer liver (high consumers), was well below the PTMI limit for Cd. The high reindeer meat and liver consumption is relevant to reindeer herders and hunters. In order to reach the PTMI for Cd when liver is the issue of concern for high consumers, an amount of 2.7 kg per month needs to be eaten. Since the PTMIs for toxic elements have been established to take into account the total intake of such elements from different sources/type of diets, it is important to take into account Cd intake from smoking and other type of diets.

Lead

The level of liver Pb from this study is comparable to those reported by Sivertsen et al. (22), which are within the range reported elsewhere (20,24). The Pb level in meat was much lower than in liver and was comparable with

Table IV. Estimated human toxic elements intake from reindeer meat and liver

	Human toxic elements intake (μg/kg body weight)				
	Meat			Liver	
Element	Low (23 g/week)	Medium (38 g/week)	High (70 g/week)	High (61 g/week)	PTWI/PTMI[a]
Cd[b]	0.002	0.003	0.006	2.293	–/25
Pb[c]	0.003	0.005	0.009	0.252	25/–
As[c]	0.005	0.007	0.014	0.013	15/–

[a]Permissible tolerable weekly intake/permissible tolerable monthly intake (μg/kg body weight).
[b]Monthly toxic element intake.
[c]Weekly toxic element intake.
Note: For the meat intake, low is (the 25 percentile), medium (the 50 percentile) and high (the 75 percentile), while the high liver intake represents the mean consumption frequency of once a month or more based on data from the SAMINOR-Study (19).

the level reported by Rintala et al. (24). The reported liver concentration of lead from north Norwegian sheep was 28% lower than the value detected from reindeer livers in the present study (28). The concentration of lead levels in livers detected in this study was comparable to those reported by Eriksson et al. (29). However, Eriksson et al. reported nearly double the maximum level (0.99 mg/kg ww) in their range than the one we detected of 522.7 ng/g ww. The median liver Pb of 247.8 ng/g ww in the present study was much lower than that of 10 μg/g ww, which was reported to be consistent with the poisoning level in most animal species (30). Lead was found to be significantly correlated with As, Co and Cu in liver and with As and Cu, in meat from Spanish cattle (31,32). Even so, we could not see such correlations in this study.

The respective meat and liver Pb concentrations of 7.6 and 247.8 ng/g ww in this study were much below the EC's ML for the meat and offal of bovine animals, sheep, pig and poultry, which were set at 0.10 and 0.50 mg/kg ww, respectively (16). The PTWI limit for Pb of 25 μg/kg body weight was recently withdrawn by FAO/WHO–JECFA. No new permissible tolerable intake limit for Pb was established. Nevertheless, weekly human Pb intake from reindeer liver, which was the highest intake compared with meat, was 100 times lower than the previous PTWI limit (Table IV).

Arsenic

Liver values reported elsewhere (20,22,33) were within our range of 0.6–156.6 ng/g ww. The extremely large arsenic range measured in this study could be due geography and need to be investigated further with more animals and involvement of more herding districts. Sivertsen et al. also reported significant inter-element correlations in reindeer between As and Co and Cu and Ni (22). Arsenic was significantly positive correlated ($r_s = 0.71$, $p < 0.01$) between meat and liver in this study. Such a correlation could be beneficial in estimating As levels in an animal body, using liver as an indicator/biomarker of As body burden. Estimated weekly As intakes from reindeer meat and reindeer liver were well below the PTWI limit for As of 15 μg/kg body weight (standard human = 60 kg) set by FAO/WHO–JECFA (15). As, which is antagonistic to the essential elements of iodine (I) and selenium (Se), accumulates in liver, kidney, skin and hair (34,35). Both organic and inorganic forms are toxic to animal.

Nickel

The median Ni liver concentration in this study was comparable with that formerly reported by Bernhoft et al. (20) and within the range formerly reported by Sivertsen et al. (22,33). The level of Ni in Finnish reindeer livers, which was lower than the level detected in our study, was found within the same range (0.01–0.03 mg/kg ww) of those from Finnish cattle and pigs (24). In previous studies, nickel concentrations in reindeer from Sør-Varanger in northern Norway were associated with atmospheric pollution from Ni smelters in 2 Russian towns (Nikel and Zapoljarnij) close to the Norwegian border in which concentrations in reindeer were higher than those in moose and sheep from the same area (22).

Vanadium

Meat, liver and bone marrow concentrations of V in this study were comparable to those reported from cattle and pigs (36). No clear regulation of V has been established in human; however, a concentration of 100 μg/day has been reported as an estimated safe daily intake (37).

Element correlation between liver and meat

Still, few studies have looked at the correlation of elements between meat–liver and meat–kidneys, despite the fact that liver and kidneys are used in food safety aspects as indicators of toxic elements in meat. Only Cd and As were statistically significantly correlated between meat and liver in the present study (Table III), whereas a significant correlation for Pb between liver and meat has been reported elsewhere (38).

Conclusions

Liver had the highest toxic element concentrations with the exception of Ni, which was highest in bone marrow. The correlations among the detected elements between liver and meat were observed for Cd and As. Therefore, liver is not a good indicator for lead in meat.

Based on the measured levels of the present studied elements and their relation to the EC's ML and the PTWI/PTMI limits, we could infer that the consumption of reindeer meat is not associated with any health risk for consumers. The Cd level exceeded the EC's ML for bovine animals in 52% of the liver samples. Nonetheless, the monthly Cd intake of 2.3 μg/kg body weight from liver was well below the PTMI of 25 μg/kg body weight set by JECFA. In the present study, Cd, Pb, As and Ni levels from reindeer seem not to differ much from previous Norwegian studies.

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Article

Concentrations and Geographical Variations of Selected Toxic Elements in Meat from Semi-Domesticated Reindeer (*Rangifer tarandus tarandus L.*) in Mid- and Northern Norway: Evaluation of Risk Assessment

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Abstract: Meat samples (n = 100) from semi-domesticated reindeer (*Rangifer tarandus tarandus L.*) were randomly collected from 10 grazing districts distributed over four Norwegian counties in 2008 and 2009. The main aim was to study concentrations and geographical variations in selected toxic elements; cadmium (Cd), lead (Pb), arsenic (As), copper (Cu), nickel (Ni) and vanadium (V) in order to assess the risk associated with reindeer meat consumption. Sample solutions were analysed using an inductively coupled plasma high resolution mass spectrometer (ICP-HRMS), whereas analysis of variance (ANOVA) was used for statistical analyses. Geographical variations in element concentrations were revealed, with As and Cd demonstrating the largest geographical differences. No clear geographical gradient was observed except for the east-west downward gradient for As. The As concentrations were highest in the vicinity of the Russian border, and only Cd was shown to increase with age ($p < 0.05$). Sex had no significant effect on the concentration of the studied elements. The concentrations of all the studied elements in reindeer meat were generally low and considerably below the maximum levels (ML) available for toxic elements set by the European Commission (EC).

Thus, reindeer meat is not likely to be a significant contributor to the human body burden of toxic elements.

Keywords: reindeer meat; toxic elements; Norway; Arctic food; risk assessment

1. Introduction

In recent years, there has been considerable concern over the extent of toxic elements in the environment and their possible negative health effects. The limited amounts of data on local sources, as well as the increased number of slaughtered reindeer, necessitate a need for the continuous monitoring of such elements in meat to secure food safety for the consumer [1–6]. The concentration of toxic elements in animal tissues depends on the animal's species, dietary concentration of the element, tissue absorption, concentrations of other elements in the animal tissue and the body's homeostatic control mechanism for the element [7]. These elements are toxic for both human and animals, and cause a range of diseases [8–12]. Furthermore, the highest concentrations have been found in tissues such as kidneys, liver and bones [1,3,13,14]. Geographical variations in the concentrations of toxic elements in meat, liver and kidneys from reindeer have previously been demonstrated [15–17].

Both natural and anthropogenic components contribute to geographical variations in the concentration of toxic elements. Moreover, the difference in exposure patterns may be due to different type of diets, as both animal diet preferences and the type of vegetation, vary from one place to another. Differences in exposure are therefore expected in areas with a different animal density and a different availability of lichens. Toxic metal concentrations in animals have been reported to be associated with the distance to pollution sources, thus districts located close to the sources have higher concentrations than other areas [18,19]. Wind frequency and direction also influence the atmospheric deposition of toxic metals [20].

The main reindeer summer/autumn feed are grasses, sedges, twigs, leaves and mushrooms [21]. Some plants (metallophytes) have the ability to absorb and accumulate more toxic elements from the soil in their tissues, even when soil concentrations are low, compared with other ones within the same geographical area. Additionally, the elements composition of plants vary within species as well as at the various stages of plant growth [22]. The decreased pH (increased acidity) of the soil as a result of acid rain affects the solubility and mobility of some toxic elements (e.g., an increase in the case of Cd) [23,24]. In this way, their uptake by plants and accumulation by animals may increase. However, areas in the southernmost part of Norway are the ones most affected by acid rain due to the long-range atmospheric transportation from Central and Western Europe compared to areas in the north of Norway [25,26]. As a consequence of this, Norwegian cervine animals—particularly reindeer—from southern Norway have previously exhibited elevated liver and kidney cadmium levels [1].

Lichens are the main reindeer winter feed, with a varied distribution among the different grazing districts, and have the ability to accumulate toxic elements from the atmosphere [27–29]. In former studies, lichens also revealed the greatest variation in metal concentrations compared with other plants collected from contaminated and reference areas in Swedish Lapland [19].

Areas close to the Norwegian-Russian border are the primary issue of concern due to the location of the two Russian towns of Nikel (nickel smeltery) and Zapoljarny (briquette industry). The town of Nikel is located 7 km from the Norwegian border, while the town of Zapoljarny is located 30 km further east. The high atmospheric level of Ni, Cu, Co and As previously measured from the Norwegian area of Svanvik close to the Russian border was reported to be due to the release from the smelting activities in Nikel and Zapoljarny [20,30]. In addition to the known Russian sources, the presence of local mining facilities and military activities in some districts acts as potential point sources, and has been an issue of concern.

The main purpose of this project was to study the concentrations and geographical variations of selected toxic elements in meat from semi-domesticated reindeer in the selected grazing districts in mid- and northern Norway in order to assess the risk associated with reindeer meat consumption.

2. Materials and Methods

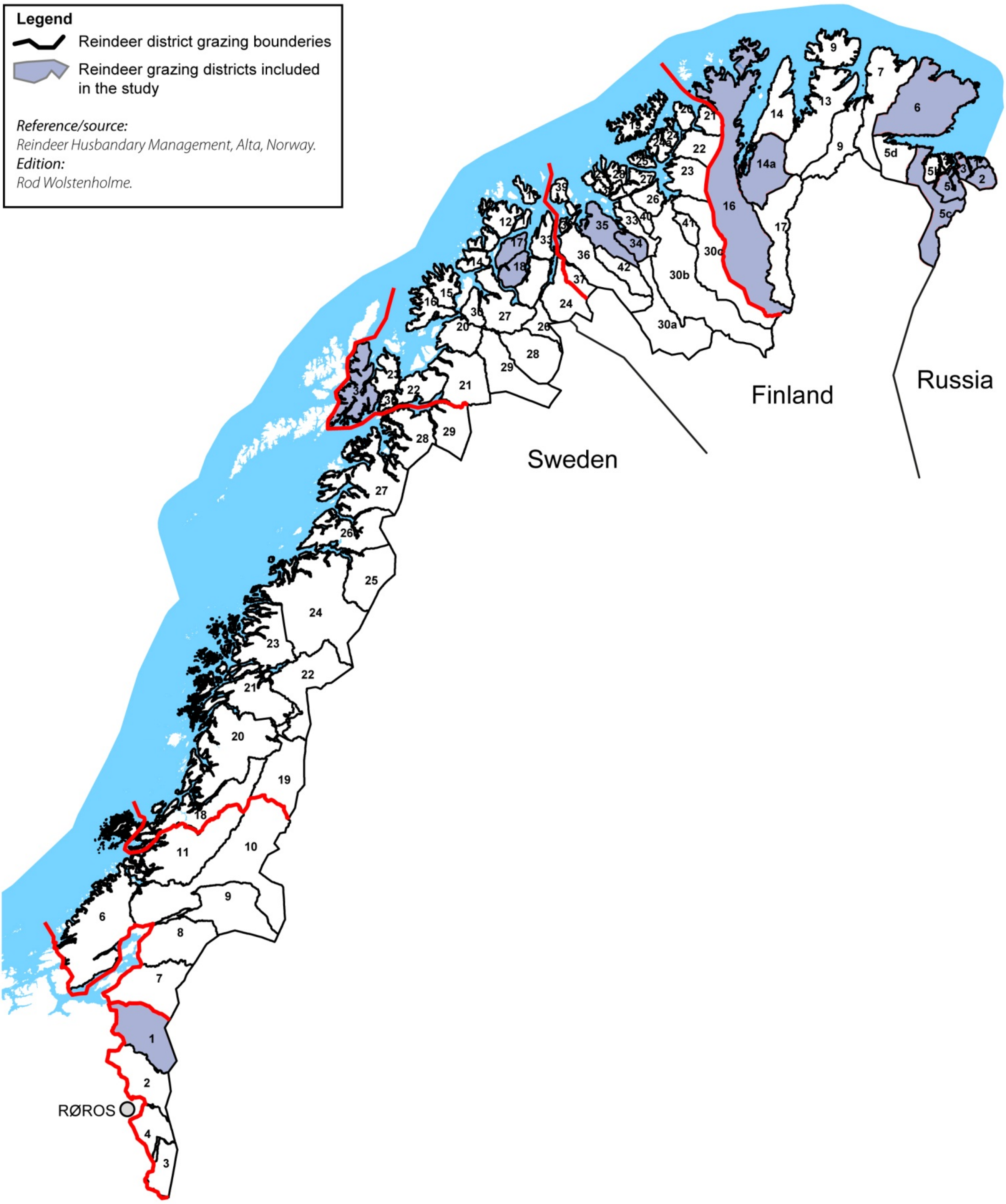
2.1. Sample Collection and Preparation

Meat samples (n = 100) from the neck-region were randomly collected from semi-domesticated reindeer in 10 different grazing districts located in northern and middle Norway in the period from October–December 2008 and September–December 2009. The samples were collected from four different counties distributed as follows: Finnmark County (seven districts), Troms County (one district), Nordland County (one district) and South-Trøndelag County (one district). The selection of the 10 districts was based on obtaining a broad geographical range and the susceptibility of certain districts to pollution from mines, smeltery and military activities (Figure 1). The selection of seven districts from Finnmark County which is the biggest and northernmost Norwegian county was based on the fact that this county has the largest number of semi-domesticated reindeer and 50% of the total number of reindeer grazing districts in Norway. The districts of Eastern Sør-Varanger, Pasvik/ Sør-Varanger and Varanger Peninsula are located near the Norwegian-Russian border, where the contamination from mines and smeltery activities is taking place. Spierttagáisá is located in a military activity area, whereas Karasjok West is a neighbouring district. There is also mining work taking place in Ábborašša, which is close to Fávrrosorda a neighbouring district.

Our focus was on young animals (1.5 years old), which represented 77% of the total samples. However, calves (approximately10 months old) and older animals (>2 years old) with the respective proportions of 12% and 11% had to be selected due to the limited availability of 1.5 year olds in some districts (n = 4). There were 52 males and 48 females of the 100 selected reindeer.

All samples were collected directly after the slaughter/dressing process and carcass weighing in acid-rinsed glasses. The glasses were labelled with sample type, carcass number, district name/number and date of sample collection. The samples were kept cool in a cooling box (at approximately 4 °C) and then moved the same day to a −20 °C freezer until they were shipped frozen to the laboratory for analysis. All of the animals from the collected samples were healthy, *i.e.*, had passed the veterinary meat inspection.

Figure 1. Map of the study area.



2.3. Chemical Analysis

The samples were separately decomposed using a microwave oven technique, with concentrated supra-pure HNO_3 (5 mL) and H_2O_2 (3 mL) being added to the decomposed sample (0.6–0.7 g) before undergoing microwave oven treatment. The following temperature regimes were subsequently used in the microwave oven: 20–50 °C (5 min), 50–100 °C (10 min), 100–180 °C (5 min) and 180 °C (15 min). After cooling the heated decomposed sample, the solution was diluted to 50 ml. The sample solution was then analysed using an inductively coupled plasma high resolution mass spectrometer (ICP-HRMS), Bremen, Germany. All standards and calibration solutions contained 1 ppb Rhenium (Re) as the internal standard, together with 1% nitric acid (HNO_3). The calibration curve was verified by standard quality control (QC) sample, National Institute of Standards and Technology (NIST, USA). The resolutions used for the elements were low (at 10) for Cd and Pb, middle (at 20) for Cu, V and Ni, and high (at 30) for As. The lens adjustment was optimized daily for maximum intensity and top separation. The analyses were done by the NILU (Norwegian Institute for Air Research) Laboratory (Kjeller, Norway). The laboratory is accredited for the methods used in the analyses according to NS-EN ISO/IEC 17025, No. TEST008. The limits of detections (LODs) for the studied toxic elements were three times standard deviation (SD) of the laboratory blanks, whereas the limits of quantifications (LOQs) were 10 times the SD of the blanks, decomposed simultaneously with the meat samples.

2.4. Statistical Analysis

STATA/SE 11.0 for Windows (STATA Corp. College Station, TX, USA) was used for the statistical analyses. Laboratory results for elements below the limit of detection (LOD) were given a numeric value at half the LOD (LOD/2) according to accepted statistical practice [31]. All toxic element concentrations, except for Cu which was normally distributed, were positively skewed (skewed to the right). Therefore, all concentrations were log transformed to obtain a normal distribution before statistical evaluation. A standardized residuals test was conducted prior to the log transformation in order to check for possible outliers (observations that were more than three standard deviations from the mean). Consequently, all outliers were removed (n = 10, details in results and discussion).

An analysis of variance and covariance (ANOVA), with the specific element as a dependent (outcome) variable and the district, age and sex as independent (explanatory) variables, was used to test for the effect of the independent variables on toxic element concentrations. Bonferroni multiple comparison tests were used to test for significant differences in toxic element concentrations among the 10 grazing districts, using the specific element as an outcome and the districts as a factor variable. A Welch test was used whenever the homogeneity of variance was violated. The level of statistical significance was set at $p < 0.05$ for all the statistical analyses.

3. Results and Discussion

The present study is unique since it is the first of its kind to include such a large number of animals to study the concentrations and geographical variability of toxic elements in meat from semi-domesticated reindeer. Moreover, the assessment was done using meat that is more relevant for human consumption. Further, the reindeer originated from 10 different grazing districts covering a large geographical area extending from the middle to the northernmost part of Norway.

The overall concentrations of toxic elements in meat samples from all animals (n = 100) are presented in Table 1. The results were presented as percentage (%) of samples above LOD, geometric mean (GM), arithmetic mean ± standard deviation (AM ± SD), range (Min-Max) and coefficient of variation (CV%). Geographical variations in toxic element concentrations between some districts were demonstrated (Table 2). Detailed result of the multiple comparison tests for concentrations among the 10 grazing districts are presented as supplementary material (Table 1S). Sex had not any significant influence on toxic element concentrations.

Table 1. The overall toxic element concentrations (ng/g ww) in reindeer meat.

Element	n	% detected	GM	AM ± SD	Min.	Max.
Cd	98	99	1.7	2.5 ± 2.5	<0.37	11
Pb	98	92	1.5	2.7 ± 3.1	<0.19	20
As	99	100	8.1	20 ± 32.1	0.7	135
Cu	98	100	1,439	1,469 ± 296	830	2,357

Legend: n = Number of observations. % detected= Percentage of samples within the limit of detection (LOD); GM = Geometric mean. AM ± SD = Arithmetic mean ± standard deviation. Min-Max= Minimum–Maximum; CV = Coefficient of variation. **Note:** The limits of detection (LODs) for the toxic elements (e.g., 0.37 and 0.19) were stipulated by calculating the mean value of all LODs for the specific element. Numbers of samples were less than 100 due to removal of outliers.

Toxic element concentrations in 10 samples were outliers and have been removed from the statistical analyses. The detected outliers were: One animal (1.5 years) from Eastern Sør-Varanger with an As concentration of 161 ng/g ww; two animals from Kanstadfjord; a 1.5 years old and an older one (>2 years) with Cd concentrations of 13 and 15 ng/g ww, respectively; two animals: a calf from Varanger Peninsula and an older one (>2 years) from Spierttagáisá with Pb concentrations of 28 and 23 ng/g ww, respectively; two animals (1.5 years) from Fávrrosorda and Karasjok West with Cu concentrations of 161 and 2837 ng/g ww, respectively; one animal (1.5 years) from Tromsdalen with Ni concentration of 19 ng/g ww; two animals (1.5 years) from Spierttagáisá and Karasjok West with V concentrations of 5.1 and 8.3 ng/g ww, respectively.

3.1. Cadmium (Cd)

Cadmium was detected in 99% of the meat samples, and had the second largest geographical variation after arsenic. The geographical variations in cadmium concentrations are presented in Tables 2 and 3.

The presence of older animals in some districts revealed a significant district × age interaction, therefore stratified data were given for Cd concentrations in districts with mixed age groups (Table 3). Calves and young animals demonstrated no significant difference in Cd concentration across geography (GM = 0.9 and 1.5, for calves and young animals respectively), while older animals (>2 years old) showed a higher Cd concentration (GM = 4.3, $p < 0.01$). The increase in Cd concentration with age was in accordance with previous studies [15,16,32]. A Finnish study on toxic metals in reindeer from four districts in Lapland has shown comparable muscle Cd concentrations ranging from 1 to 6 ng/g ww [15]. Moreover, Cd concentrations from the present study were up to 100 times lower than those found in reindeer muscle (mean = 580 ng/g ww) originating from Karelia in the Russian Federation [33].

Cd concentrations in some districts from the present study were higher than those from reindeer muscles collected from three Greenlandic districts, whereas concentrations from the districts of Essand/Røros, Ábborašša, Kanstadfjord and Fávrrosorda were comparable to that reported from one district (GM = 3 ng/g ww) [17]. The high Cd concentration in Kanstadfjord was due to presence of some older animals (n = 4). Furthermore, high Cd concentration in Fávrrosorda might be due to atmospheric deposition from the neighbouring district Ábborašša with its gold mining activity. The high concentration in Essand was probably a result of acid rain due to atmospheric transport of industrial pollution from Europe which has previously been reported to affect southern Norway to a greater extent than northern part of the country [34–36].

3.2. Lead (Pb)

Pb was detected in 92% of the meat samples. Concentrations of the Pb among the various grazing districts did not vary to the same degree as for As and Cd (Table 2).

The study on Finnish reindeer by Rintala and colleagues had also reported little differences in concentrations of Pb among different Lapland areas [15]. Even so, the Pb concentrations detected in this study were much lower (10 to 20 times) than those reported in reindeer meat by the same reference above. The Pb concentration from Fávrrosorda in the present study (GM= 7.4, AM= 8.6 ng/g ww) was an exception and could be compared to the concentration from the southern, western and eastern parts of Finnish Lapland, in which the Pb concentration was half of that detected from northern Lapland [15]. The mean Pb concentration of 2.14 µg/g ww previously measured from the Karelian reindeer muscle in Russia was 289 times higher than the greatest level of GM= 7.4 ng/g ww measured in the present study [33].

The Pb concentration was five times higher ($p < 0.01$) in Fávrrosorda (a neighbouring district) compared to Ábborašša (gold mining facilities). This suggests that the deposition of lead occurs in the neighbourhood of the mining area rather than the mining area itself, which is based on the wind direction and is in accordance with results presented elsewhere [19]. The Pb concentration measured from Fávrrosorda in this study was in good agreement with those from unknown point sources in Greenlandic caribou and reindeer muscles from Isortoq (GM = 7 ng/g ww) in the north and Akia (GM = 6 ng/g ww) in the middle part of the country [17]. However, Pb concentrations in the remaining districts in the present study were two to four times lower than those reported by the Greenlandic study.

As for Cd, high Pb concentration in Fávrrosorda might be due to atmospheric deposition from the neighbouring district Ábborašša with its gold mining activity.

Table 2. Concentrations of toxic elements (ng/g wet wt) in reindeer meat (n = 90) from the ten different grazing districts.

		District									
		ES-Varanger	Pasvik	Varanger P	Spierttagáisá	Karasjok	Ábborašša	Fávrrosorda	Tromsdalen	Kanstadfjord	Essand
Cd	GM	0.9	1.5	1.4	0.9	0.9	2.2	4.3	0.9	5.1	3.6
	AM ± SD	0.9 ± 0.7	1.9 ± 2	2.9 ± 3.5	0.9 ± 0.5	1.1 ± 0.6	2.9 ± 2.5	4.8 ± 1.9	0.9 ± 0.3	4.7 ± 2.9	4.4 ± 2.8
	Min-Max	0.5–2.8	0.8–7.6	0.4–10.4	0.4–2.1	0.6–2.6	0.9–8.2	1.5–7.4	<0.37–1.4	1.6–10.8	1.1–8.5
	CV%	69	103	121	53	54	85	41	31	63	64
	n	10	10	10	10	10	10	10	10	8	10
Pb	GM	0.4	1.9	1.6	1.2	1	1.6	7.4	0.9	1.7	2.7
	AM ± SD	0.7 ± 0.5	2.3 ± 1.9	2.1 ± 1.4	1.9 ± 2.1	2.2 ± 2.5	1.7 ± 1.6	8.6 ± 4.9	1.9 ± 2.5	2.5 ± 1.9	2.9 ± 1.2
	Min-Max	<0.23–1.5	1–7.8	0.3–4.4	<0.22–5.8	<0.12–8.3	0.18–4.6	3.1–20	<0.1–7.3	0.27–5.6	1.6–5.9
	CV%	81	84	68	111	114	96	58	128	79	42
	n	10	10	9	9	10	10	10	10	10	10
As	GM	106	48	10	5.9	8.8	6.9	1.9	1.5	3.9	7.2
	AM ± SD	107 ± 17	52 ± 19	11 ± 3.3	6.4 ± 2.5	8.9 ± 1.5	6.9 ± 1.2	2.2 ± 1.1	1.6 ± 1.8	4.1 ± 0.7	7.3 ± 1.7
	Min-Max	84–135	27–79	7–16	2.5–11	6.3–11	4.9–8.4	0.9–3.9	0.7–2.9	2.9–4.9	4.7–10
	CV%	16	38	30	39	17	17	49	48	17	24
	n	9	10	10	10	10	10	10	10	10	10
Cu	GM	1,700	1,700	1,300	1,400	1,500	1,600	1,200	1,300	1,300	1,600
	AM ± SD	1,700 ± 292	1,702 ± 296	1,326 ± 194	1,444 ± 188	1,549 ± 276	1,564 ± 231	1,208 ± 274	1,306 ± 167	1,287 ± 275	1,608 ± 295
	Min-Max	1,300–2,100	1,300–2,100	1,100–1,600	1,300–1,900	1,200–2,100	1,300–2,100	800–1,700	1,100–1,600	800–1,600	1,300–2,400
	CV%	17	17	15	13	18	15	23	13	21	18
	n	10	10	10	10	9	10	9	10	10	10
Ni	GM	<6.7	<7.2	0.8	<9.5	<11	<7.1	1.2	1.3	<5.7	<1.5
	AM±SD	<6.7	<7.2	1.2±1.1	<9.5	<11	<7.1	2.7±2.5	2.1±4.5	<5.7	<1.5
	Min.-Max	<6.7–<14	<7.2–<13	<0.4–3.6	<9.5–<39	<11–11	<7.1–<18	<0.1–7.1	<0.9–11	<5.7–<20	<1.5–5.8
	CV%			93				93	162		
	n	10	10	10	10	10	10	10	9	10	10
V	GM	<0.01	<0.02	<2.7	0.2	<0.26	<0.24	<1.9	<0.9	<0.09	<0.02
	AM ± SD	<0.01	<0.02	<2.7	0.3 ± 0.6	<0.26	<0.24	<1.9	<0.9	<0.09	<0.02
	Min-Max	<0.01–0.2	<0.02–0.3	<2.7–<3.8	<0.09–3.8	<0.02–8.3	<0.24–0.39	<1.9–<3.6	<0.9–3.5	<0.09–0.2	<0.02–<0.2
	CV%				197						
	n	10	10	10	9	9	10	10	10	10	10

Legend: GM = Geometric mean. AM = Arithmetic mean. SD = Standard deviation. Min.-Max. = Minimum - Maximum. < X = below limit of detection (LOD). CV = Coefficient of variation. n= number of observations; **Note:** Numbers of observations less than 10 in some districts (n = 4 districts) were due to exclusion of outliers.

Table 3. Age stratified Cd concentration (ng/g ww) in reindeer meat from the grazing districts with mixed age groups.

	District	
	Eastern Sør-Varanger	
Cd Concentration	Calves/Young (n = 8) (10 months–1.5 years)	Old (n = 2) (>2 years)
GM	0.74	1.5
AM	0.79	1.8
Min-Max	0.5–1.5	0.8–2.8
	Varanger Peninsula	
Cd Concentration	Calves/Young (n = 6) (10 months–1.5 years)	Old (n = 4) (>2 years)
GM	0.57	5.7
AM	0.58	6.3
Min-Max	0.4–0.7	2.8–10
	Kandstadfjord/Western Hinnøy	
Cd Concentration	Calves/Young (n = 6) (10 months–1.5 years)	Old (n = 4) (>2 years)
GM	3.4	5.2
AM	4.3	5.2
Min-Max	1.6–11	4.1–6.2

Note: The district Pasvik/Sør-Varanger: Calves/Young (n = 9): GM = 1.3, AM = 1.4, Min-Max: 0.8–2.1 ng/g ww; Old (n = 1): 7.6 ng/g ww.

3.3. Arsenic (As)

As was detected in all meat samples (100%). The As was the element that showed most of the geographical variations (Table 2) among the studied elements. An east-west downward geographical gradient was observed for As, with the highest concentrations measured in the east (the three districts in the vicinity of the Russian border). However, a north-south trend (highest in the south) for As, Cd and Pb has previously been found in Norwegian surface soils, coniferous forest ecosystems and some herbivorous animals [2,26,37–39].

Due to the wind frequency towards the districts of Eastern Sør-Varanger and Pasvik/Sør-Varanger, the high As concentration in these districts could be explained in this study by the As being released from the smelter activity in the Russian town of Nikel, which was further reported to be higher during summer as compared to winter [20]. Furthermore, the wind from the east (E) brings waste from the town of Nikel towards the direction of Svanvik/Passvik, while the wind from the north (N) and north-east (NE) brings waste from Zapoljarny during summer. The dominating wind in winter from the south (S) and south-west (SW) brings waste from Nikel to Jarfjord. However, the elevated As and Ni concentrations below the LOD in these two districts indicates additional As sources such as mining and geogenic sources in this area. The As concentrations in meat from Eastern Sør-Varanger (GM = 106.1 ng/g ww) and Pasvik/Sør-Varanger (GM = 47.9 ng/g ww) from this study were in agreement with those formerly revealed in liver samples from reindeer in the same area when compared with samples from other areas in the County [40]. By comparison, Bernhoft and colleagues reported median As concentration of 0.035 µg/g ww in reindeer liver collected from north western Russia [32].

The district of Ábborašša (gold mining activity) displayed As concentration (GM) three times higher than that found in the neighbouring district of Fávrrosorda, which could be explained by pollution from the mining work in the district, as As has been reported to be associated with gold mineralization [41,42]. In addition, the district of Spierttagáisá (military activity) revealed a lower As concentration (GM = 5.9 ng/g ww) than that (GM = 8.8 ng/g ww) found in the neighbouring district of Karasjok West, although the difference was not statistically significant. The As concentration in samples from Essand/Røros was similar to those detected in Ábborašša (gold mining activity) and Spierttagáisá (military activity). This could be due to the long-range atmospheric pollution from Europe, as a previous study has demonstrated that southern Norwegian areas are more prone to atmospheric pollution from Europe than the northern areas [43]. Contribution from soil due to geogenic sources could also lead to elevated As level in the surrounding environment. In accordance with previous study, the reindeer's age and sex had no effect on the arsenic concentration [32].

3.4. Copper (Cu)

Cu was detected in all meat samples (100%) and had the highest concentration among all the studied elements (GM = 1,439 ng/g ww). Cu concentrations did not vary much among the districts (Table 2). Study on Finnish reindeer had also reported little differences in concentrations of Cu among different Lapland areas [15].

Cu concentrations detected in this study were in agreement with those reported from Finnish Lapland and Russian Karelia, and were half of those reported from Greenlandic reindeer muscle [15,17,33]. The results for the effect of age and sex on Cu concentrations from this study stand in contradiction to those reported by Bernhoft and colleagues, in which hepatic Cu concentration was higher in reindeer calves than in adult females, and higher in adult males than in adult females [32].

Districts that displayed relatively high Cu concentrations could be due to contamination from local point sources (gold mining in Ábborašša) and atmospheric transportation in Pasvik and Eastern Sør-Varanger (Russian towns of Nikel with its nickel smeltery and Zapoljarny with its briquette industry) [16].

3.5. Nickel (Ni)

Ni was detected in samples from five of the 10 districts (Fávrrosorda, Tromsdalen, Varanger Peninsula, Karasjok West and Essand/Røros) and in 20% of the total meat samples (n = 100). The Ni detection percentage within these five districts varied as follows: 80% (Fávrrosorda), 60% (Tromsdalen), 40% (Varanger Peninsula) and 10% in the districts of Karasjok West and Essand/Røros.

The districts of Varanger Peninsula, Fávrrosorda and Tromsdalen had geometric/arithmetic mean Ni concentrations above the LOD (Table 2), in which the Ni concentrations were comparable.

Previous studies on human Ni exposure along the Norwegian-Russian border have shown that urinary Ni concentrations in this area were no higher than the ones exhibited in other populations [44]. No data were available on Ni and V from reindeer muscle other than that of the Ni concentration from the Karelian Russian reindeer, which was reported to be below the LOD [33]. Nonetheless, the Ni concentrations formerly reported in liver and kidney samples from reindeer originating from the

Norwegian-Russian border (Sør-Varanger, north eastern Norway and Rybatsjy Ostrov, north western Russia) had exhibited geographical variations and were much higher in levels than those documented in this study due to tissue differences [16,32].

3.6. Vanadium (V)

V was detected in 21% of the total meat samples (n = 100) and in seven of the 10 districts (Kanstadfjord, Tromsdalen, Pasvik, Spierttagáisá, Karasjok West, Eastern Sør-Varanger and Ábborašša). The detection percentages within these seven districts varied as follows: 50% (Spierttagáisá), 40% (Kanstadfjord), 30% (Eastern Sør-Varanger, Pasvik and Ábborašša) and 10% in Tromsdalen and Karasjok West. The district Spierttagáisá was the only one that had geometric/arithmetic mean V concentration (0.2/0.3 ng/g ww) above the LOD.

V has been described as a useful environmental pollution marker for the potential release of toxic elements from fossil fuels and oil refinery processes [45]. Results on V from Canadian (Yukon) caribou kidney reported an average concentration of 0.42 µg/g dry weight, 79.9% moisture [3].

3.7. Risk Assessment of Toxic Elements from Reindeer Meat Consumption

Reindeer meat is consumed as fresh, smoked or dried products in Norway, with the highest consumption among the indigenous Sami people, particularly reindeer herders and their families, compared to ethnic Norwegians [46]. The average consumption is generally low compared to other meat types and constitute approximately 23 g and 70 g/week for low and high consumers in areas with both Sami and ethnic Norwegians [47,48]. The estimated human toxic elements intake from reindeer meat based on the high consumption (70 g meat/week) were 0.01 and 0.01 µg/kg human body weight for Cd and As, respectively. These estimations (monthly for Cd and weekly for As) were much lower than permissible tolerable monthly intake (PTMI) for Cd (25 µg/kg human body weight) and weekly intake (PTWI) of 15 µg/kg human body weight for As [49,50]. The FAO/WHO-JECFA has recently withdrawn the PTWI limit of 25 µg/kg human body weight for Pb due to its association with a decrease of at least three intelligence quotient (IQ) points in children and an increase in systolic blood pressure of approximately 3 mmHg in adults [49]. No new PTWI limit was established for Pb. Nevertheless, weekly human Pb exposure from reindeer meat of 0.002 µg/kg human body weight was 12,500 times lower than the previous PTWI limit. The estimated daily Cu intake from reindeer meat in this study was about 0.0003 mg/kg body weight which was well below the acceptable daily intake (ADI) of 0.5 mg/kg body weight [51]. There are no established PTWI/PTMI limits for Ni and V. Nevertheless, concentrations of Ni and V detected in the present study were considerably lower than tolerable upper intake levels of 1 mg and 1.8 mg per day that have been reported elsewhere for Ni and V, respectively [52].

Estimation of human exposure to toxic elements through reindeer meat was previously done based on dietary data from a questionnaire on the Population-based Health and Living Conditions in areas with Sami and Norwegian populations–The SAMINOR Study and the equation described in one of our previous studies [47,48]. The dietary data from the questionnaire and calculations based on the equation mentioned above have revealed considerably low human exposure to toxic elements through meat and other edible tissues from reindeer.

The presence of individual animals with elevated toxic element concentrations (outliers) was investigated further by relating these concentrations to concepts such as maximum levels (ML), acceptable daily intakes (ADI), professional tolerable intakes (PTI) and healthy animals' parameters [12,49,50,53]. For instance, the elevated concentrations of outliers in cases of cadmium (13 and 15 ng/g ww) and lead (23 and 28 ng/g ww) have been estimated to constitute 20% and 40% of the maximum levels (ML) set for Cd, and 20% and 30% of the ML set for Pb [53]. Consequently, the elevated concentrations measured in this study should not be an issue of concern to consumers.

4. Conclusions

Arsenic and cadmium were the elements that exhibited most of the geographical differences. No clear geographical trend was observed except for the east-west gradient for As, with the highest concentrations measured in the east (near the Russian border). The presence of older animals (>2 years) displayed an age effect as animals more than 2 years old demonstrated higher cadmium concentration than ones <2 years old, whereas sex had no significant effect on toxic element concentrations. The concentrations of the toxic elements detected in this study were low and considerably below the maximum levels (ML) and permissible tolerable weekly/monthly intake (PTWI/PTMI) limits available for hazardous toxic elements. This suggests that the use of reindeer meat as human food is safe in relation to toxic elements, even along the Norwegian-Russian border where previous studies have revealed elevated concentrations in liver and kidneys from reindeer [16]. Based on the result from the present study, we have no reason to warn people against eating reindeer meat. Further investigations regarding arsenic findings are needed.

Conflict of Interest

The authors declare no conflict of interest.

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Supplemental Material

Table S1. Geographical differences in concentrations of the studied toxic elements among the ten grazing districts.

Toxic element	n	District X: District Y (P-value)			
Cd	98				
		Kanstadfjord: Tromsdalen **	**Kanstadfjord:** Spierttagáisá **	**Kanstadfjord:** Karasjok **	**Kanstadfjord:** E. S-Varanger **
		Tromsdalen**: Fávrrosorda** **	Tromsdalen**: Essand** **	**Fávrrosorda:** Varanger P **	**Fávrrosorda:** Pasvik *
		Fávrrosorda: Spierttagáisá **	**Fávrrosorda:** Karasjok **	**Fávrrosorda:** E. S-Varanger **	Spierttagáisá**: Essand** **
		Karasjok**: Essand****	E. S-Varanger**: Essand** **		
Pb	98				
		Tromsdalen**: Fávrrosorda** **	**Fávrrosorda:** Spierttagáisá *	**Fávrrosorda:** Karasjok **	**Fávrrosorda:**E. S-Varanger **
		Fávrrosorda: Ábborašša **	**Pasvik:** E. S-Varanger *	E. S-Varanger**: Essand** **	
As	99				
		Kanstadfjord: Tromsdalen **	**Kanstadfjord:** Fávrrosorda **	Kanstadfjord**: Varanger P** **	Kanstadfjord**: Pasvik** **
		Kanstadfjord: Karasjok **	Kanstadfjord**:E. S-Varanger** **	Kanstadfjord**: Essand** *	Kanstadfjord**: Ábborašša** *
		Tromsdalen**: Varanger P** **	Tromsdalen**: Pasvik** **	Tromsdalen**: Spierttagáisá** **	Tromsdalen**: Karasjok** **
		Tromsdalen**: E. S-Varanger** **	Tromsdalen**: Essand** **	Tromsdalen**: Ábborašša** **	Fávrrosorda**: Varanger P** **
		Fávrrosorda**: Pasvik** **	Fávrrosorda**: Spierttagáisá** **	Fávrrosorda**: Karasjok** **	Fávrrosorda**:E. S-Varanger** **
		Fávrrosorda**: Essand** **	Fávrrosorda**: Ábborašša** **	Varanger P**: Pasvik** **	**Varanger P:** Spierttagáisá *
		Varanger P**:E. S-Varanger** **	**Pasvik:** Spierttagáisá **	**Pasvik:** Karasjok **	Pasvik**: E. S-Varanger** **
		Pasvik: Essand **	**Pasvik:** Ábborašša **	Spierttagáisá**: E. S-Varanger** **	Karasjok**: E. S-Varanger** **
		E. S-Varanger: Essand**	**E. S-Varanger:** Ábborašša**		
Cu	98				
		Kanstadfjord: **Pasvik** *	Kanstadfjord**: E. S-Varanger** *	Fávrrosorda**: Pasvik** **	Fávrrosorda**: E. S-Varanger** **
		Fávrrosorda**: Essand** *	Fávrrosorda**: Ábborašša** *		

Legend: n = number of observations. Level of significance *: $0.01 < p < 0.05$, **: $p < 0.01$. **Note:** Districts in **bold** indicate the highest concentrations.

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Article

Selected Vitamins and Essential Elements in Meat from Semi-Domesticated Reindeer (*Rangifer tarandus tarandus L.*) in Mid- and Northern Norway: Geographical Variations and Effect of Animal Population Density

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Abstract: Meat samples (n= 100) were collected from semi-domesticated reindeer originating from 10 grazing districts in Norway. We aimed at studying concentrations, correlations, geographical variations and effect of animal population density on vitamins A, B3, B7, B12 and E, and calcium, iron, zinc, selenium, chromium and cobalt. Mean concentrations of vitamins A, B3, B7; B12 and E were < 5µg, 6.6 mg, < 0.5 µg, 4.7 µg and 0.5 mg/ 100 g wet weight, respectively. Concentrations of calcium, iron, zinc, selenium, chromium and cobalt were 4.7 mg, 2.8 mg, 6.4 mg, 19.4 µg, 1.7 µg and 0.5 µg/ 100 g wet weight, respectively. Vitamin E and selenium were the nutrients that exhibited the largest geographical variations ($p< 0.05$), although no geographical gradient was observed for any of the studied nutrients. Age had a significant effect on zinc and selenium concentrations. Iron was significantly positive correlated with calcium ($r= 0.3416$, $p< 0.01$) and vitamin B12 with zinc ($r= 0.35$, $p< 0.05$). Reindeer from districts with low animal population density had significantly higher selenium concentration than those from districts with medium and high population densities ($p< 0.01$). Reindeer meat contained higher vitamin B12, iron, zinc and selenium concentrations when compared to Norwegian beef, lamb, mutton, pork and chicken meat.

Keywords: Sami; reindeer; meat; vitamins; essential elements; geography; animal population density

1. Introduction

Red meat consumption has previously been stated as a risk factor for cardiovascular diseases (CVD) and colon cancer due to the fatty acids composition [1]. In contrast to this, reindeer meat has a desirable fatty acid profile. Moreover, the limited data on reindeer meat has revealed higher nutrient contents (*e.g.*, vitamin B12 and iron) when compared to other red and white meat types [2]. Reindeer as ruminant animals preserve the ability of synthesizing a large amount of vitamin B12 (when cobalt is present) as a result of the rumen microbial activity. The vitamin B12 is then stored in liver and meat, and represents three to five times the amount found in meat from mono-gastric animals such as pigs and poultry [3].

Reindeer husbandry in Norway is restricted by law to the Sami indigenous people and is based on a free range herding system all the year around [4]. The reindeer moves between different grazing districts during the year due to the varying natural conditions and nutritional demands [5]. These movements are man-controlled as well as season-dependent (summer vs. winter). The natural conditions such as geology, degrees of snow depth and formation of ice crusts, length of summer season, presence and intensity of parasites especially warble flies (*Hypoderma tarandi*) and possible replacement of vegetation system with species that are not favoured by reindeer vary across geography [6]. Furthermore, the varying density of reindeer population and grazing areas available for reindeer pasture (capacity) may as well as result in different degrees of supplement-feed (*e.g.,* pellet concentrates and hay) practice as districts suffered overgrazing are more likely to rely on such practice more often than ones less affected. These variations may lead to different pasture qualities and varied degrees of pasture utilities by reindeer among grazing districts. Thus, varied nutrient concentrations in meat from reindeer are expected between districts as indicated in our previous study [2a].

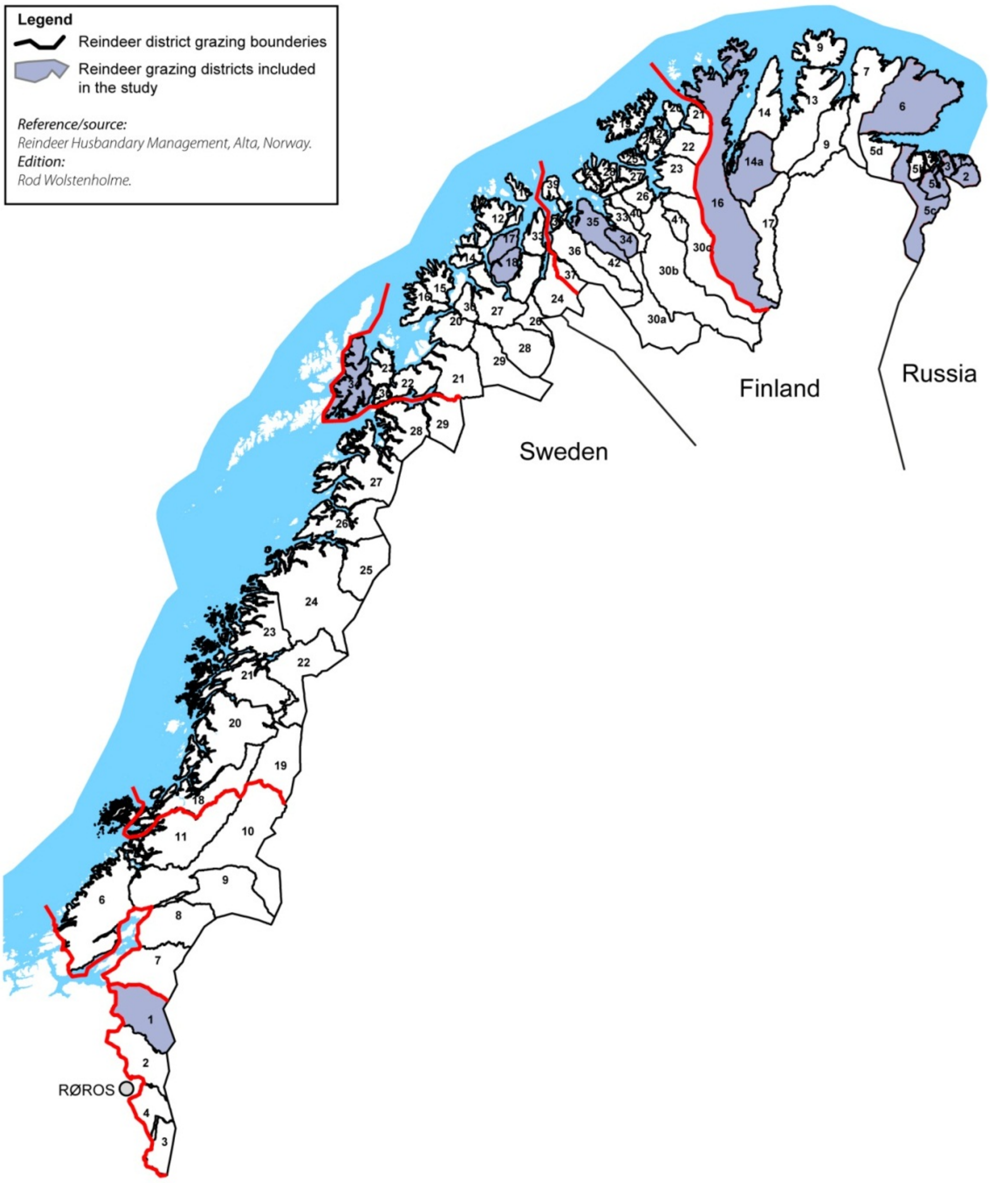
The main purpose of this work was to increase knowledge about nutrients in reindeer meat by studying geographical variations and effect of animal population density on selected vitamins and essential elements in meat from semi-domesticated reindeer originating from mid- and northern Norway.

2. Experimental Section

2.1. Geographical area

The meat samples (n=100) were collected from ten grazing districts (equal number, n=10) from four different Counties distributed as follows: Finnmark County (7 districts), Troms County (one district), Nordland County (one district) and Sør-Trøndelag (one district). The selection of the ten districts was based on obtaining broad geographical and animal population density ranges (Figure 1). The selection of seven districts from Finnmark which is the biggest and the northernmost Norwegian County was based on the fact that this County has the largest number of semi-domesticated reindeer and 50% of the total number of the reindeer grazing districts in Norway.

Figure 1. Map of the study area



2.2. Sample collection and preparation

Meat samples (n=100) from the dorsal neck region (pure muscles) were randomly collected from reindeer in ten different grazing districts located in northern and middle of Norway in the period from October-December 2008 and September-December 2009. The randomization was based on the fact that reindeer from different herds within a same district were transported together to the slaughterhouse and slaughtered at a same day. Thus, reindeer from almost all herds within the same district were represented in sampling process. For comparison reasons we aimed at collecting samples from young animal (1.5 years old). However, due to limited availability of 1.5 years old in some districts (n= 4), calves (approximately10 months old) and old animals (> 2 years old) with the respective proportions of 12% and 11% were selected. Thus, 77% of the total samples consisted of young animals. Age of the reindeer was obtained directly from the tags attached to animals' carcasses when they passed the weighing post in the slaughterhouses. There were 52 males and 48 females of the 100 selected reindeer. All samples (composed of all dorsal neck muscles) were collected directly after the slaughter/ dressing process and carcass weighing in pre-marked plastic bags prior to further division in dedicated glass containers. Glass containers with samples for vitamins analyses were covered with aluminium foil to prevent them from being exposed to light. The samples were kept cool in a cooling box (approximately 4° C) immediately after collection and division, and then moved the same day to a –20° C freezer until they were freighted frozen to the laboratory for analysis. All animals from which samples were collected were healthy ones, *i.e.*, passed the ante- and post mortem inspection.

Due to the high cost of vitamin analysis, pooled samples of raw meat (with aliquots of muscles) from each five animals originated from the same district (two pooled samples per district) were prepared.

2.3. Chemical analyses

2.3.1. Vitamins

The analyses of vitamins were done by GBA-Food, Hamburg, Germany according to methods of German Food Act LMBG § 35, LFGB § 64 and standard methods of Association of Official Analytical Chemists (AOAC) [7]. The laboratory is accredited for the methods used in the analyses according to Staatliche Akkreditierungsstelle Hannover, AKS-P-20213-EU. The vitamin E concentration is composed of all tocopherols (α, β, γ and Δ tocopherols), whereas vitamins A and B3 concentrations refer to retinol and niacin, respectively. Vitamins A, B3, B12 and E were analysed using normative reference § 35 LMBG/ DIN EN 12823, AOAC 944.13, AOAC 952.20 and § 64 LMBG/ DIN EN12822, respectively. For vitamin B7, an internal method was employed using hot acid (HCl) hydrolysis in the extraction, and microbiological/ turbidimetric method in detection and external standard in quantification. Measurement of uncertainty for vitamins analyses were given as extensive uncertainty measurement according to (Guide to the expression of uncertainty in measurement, ISO, Geneva, Switzerland) estimated by a covering factor of 2 (95% confidence interval). The selection of the studied vitamins and essential elements was based on data from a previous study by the same authors [2a].

2.3.2. Essential elements

The samples were separately decomposed using a microwave oven (Ethos Plus, Milestone Inc., Shelton, CT, USA) and concentrated supra-pure HNO_3 (5 ml) and H_2O_2 (3 ml) were added to the decomposed sample (0.6 – 0.7 g) before undergoing the microwave oven treatment. Hence, the following temperature regimes were subsequently used in the microwave oven: 20-50° C (5 min.), 50-100° C (10 min), 100-180° C (5 min.) and 180° C (15 min.). After cooling down the heated decomposed sample, the solution was diluted to 50 ml. The sample solution was analysed using an inductively coupled plasma high resolution mass spectrometer (ICP-HRMS), Bremen, Germany. All standards and calibration solutions contained 1 ppb Rhenium (Re) as the internal standard, together with 1% nitric acid (HNO_3). The calibration curve was verified by standard quality control (QC) sample (Spex Standard, Ultra Scientific, North Kingston, RI, USA) in compliance with ANSI/NCSLZ-540-1 and ISO 90001. The QC material SRM-1566a (Oyster tissue) was obtained from the National Institute of Standards and Technology (NIST), Maryland, USA. The resolutions used for essential elements were low (10) for (Zn), middle (20) for (Ca, Fe), and high (30) for (Se). Lens-adjustment was optimized daily to ensure maximum intensity and top separation. The analyses were done by the NILU

(Norwegian Institute for Air Research) Laboratory, Kjeller, Norway. The laboratory is accredited for the methods used in the analyses according to NS-EN ISO/IEC 17025, No. TEST008. The limits of detection (LODs) for the studied essential elements were three times standard deviation (SD) of the laboratory blanks, whereas the limits of quantifications (LOQs) were 10 times the SD of the blanks, decomposed simultaneously with the meat samples.

2.4. Statistical Procedures

STATA/SE 12.0 for Windows (STATA Corp. College Station, TX, USA) was used for statistical analyses. Laboratory results for vitamins and essential elements below the limit of detection (LOD) were given a numeric value of zero. A standardized residuals test was conducted in order to check for possible outliers in essential elements concentrations (observations that were more than 3 standard deviations from the mean). All outliers were removed (n= 7, details in results and discussion parts).

Analysis of variance and covariance (ANOVA), with the specific essential element as a dependent variable and districts/animal density, age and sex as independent variables, was used to test for the effect of animal population density on essential element concentrations. Animal population density was categorized, on the basis of the 10 districts included in the study, into 3 groups; low (0.8-1.9, n= 5 districts), medium (3-5.3, n= 4 districts) and high (6-13.7, n= 1 district) animals/ km^2. The animal population density (animal/ km^2) for each reindeer grazing district was calculated according to available data and based on the following formula:

Animal population density = Number of reindeer in a specific district/ total area of the district.
The district areas were given in square kilometre (km^2).

For vitamins, the final model consisted of the specific vitamin as dependent variable and animal density as an independent variable. Due to presence of some pooled samples (for vitamins analyses) with mixed age and sex groups from 4 districts (n= 8 pooled samples), additional statistical analyses were done to look into the effect of age and sex on vitamin concentrations using pooled samples from the homogenous age and sex groups (more details on statistical analysis for vitamins in discussion part). The relationship between vitamins B3 and B12, and animal population density was U-formed (non-linear), therefore we log transformed the animal density variable and performed a regression analysis on vitamins B3 and B12.

Bonferroni multiple comparison tests were used to test for significant differences in concentrations of vitamins and essential elements among the grazing districts using the specific vitamin/ essential element as a response (dependent) and the districts as a factor variable. Welch test was used whenever homogeneity of variance was violated. The overall vitamin concentrations were given as grand mean (mean of the different pooled samples, n= 20 pooled samples composed of 100 individual samples, *i.e.*, 5 individual samples in each pooled sample originating from the same grazing district). Pairwise correlation tests with Bonferroni-adjusted significance level were used to test for possible correlations within/ between vitamins and essential elements. The level of statistical significance was set at $p < 0.05$ for all the statistical analyses.

3. Results

Results of essential element concentrations were presented as mean per 100 g raw meat, standard deviation (SD) and Minimum-Maximum. For vitamin concentrations in samples from the different districts, the results were presented as concentrations of the two pooled samples from each district. Furthermore, the overall vitamin concentrations were given as grand mean (mean of the 20 pooled samples).

3.1. Vitamins

Vitamin A was below the limit of detection (< 5 µg/100 g) in all pooled samples, except for one from Essand (Røros) with a concentration slightly above the limit of detection (5.7 µg/100 g), whereas vitamins B3, B12 and E were above the limits of detection (LOD) in all pooled samples. Vitamin B7 was detected (> 0.5 mg/ 100 g) in 5 pooled samples from five districts with a minimum concentration equal to the LOD and a maximum one of 0.3 µg above the LOD. The overall vitamin concentrations are presented in Table 1.

Table 1. The overall grand mean concentrations of the studied vitamins per 100g raw meat (n= 20 pooled samples composed of 100 individual meat samples)

	Mean	SD	Minimum	Maximum	% PSALOD	LOD
Vitamin A (µg)	<5	--	<5	5.7	5%	5
Vitamin B3 (mg)	6.6	0.8	4.7	7.7	100%	0.2
Vitamin B7 (µg)	<0.5	0.1	<0.5	0.8	25%	0.5
Vitamin B12 (µg)	4.7	1.7	1.7	8.8	100%	0.1
Vitamin E (mg)	0.5	0.2	0.3	0.8	100%	0.01

Legend: LOD= Limit of detection. SD= Standard deviation. % PSALOD = Percentage of pooled samples (n= 20) above the LOD. **Note:** Standard deviation (SD) is not given for vitamin A as only one pooled sample (n=5 individual samples) had a concentration above the limit of detection.

No further statistical analyses were done on vitamin A and B7 due to low percentage of samples above the LOD. A significant positive correlation was observed between vitamin B12 and Zn ($r = 0.35$, $p < 0.05$).

3.2. Essential elements

The studied essential elements were detected in all meat samples (100%), except for cobalt (Co) in which two samples had concentrations below the limit of detection (<0.12 µg/ 100g). The overall mean concentrations of essential elements are presented in Table 2. Concentrations of selenium (Se), cobalt (Co), zinc (Zn) and chromium (Cr) were significantly different ($p < 0.05$) between some of the districts. Sex had not significant effect for any of essential elements, whereas age had a significant effect on Zn concentrations ($F = 3.26$, $p < 0.05$).

Table 2. Overall mean concentrations of the selected essential elements per 100g raw meat

	n	Mean	SD	Minimum	Maximum	LOD	LOQ
Ca (mg)	99	4.7	1.3	1.9	10.8	0.07	0.24
Fe (mg)	99	2.8	0.7	1.5	4.6	0.01	0.03
Zn (mg)	100	6.4	1.6	2.6	10.7	0.01	0.03
Se (µg)	98	19.4	10.1	7.1	51.5	1.55	5.15
Cr (µg)	99	1.7	2.9	0.1	16.2	0.10	3.45
Co (µg)	98	0.5	0.3	<0.1	1.7	0.09	0.31

Legend: SD = Standard deviation. LOD = Limit of detection. LOQ = Limit of quantification.
Note: Number of samples (n) less than 100 for some essential elements were due to exclusion of outliers (n= 7) from statistical analyses.

Essential element concentrations in some samples (n= 7) were outliers and have been removed from the statistical analyses. The detected outliers were: One animal (1.5 year) from Karasjok West with a calcium concentration of 56.9 mg/ 100g; one animal (calf) from Pasvik with a Fe concentration of 60.8 mg/ 100g; two animals (1.5 year) from Eastern Sør-Varanger with Se concentrations of 68.1 and 68.4 µg/ 100g; one animal (1.5 year) from Karasjok West with a Cr concentration of 82.4 µg/ 100g; two animals (1.5 year) from Spierttagáisá with Co concentrations of 21.3 and 22.6 µg/ 100g.

Calcium concentrations were in the range of 3.8 - 5.4 mg/ 100g, whereas iron concentrations were in the range of 2.2 - 3.5 mg/ 100g. The interaction between the districts and age in zinc and selenium models were significant ($F= 2.59$, $p< 0.05$ and $F= 6.47$, $p< 0.01$, respectively), therefore stratified analyses were provided in Tables 3 and 4.

Table 3. Age stratified Zn Concentration (mg/ 100g ww) in reindeer meat from districts with mixed age groups

District	Mean ± SD Zinc Concentration (Minimum- Maximum)			
	Calves	n	Young and Older	n
Varanger Peninsula	5.5±1 (4.4-6.5)	3	8.4±1.5 (6.8-10.1)	7
Eastern Sør-Varanger	-- [a] -- [a]	1	6.9±1.7 (4.7-10.4)	9
Pasvik/Sør-Varanger	4.2±0.8 (2.8-5.1)	7	4.4±2.3 (2.6-7)	3
Kanstadfjord	-- [b] -- [b]	1	6.8±0.5 (6.3-7.7)	9

[a] One calf with Zn concentration of 5.1 mg/100g.
[b] One calf with Zn concentration of 5.7 mg/100g.

Table 4. Age stratified Se Concentration (µg/ 100g ww) in reindeer meat from districts with mixed age groups

District	Mean ± SD Se Concentration (Minimum- Maximum)					
	Calves and older animals		n	Young animals		n
Varanger Peninsula	16.9±5	(10.7-22.3)	7	20.5±4.1	(17.1-24.9)	3
Eastern Sør-Varanger	43.4±7.2	(37.9-51.5)	3	44.4±4.5	(40.1-51.5)	5
Pasvik/Sør-Varanger	30.2±5.9	(24.6-43.4)	8	17.2±3.4	(14.7-19.6)	2
Kanstadfjord	25.3±4.7	(17.1-28.9)	5	28.4±2.2	(25.6-30.7)	5

Young animals (1.5 years) and older ones (> 2 years) demonstrated no significant difference in Zn concentration between districts (Mean= 6.6 and 7.1 for young and older animals, respectively), while calves showed a lower mean Zn concentration (4.72, $p < 0.01$). Moreover, there was no significant difference in Se concentrations between calves and older animals (25.6 and 25.8 µg/ 100g, respectively), whereas young animals (1.5 year) had a significantly lower Se concentration (16.6 µg/ 100g, $p < 0.05$).

Chromium and cobalt were the least abundant essential elements in reindeer meat and had mean concentrations of 1.7 and 0.5 µg/ 100g, respectively. Significant positive correlations were revealed only between calcium and iron ($r = 0.34$, $p < 0.05$), and zinc and vitamin B12 ($r = 0.35$, $p < 0.5$).

3.2 Geographical variations

Geographical variations were revealed in concentrations of vitamins B3, B12, E, zinc, selenium, cobalt and chromium, with vitamin E and selenium demonstrating the largest geographical variations. None of the studied nutrients demonstrated a geographical gradient as could be seen in Tables 5 and 6.

The district Essand (Røros) was distinguished by being the only district from which vitamin A concentration was above the LOD (one pooled sample). Additionally, Eastern Sør-Varanger, Karasjok West, Ábborašša, Tromsdalen and Essand were the districts that had vitamin B7 concentrations (from one pooled sample each) above the LOD. The district Tromsdalen was distinguished by its high vitamins B3, E and Cr, whereas Eastern Sør-Varanger, Pasvik and Kanstadfjord by their high Se concentrations.

Furthermore, the districts Eastern Sør-Varanger, Pasvik, Fávrrosorda and Spierttagáisá were distinguished by their high Co concentrations.

Table 5. Concentration of vitamins per 100g reindeer meat (ww) from the different districts

	Pooled sample 1- Pooled sample 2 (n= 20 pooled samples)			
District	B3 (mg)	B7 (µg)	B12 (µg)	E (mg)
Eatern Sør-Varanger	5.1-6.6	<0.5-0.5	5-5.7	0.5- 0.6
Pasvik	7.2-7.6	< 0.5-<0.5	3.3-5	0.5-0.5
Varanger Peninsula	4.7-6.4	<0.5-<0.5	3.2-8.8	0.4-0.8
Spierttagáisá	5.8-6.7	<0.5-<0.5	3.9-4.1	0.5-0.7
Karasjok West	6.7-7.1	<0.5-0.7	2.7-7.8	0.3-0.3
Ábborašša	6.7-7.2	<0.5-0.6	4.7-5	0.4-0.5
Fávrrosorda	5.9-7	< 0.5-<0.5	5.9-6.1	0.4-0.5
Tromsdalen	7.5-7.7	<0.5-0.5	1.7-2.1	0.8-0.8
Kanstadfjord	6.5-7.4	< 0.5-<0.5	3.9-4.5	0.6-0.7
Essand (Røros)	6.1-6.6	<0.5-0.8	5-5.5	0.3-0.3

Note: Pooled sample: samples from 5 individual animals originating from the same district.
Vitamin A concentrations were below the limit of detection (< 5 µg) in samples from all districts, except for the district Essand (Røros) in which one pooled sample was within detection limit (5.7 µg/100 g).

Table 6. Concentration of the selected essential elements per 100 g (ww) across grazing districts

	Mean±SD (Minimum-Maximum)	n = 10 samples per district				
District	Ca (mg)	Fe (mg)	Zn (mg)	Se (µg)	Cr (µg)	Co (µg)
Eastern Sør-Varanger	4.6±0.6	3.1 ±0.6	6.7±1.7	44±5.2	0.6±0.7	1±0.4
	(4.1 -5.9)	(2.2- 4.2)	(4.7-10.4)	(37.9-51.5)	(0.2-2.4)	(0.6-1.7)
Pasvik/Sør-Varanger	4.9±0.9	3.5±0.7	4.3± 1.3	27.6±7.7	0.9±0.4	0.6±0.3
	(4.3-6.5)	(2.3-4.6)	(2.6-7)	(14.7-43.4)	(0.4-1.6)	(0.3-1.1)
Varanger Peninsula	5.1±1.2	2.8±0.7	7.6±1.9	17.9±4.9	0.4±0.3	0.4±0.2
	(3.8-7.5)	(2-4.3)	(4.4-10.1)	(10.7-24.9)	(0.1-1.1)	(0.1-0.9)
Spierttagáisá	4.9±1.1	2.2±0.5	5.7±0.9	12.1±2.5	3.1±4.5	0.7±0.2
	(3.9-7.4)	(1.7-3.3)	(4.8-7.9)	(9.3-17.6)	(0.1-12.5)	(0.5-1.1)
Karasjok West	4.7±1.3	2.9±0.6	6.9±1.5	13.4±3.3	1.2±1.3	0.4±0.2
	(3.6-7.9)	(1.8-3.9)	(4.31-9.4)	(7.6-18.4)	(0.3-4.4)	(0.1-0.9)
Ábborašša	4.9±0.8	2.8±0.6	6.4±1.1	11.7±1.4	1.7±1.9	0.6±0.2
	(3.6-7.3)	(2-4.1)	(4.5-7.6)	(8.1-12.9)	(0.2-5.4)	(0.3-0.9)
Fávrrosorda	3.8±1.7	2.6±0.9	7.1±2.3	12.9±2.8	2.1±3.4	0.7±0.4
	(1.9-8.3)	(1.5-4)	(4-10.7)	(9.7-17.7)	(0.3-11.6)	(0.2-1.3)
Tromsdalen/ Andersdalen-Stormheimen	4.6±1.2	2.9±0.8	5.4±0.7	12.9±2.8	5.1±5.8	0.3±0.3
	(3.3-7.3)	(1.7-4.2)	(4.4-6.2)	(7.1-16.5)	(0.3-16.2)	(<0.1-0.7)
Kanstadfjord/ Westren Hinnøy	5.4±2.2	2.8±0.5	6.7±0.6	26.9±3.8	1.2±0.9	0.3±0.1
	(3.8-10.8)	(2.1-3.6)	(5.7-7.7)	(17.1-30.7)	(0.2-3.2)	(0.2-0.4)
Essand/ Røros	3.6±0.4	2.8±0.3	7.2±0.9	18.8±4.7	0.3±0.2	0.4±0.2
	(2.9-4.4)	(2.5-3.5)	(5.1-8.6)	(8.1-23.4)	(0.1-0.7)	(0.1-0.6)

Note: Number of samples (n) less than 10 per district (due to exclusion of outliers) for some essential elements:
Eastern Sør-Varanger: n= 8 for selenium. Karasjok West: n= 9 for calcium and chromium. Pasvik: n= 9 for iron.
Spierttagáisá: n= 8 for cobalt.

3.3 Animal population density

No significant effect for animal population density on concentrations of the studied nutrients could be observed, except for that on selenium concentration. Reindeer originating from districts with low animal population density (0.8 -1.9 animals/km^2) had on average 12.4 µg/ 100g higher Se ($p< 0.01$) than those originating from districts with medium (3-5.3 animals/km^2) and high (6 -13.7 animals/km^2) animal population densities.

4. Discussion

Reindeer meat contained higher vitamin B12, Fe, Zn and Se concentrations when compared to Norwegian beef, lamb, mutton, pork and chicken meat [2c]. The geographical differences revealed in this study were not large and will most likely have no impact for consumers. Vitamin E and Selenium demonstrated relatively large geographical variations. Calves had a significant lower Zn concentration than young and older animals, whereas young animals had a significant lower Se concentration than calves and older animals. Positive correlations were revealed between iron and calcium, and vitamin B12 and zinc. Animals originating from districts with low animal population density had on average higher selenium concentration than those from districts with medium and high population densities.

4.1. Concentrations and geographical variations

Reindeer meat contained a concentration of vitamin B12 that are nearly four, five, nine and twelve times higher than those of lamb meat, beef, pork and chicken, respectively. Iron concentration in reindeer meat was twice higher than those of lamb meat and beef, and four times higher than those of pork and chicken. Furthermore, Zinc concentration was twice higher than that of beef, three times higher than those of lamb and pork, and five times higher than that of chicken, whereas selenium concentration was twice higher than those of pork and chicken, seven times than that of lamb and five times than that of beef [2c].

Vitamin A was detected in only one pooled sample originating from Essand/Røros. Vitamin E and selenium were the nutrients that demonstrated the largest geographical variations, whereas no geographical differences were found for vitamin B7, calcium and iron concentrations. Calves (10 months) had a significant lower Zn concentration than young (1.5 years) and older animals (> 2 years), whereas young animals had a significant lower Se concentration than calves and older animals. Iron was positively correlated with calcium, and vitamin B12 was positively correlated with zinc. Districts with medium and high animal population density (3-5.3 and 6-13.7 animals/km^2, respectively) had an average 12.4 µg/ 100g raw meat lower selenium than those with low population density (0.8 -1.9 animals/km^2).

Concentration of vitamin A detected from the pooled sample originating from Essand (Røros) in this study (5.7 µg/100g) was comparable to those reported from reindeer meat in Finland, twice higher than that from Sweden and four times lower than those previously reported from Norway [2a, 8]. Furthermore, vitamin A concentration reported from Arctic Canadian caribou (93.5 µg/100g ww) was much higher than concentration detected in the present study [9].

Concentrations of vitamin B3 detected in this study (6.6 mg/100g) was comparable to that found in US caribou, slightly higher than that previously reported from Norway (4.3 mg/100g) and slightly lower than those reported from Finland (8.6 mg/100g) and Canadian caribou, 10.9 mg/100g [2a, 8b, 10]. No data on vitamin B7 in meat from reindeer or caribou were available for comparison other than that of 1.2 µg/100 g from Norway which was twice higher than the value of 0.6 µg/100 g detected in the present study [2a]. Vitamin B12 concentration of 4.7 µg/100 g detected in the present study was comparable to that previously reported from Norway (3.3 µg/100 g) and slightly lower that of 6.31 µg/100 g reported from US caribou [2a, 10a]. Additionally, vitamin B12 concentration in reindeer meat was found to be higher when compared to concentrations in meat from other ruminant animals (*e.g.,* mutton and beef) [2]. This could be due to reindeers' feeding on lichens as lichens have previously been found to improve microbial activity in reindeers' rumens [11].

Concentration of vitamin E (α, β, γ and Δ tocopherols) detected in the present study (0.5 mg/100 g) was comparable to those previously reported by same authors [2a]. Additionally, vitamin E concentrations in reindeer meat based on varied tocopherols have been previously reported from Norway (0.6 mg/100g α-tocopherol), Sweden (3.79 and 0.09 µg/g α- and γ-tocopherols, respectively), Finland (0.84 mg/100g α-tocopherol) and Arctic Canada (0.15 mg/100g α-tocopherol) [8b, c, 9, 12].

Calcium concentration of 4.7 mg/100g detected in this study was comparable to those reported from Norway, Finland, and Arctic Canada [2a, 8b, 13]. However, Ca concentration of 17 mg/100g previously reported from US caribou was nearly four times higher than one detected in the present study [10a].

Iron concentration in the present study was comparable to those of 3.3 and 3 mg/100g formerly reported from Norway and Finland, respectively [2a, 8b]. However, Fe concentration from this study was nearly 50% lower than those reported from Russian reindeer, Arctic Canadian and US caribou [10a, 13-14]. The highest Fe concentration of 4.6 mg/100g measured from this study agreed well with that newly reported from a Norwegian study on reindeer meat by Triumf and colleagues [12]. Zinc concentration detected in the present study was in agreement with that previously reported from Norway and nearly twice higher than values reported from Greenlandic reindeer, Arctic Canadian and US caribou [2a, 10a, 13, 15].

Selenium concentration in the present study was characterized by a wide range (7.1 – 51.5 µg/100g). This was due to geographical variation in Se concentration which has also been demonstrated in our previous study on meat, liver, tallow and bone marrow from reindeer [2a]. Se concentration detected in this study was comparable to that reported from Finland (24 µg/100g), twice of that of 10.2 µg/100g reported from US caribou and much higher than that of 3 µg/100g reported from a previous Norwegian study [2a, 8a, b, 10a]. Furthermore, Se concentrations from Greenlandic reindeer ranged from 0.3 to 2.52 µg/100g while Canadian Arctic caribou revealed much lower concentration (0.01 µg/100g) [13, 15]. No data were available for comparison regarding Cr and Co concentrations in meat from reindeer and caribou. However, Sivertsen and colleagues have reported Cr concentration of 2 µg/100g and Co concentrations ranged from 7 to 11 µg/100g from reindeer liver in Norway [16]. Similar Co concentrations as detected by Sivertsen et al. and a higher Cr (8 µg/100g) have been detected from Russian reindeer liver [17].

Reindeer meat contained higher vitamin B12, Fe, Zn and Se concentrations when compared to Norwegian beef, lamb, mutton, pork and chicken meat [2c]. Carcass cuts have been taken into consideration when the previous mentioned comparison was done. This has been conducted either by using the same carcass cut (e.g., neck cutlets) or cuts that are anatomically relevant to neck muscles (e.g., saddle muscles). The geographical differences for nutrient concentrations revealed in the present study were generally not large and will most likely have no impact for consumers. The evaluation of the vitamin and essential element concentrations in terms of high or low should be looked at in the light of the recommended dietary intake/ allowance (RDI/RDA) values for each vitamin or essential element as set by the Nordic Council of Ministers/ the US National Research Council [18].

The wide Se concentration range (µg/100g raw reindeer meat) in the present study contributes to 18-100% and 14-100% of the RDI for adult women and men, respectively. Furthermore, the detected Zn range contributes to 38-157% and 29-122% for women and men, respectively. The detected vitamin B12 range of 1.7-8.8 µg/100g raw reindeer meat contribute to 85 -440% of the RDI for both women and men. There are no established RDI/RDA for vitamin B7, cobalt and chromium. However a US recommendation for vitamin B7 of 30 µg per day was set as an adequate intake (AI) for adults [19]. Furthermore, chromium concentrations of 25 and 35 µg/ day have been reported as adequate intakes (AI) for young women and men, respectively [20].

Vitamin B12 synthesis in ruminant animals (*e.g.,* reindeer) depends on the presence of cobalt [21]. Hence, we were expecting vitamin B12 to correlate positively with cobalt, but such correlation was not observed in the present study.

4.2 Animal population density

Animals originating from grazing districts with low animal population density had on average higher Se concentrations than those from districts with higher animal population densities. This could be due to the fact that animals from grazing districts with high animal population density and limited lichens availability are more likely to experience competition on lichen sources. The lichens have previously been found to contain higher selenium when compared to other plant groups [22].

4.3 Pooled vitamin samples

Due to presence of some pooled vitamin samples with mixed age groups (n= 8 pooled samples) from some districts (n= 4 districts), additional statistical analyses were performed in order to see whether age had an effect on vitamin concentrations or not before we could join all data together prior to the statistical analyses. This was done by dividing data into two sets; one with districts (n= 6 districts) that had homogenized age group (n= 60 animals) and the other with those (n= 4 districts) with mixed age group (n= 40 animals). We have done the same statistical analyses as described in the statistical analysis part on both data sets and no effect for age on vitamin concentrations could be observed (results not presented). However, reindeer calves had previously been reported to have higher (7–10 %) vitamin concentrations than those of adult reindeer [8a, 23]. Advantages and disadvantages of pooled samples were discussed elsewhere [2a]. The ideal situation in case of pooled samples is that samples need to be originated from same districts and consist of homogenized age and sex groups. However, looking into differences in vitamin concentrations within districts would not be possible in such case as concentrations of pooled samples were based on mean of the individual samples from which the pooled sample consisted (*i.e.*, not obtained from individual concentrations separately).

5. Conclusions

Reindeer meat contained higher vitamin B12, Fe, Zn and Se concentrations when compared to Norwegian beef, lamb, mutton, pork and chicken meat (twice to twelve times higher depending on the nutrient). Thus, despite the low reindeer meat consumption in Norway compared to other meat types, the little amount consumed could significantly contribute to the recommended intake of such nutrients. The geographical differences revealed in this study were not large and will most likely have no impact for consumers. Vitamin E and Selenium demonstrated relatively large geographical variations, although no geographical gradient was observed for any of the studied nutrients. Sex had not significant effect in any of the essential elements, and no significant age effect was observed on Ca, Fe, Cr and Co concentrations. Calves had a significant lower Zn concentration than young and older animals, whereas young animals had a significant lower Se concentration than calves and older animals. Iron was positively correlated with calcium and vitamin B12 was positively correlated with zinc. Animals originating from districts with low animal population density had on average higher selenium concentration than those from districts with medium and high densities. There is a need for data on grazing districts' qualities, lichens distribution and summer flies intensity across the different grazing districts in order to get a better explanation for the observed geographical variations.

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Conflict of Interest

The authors declare no conflict of interest.

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Appendix I

Accompanying Form

Reindeer as Food Substance
Centre for Sami Health Research
Department of Community Medicine
Faculty of Health Sciences
University of Tromsø

Slaughterhouse name:
District name and number:
Sample type:
Sampling date:

No.	**Carcass No.**	**Carcass weight (Kg)**	**Age (Year)**	**Sex**	**Notes**
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Appendix II

Overview over sample types employed in the laboratory analyses

Sample type	Variable	Sample weight	Container
Muscle	Lipids and fatty acids	50 g	Glass covered with aluminum foil
	Essentials and toxic elements	20 g	Acid-rinsed plastic tube
	Vitamins	50 g	Plastic boxes covered with aluminum foil
For vitamin samples, the 50 grams are composed of 5 individual samples (10 g each) mixed together to make one pooled sample.			

Sample type	Variable	Sample weight	Container
Liver	Lipids and fatty acids	50 g	Glass covered with aluminum foil
	Essentials and toxic elements	20 g	Acid-rinsed plastic tube
	Vitamins	20 g	Plastic boxes covered with aluminum foil
For vitamin samples, the 20 grams are composed of 5 individual samples (4 g each) mixed together to make one pooled sample.			

Sample type	Variable	Sample weight	Container
Tallow	Lipids and fatty acids	2 g	Glass covered with aluminum foil
	Essentials and toxic elements	5 g	Acid-rinsed plastic tube
	Vitamins	20 g	Plastic boxes covered with aluminum foil
For vitamin samples, the 20 grams are composed of 5 individual samples (4 g each) mixed together to make one pooled sample.			

Sample type	Variable	Sample weight	Container
Bone marrow	Lipids and fatty acids	2 g	Glass covered with aluminum foil
	Essentials and toxic elements	10 g	Acid-rinsed plastic tube
	Vitamins	20 g	Plastic boxes covered with aluminum foil
For vitamin samples, the 20 grams are composed of 5 individual samples (4 g each) mixed together to make one pooled sample.			

Containers dedicated to samples for different types of laboratory analyses

Container used for samples intended for lipids and fatty acids laboratory analyses

Container used for samples intended for essential and toxic elements laboratory analyses

Container used for samples intended for vitamins laboratory analysis

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