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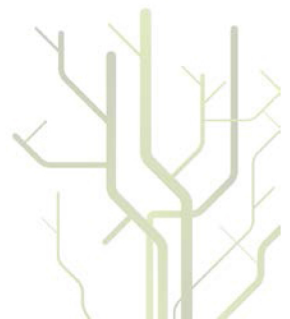DEPARTMENT OF COMMUNITY MEDICINE

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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NUTRIENTS AND TOXIC ELEMENTS IN SEMI-DOMESTICATED REINDEER IN NORWAY

Nutritional and Food Safety Aspects

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A dissertation for the degree of Philosophiae Doctor (PhD)
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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.

Acknowledgements

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 deaŧalaš oassi sámi kultuvrras ja maiddái deaŧalaš 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, deaŧalaš 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.

TABLE OF CONTENTS

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 ($\mu g/kg = ng/g$)
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
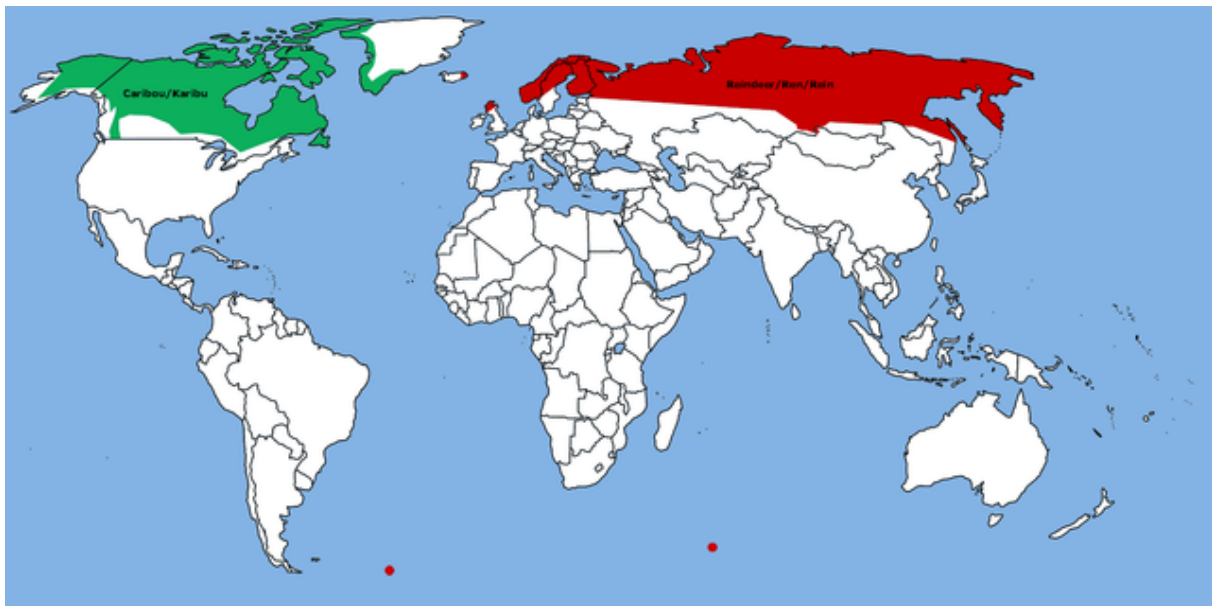
Source: Tom Bjornstad [6].

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
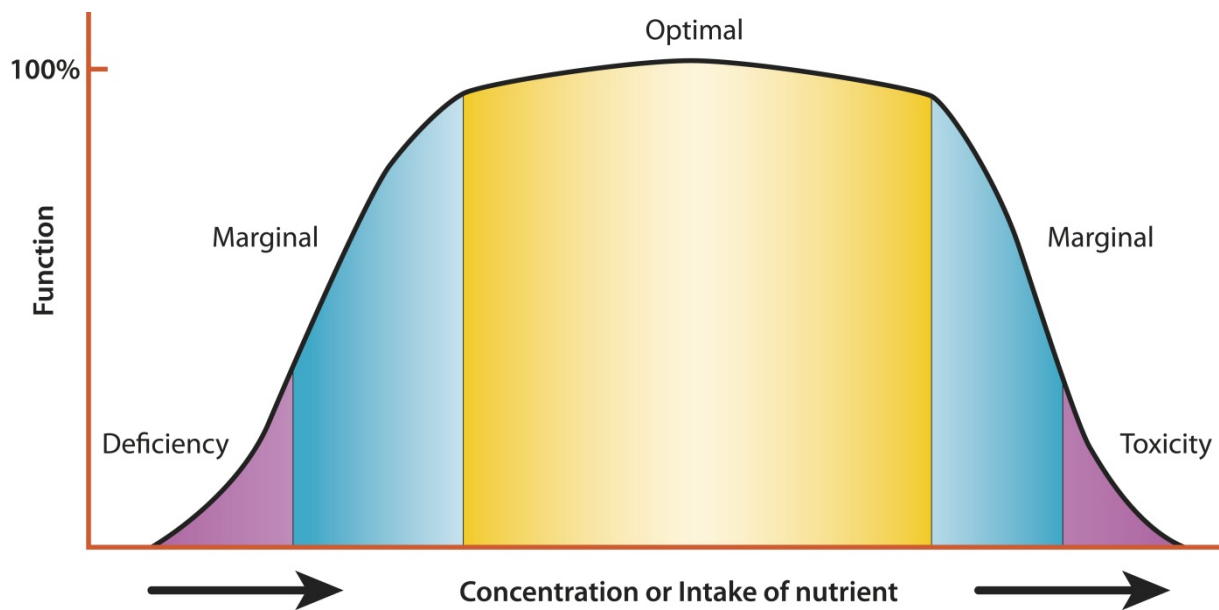
Reindeer meat has been known of its high vitamin contents when compared to meat from

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
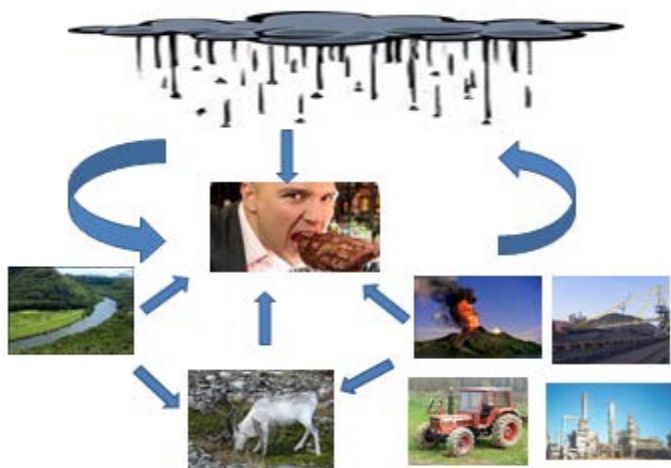
1.8 Effect of toxic elements on human health

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 $\sum$PUFA n-6/ $\sum$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

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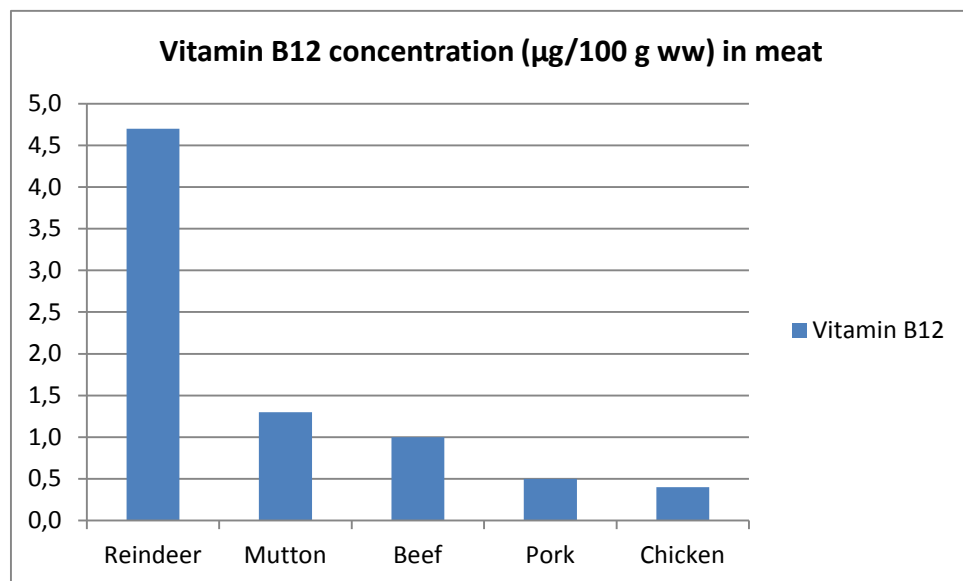
References: Reindeer, mutton, beef, pork and chicken [83, 84].

Figure 4. Iron and zinc concentrations (mg/100g ww) in meat from reindeer and other domestic animals
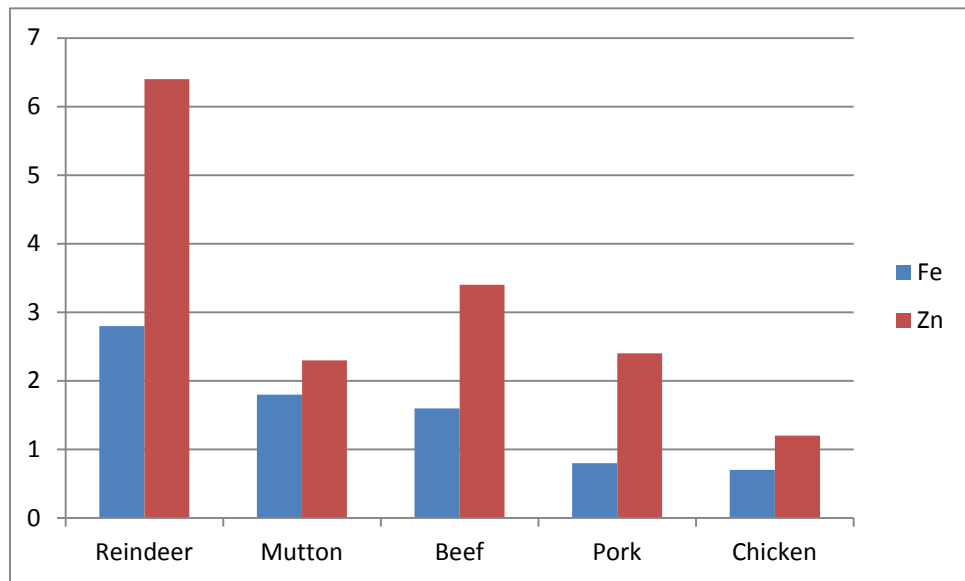
References: Reindeer, mutton, beef, pork and chicken [83, 84].

Figure 5. Selenium concentration in meat from reindeer and other domestic animals
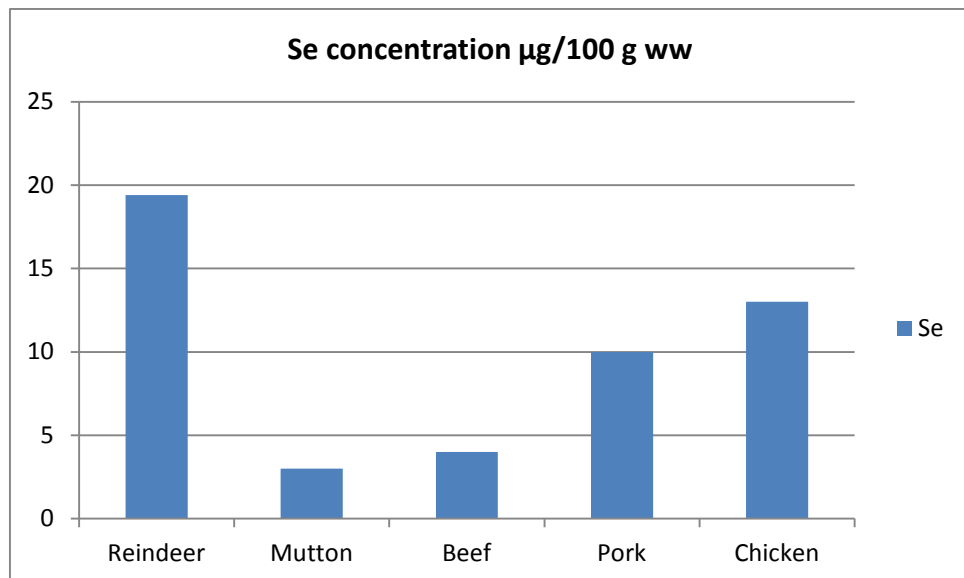
References: Reindeer, mutton, beef, pork and chicken [83, 84].

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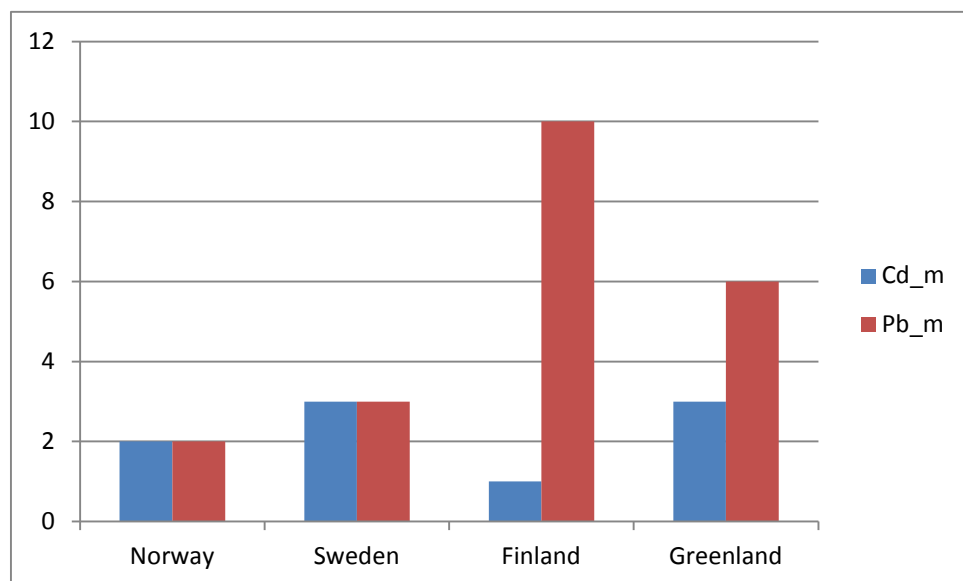
References: Norway, Sweden, Finland and Greenland [50, 52, 54, 90].

Figure 7. Levels of cadmium and lead (ng/g ww) in reindeer liver
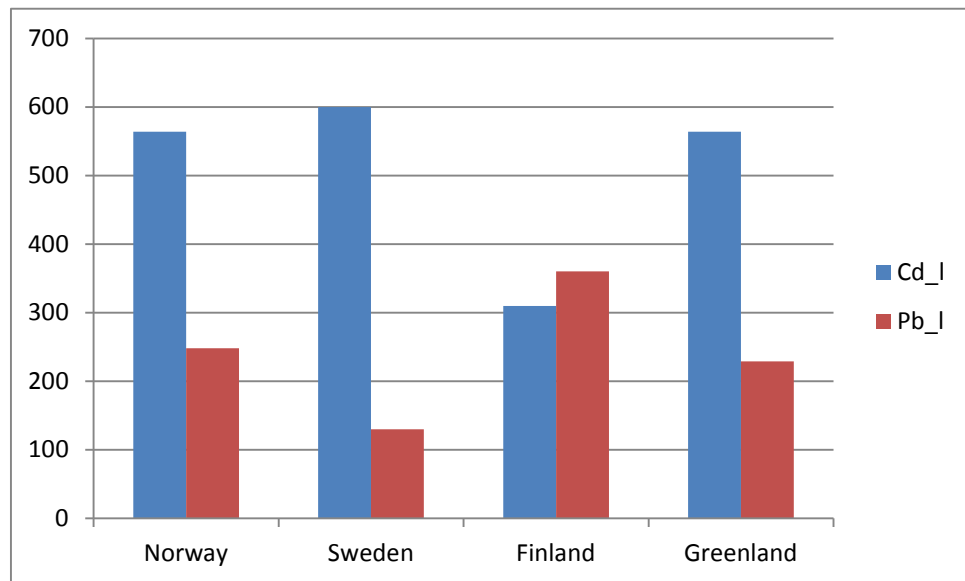
References: Norway, Sweden, Finland and Greenland [50, 52, 54, 90].

Figure 8. Levels of cadmium, lead and arsenic (ng/g ww) in liver of Norwegian and Russian reindeer
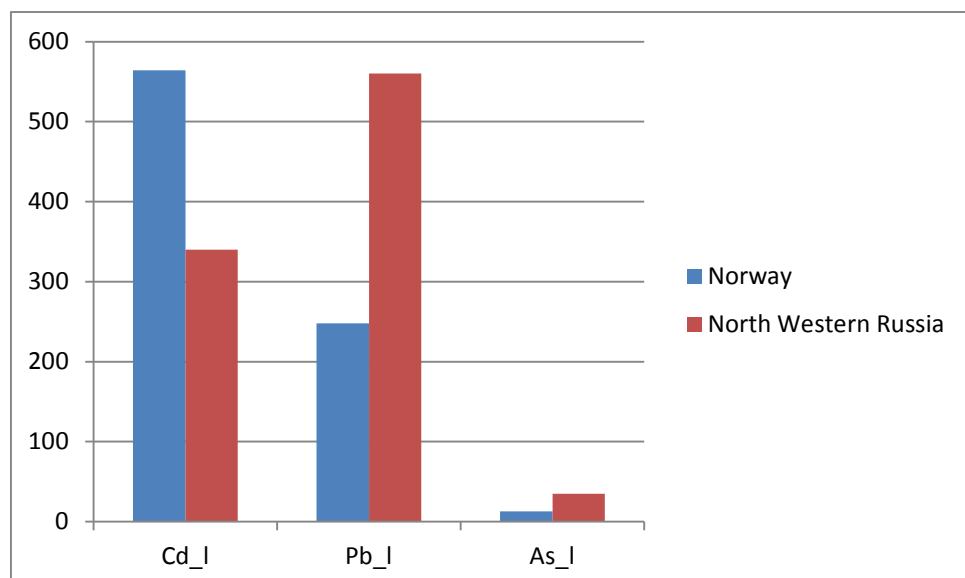
References: Norway and Russia [90, 91].

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.''