Fatty Acids and Antioxidants in Reindeer and Red Deer

Emphasis on Animal Nutrition and Consequent Meat Quality

Sabine Sampels
Faculty of Natural Resources and Agricultural Sciences
Department of Food Science
Uppsala

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Abstract

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The aim of this thesis was to investigate importance of dietary fatty acids (FA) and animal age and sex on FA metabolism. In addition relation between FA and antioxidants on the consequent nutritional and technological quality of reindeer and red deer meat were addressed.

A diet rich in polyunsaturated FA (PUFA) especially long chained n-3 FA (≥C20) has beneficial effects on human health, *e.g.* in prevention of arteriosclerosis. Game meat is a potential food source that is both lean and rich in n-3 PUFA, however different animal production systems can affect its natural FA composition.
In the present studies on reindeer and red deer, that were either grazing or fed with pellets, meat from grazing animals had higher amounts of n-3 PUFA and lower n-6/n-3 ratio. Our results from feeding studies indicate that reindeer are unable to sufficiently elongate and desaturate towards 22:5n-3 and 22:6n-3, and indicate that dietary intake of C 22 PUFA is necessary for reindeer.

The differences in FA composition between grazing reindeer of different sex and age were ascribed mainly to fatness.
FA composition of the meat influences not only its nutritional quality but also other quality aspects such as shelf life and processing stability. The higher degree of unsaturation of FA is, the more prone to oxidation they are. Therefore the antioxidant status in the animals is important for the protection of the meat against oxidation.
The studies on processed and stored meat showed that changes in FA composition and antioxidant content influenced its processing stability and shelf life. α-Tocopherol status had a greater impact on lipid oxidation and colour stability than did changes in FA composition. In contrast to lipid oxidation, decreasing colour stability and increasing amounts of free FA were found to be the first indicators of quality deterioration.

The knowledge from the present thesis adds valuable information on lipid metabolism of ruminants, demonstrated in reindeer.

Keywords: α-tocopherol, *Cervus elaphus* L., desaturation, human nutrition, lichen, linseed, lipid classes, phospholipids, *Rangifer tarandus tarandus* L., squalene, n-6/n-3 ratio.

Author's address: Sabine Sampels, Department of Food Science, P.O. Box 7051, Swedish University of Agricultural Sciences (SLU), SE-75007 Uppsala, Sweden. E-mail address: Sabine.Sampels@lmv.slu.se

Sammanfattning

I denna avhandling undersöktes fettsyrasammansättningen och halten av antioxidanter i foder och kött från renar och hjortar. Syftet var att analysera effekterna av fodrets sammansättning, djurens ålder och kön på renars fett- och antioxidantmetabolism å ena sidan och på några kvalitetsaspekter i ren- och hjortkött å andra sidan.

En diet med en hög halt fleromättade fettsyror, speciellt långkedjiga omega-3 (n-3) fettsyror, har gynnsamma effekter på människans hälsa, bl.a. för förebyggandet av hjärt- och kärlsjukdomar. Kosten har varit rik på n-3 fettsyror under evolutionens gång och människan är alltså anpassad till ett högre intag av n-3 fettsyror än vad vi idag konsumerar. Efter industrialiseringen inom jordbruket har konsumtionen av spannmål, som är rika på n-6 fettsyror, ökat mycket och därmed också kvoten n-6/n-3. Viltkött är i regel magert och har ett högt innehåll av n-3 fleromättade fettsyror, men utfodring med spannmålsbaserade pellets kan leda till en minskad n-3 halt i köttet.

I våra försök hade betande renar och hjortar högre halter av n-3 fettsyror och en lägre n-6/n-3 kvot i köttet än pelletsutfodrade djur. Renar som fick pellets med en tillsats av linfrökaka (som är rik på 18:3n-3) ökade mängden 18:3n-3 och 20:5n-3 i muskulaturen men fick ingen ökning av 22:5n-3 och 22:6n-3. Dessa resultat tyder på att renar inte kan desaturera och elongera fettsyror till C22 fettsyror, utan att de måste ta upp dessa genom fodret. Skillnader i fettsyrasammansättningen mellan betande renar av olika ålder och kön visade sig i huvudsak bero på skillnader i djurens fettansättning.

Fettsyrasammansättningen och antioxidanthalten i köttet påverkar inte bara köttets nutritionella värde utan också andra kvalitetsparametrar som hållbarhet och processtabilitet. Fettsyror oxiderar lättare ju mera omättade de är, därför är halten av antioxidanter i köttet viktig för att förhindra oxidation. Våra studier visar att fodrets α-tokoferolhalt bara delvis påverkade halten av α-tokoferol i köttet. Vi antar att en annan faktor som kan påverka halten av α-tokoferol i köttet är en möjlig mättnadsgrad av α-tokoferol i muskeln. Bioaktiva ämnen från laven (som renarna betar) med antioxidativa egenskaper eller förmågan att öka α-tokoferolens biotillgänglighet kan också bidrar till den slutliga α-tokoferolhalten i köttet.

Studier på förädlat/lagrat kött har visat att förändringar i fettsyrasammansättningen och halten av antioxidanter påverkar processtabiliteten och hållbarheten. En minskad α-tokoferolhalt ökade fettoxidationen och försämrade färgstabiliteten mer än vad en högre andel fleromättade fettsyror gjorde. Inte mängden oxidationsprodukter, utan försämrad färgstabilitet och en ökad mängd fria fettsyror visade sig vara de första indikatorerna på en kvalitetsförsämring.

Resultaten ur denna avhandling bidrar med nya kunskaper om fettmetabolism hos idisslare, här undersökt på ren.

Zusammenfassung

In der vorliegenden Studie wurden die Fettsäurezusammensetzung und der Gehalt an Antioxidantien in Futter und Fleisch von Hirschen und Rentieren untersucht. Das Ziel war, Effekte zwischen Futter, Alter und Geschlecht der Tiere einerseits auf den Fettsäuremetabolismus, und andererseits auf einige ernährungsphysiologische und technologische Qualitätsaspekte des Fleisches zu untersuchen.

Eine Diät reich an mehrfach ungesättigten Fettsäuren insbesondere langkettigen Omega-3 (n-3)-Fettsäuren, ist förderliche für die Gesundheit des Menschen, da sie zum Beispiel Arteriosklerose vorbeugt. Im Vergleich zur Ernährung des Menschen in prähistorischer Zeit, ist die heutige gekennzeichnet durch eine deutlich niedrigere Einnahme von n-3-Fettsäuren. Der erhöhten Verzehr von Getreideprodukten seit Einführung des Ackerbaus führte zu einem Anstieg der Omega-6 (n-6)-Fettsäuren und damit der n-6/n-3 Quote in der Nahrung. Da Wildfleisch natürlich fettarm und reich an n-3-Fettsäuren ist, kann dessen Verzehr somit die n-6/n-3 Quote in der menschlichen Nahrung positive beinflussen. Durch Fütterung von Getreide kann jedoch der natürliche Gehalt an n-3-Fettsäuren im Wildfleisch sinken.

In den vorliegenden Studien zeigen Vergleiche von grasenden Rentieren und Hirschen mit ihren mit Pellets gefütterten Artverwandten einen höheren Anteil von n-3-Fettsäuren und eine geringere n-6/n-3 Quote im Fleisch der grasenden Tiere. Bei Rentieren, die mit Pellets gefüttert wurden denen gequetschte Leinsamen (einer Quelle reich an 18:3n-3) zugesetzt worden waren, wurden erhöhte Mengen der Fettsäuren 18:3n-3 und 20:5n-3 im Fleisch gefunden. Dagegen konnte keine Erhöhung von 22:5n-3 und 22:6n-3 festgestellt werden. Die vorliegenden Ergebnisse zeigen, dass die Rentiere nicht in der Lage sind, Fettsäuren bis hin zu C22 zu verlängern und desaturieren, so dass die Tiere diese Fettsäuren mit der Nahrung aufnehmen müssen. Unterschiede in der Fettsäurezusammensetzung des Fleisches von grasenden Rentieren verschiedenen Alters und Geschlechts konnten hauptsächlich Differenzen in der Fettanlagerung zugeschrieben werden.

Neben der ernährungsphysiologischen Qualität werden auch Haltbarkeit und Verarbeitungsstabilität durch die Fettsäurenzusammensetzung und der Gehalt an Antioxidantien des Fleisches beeinflusst. Hier kann ein höherer Anteil ungesättigter Fettsäuren im Fleisch zu gereingerer Haltbarkeit führen, da diese leichter als gesättigte oxidiert werden. Daher spielt der Gehalt an Antioxidantien im Fleisch eine wichtige Rolle zum Schutz vor Oxidation.

Unsere Studien zeigen, dass der Gehalt von α-Tocopherol im Futter den Gehalt im Fleisch der Hirsche und Rentiere nur teilweise beeinflusst. Wir schlagen vor, dass ein maximaler Gehalt an α-Tocopherol im Muskel nicht überstiegen werden kann. Ausserdem könnten bioaktive Substanzen aus den Flechten (Winterfutter der Rentiere) als Antioxidatien wirken oder die biologische Verfügbarkeit von α-Tocopherol erhöhen und so den α-Tocopherolgehalt im Fleisch beeinflussenden.

Die Studien an verarbeitetem und gelagertem Fleisch bestätigen, dass Änderungen in der Fettsäurenzusammensetzung und dem Gehalt an Antioxidantien die Verarbeitungs- und Lagerungsstabilität beeinflussen. Es konnte gezeigt werden, dass der Gehalt an α-Tocopherol im Fleisch einen stärkeren Einfluss auf Oxidation und Farbstabilität als die Fettsäurenzusammensetzung hatte. Als erste Indikatoren einer beginnenden Qualitätsminderung wurde nämlich nicht ein Anstieg an Oxidationsprodukten, sondern verminderte Farbstabilität und eine ansteigende Menge von freien Fettsäuren im Fleisch festgestellt.

Die Ergebnisse aus unseren Studien erweitern das Wissen über den Fettsäuremetabolismus von Wiederkäuern am Beispiel von Rentieren.

Contents

Appendix

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

I. Sampels, S., Pickova, J. & Wiklund, E. (2004). Fatty acids, antioxidants and oxidation stability of processed reindeer meat. *Meat Science, 67,* 523-532.

II. Wiklund, E., Sampels, S., Manley, T.R., Pickova, J. and Littlejohn, R.P. Effects of feeding regimen and chilled storage on water holding capacity, colour stability, pigment content and oxidation in red deer (*Cervus elaphus*) meat. *Journal of the Science of Food and Agriculture,* accepted.

III. Sampels, S., Pickova, J. & Wiklund, E. Fatty acid composition and vitamin E and A content of *M. longissimus dorsi* from reindeer of different age and sex grazing on natural pasture and fed two different diets. *Submitted*.

IV. Sampels, S., Pickova, J., & Wiklund, E. Influence of production system, age and sex on carcass parameters and some biochemical meat quality characteristics of reindeer (*Rangifer tarandus tarandus* L.). *Submitted*.

The author's contribution to the studies

For all studies I planned the research jointly with the co-authors.

Study I: I carried out the analyses, processed the data, and was responsible for compiling the manuscript.

S**tudy II**: I participated in the fieldwork, collection of samples and analyses of water holding capacity and colour stability. I was responsible for the analyses of pigment content, the lipid class and the TBARS analyses, and participated actively in compiling the manuscript.

S**tudies III** and **IV**: I took part in collecting the samples, was responsible for the analyses of vitamin E and A, lipid class composition and fatty acid composition, processed the data and was responsible for compiling the manuscripts.

List of abbreviations used in the text

Δ	Delta
BHT	Butylated hydroxytoluene
CPD	Conventional pellet diet
DM	Dry matter
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acids
FAME	Fatty acid methyl esters
FFA	Free fatty acids
GC	Capillary gas liquid chromatography
GC-MS	Gas mass spectrometry
HPLC	High performance liquid chromatography
IMF	Intramuscular fat
LC	Long chain
LPD	Linseed pellet diet
MDA	Malondialdehyde
MUFA	Monounsaturated fatty acids
N	Neutral lipids
PL	Polar lipids
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
SPE	Solid-phase-extraction
TAG	Triacylglycerols
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TLC	Thin layer chromatography

Introduction

In pre-agricultural times, the foods available to humans were game meat, fish, green leafy vegetables, fruits, berries, honey and nuts (Simopoulos, 2003). This diet, containing higher amounts of n-3 and lower amounts of n-6 polyunsaturated fatty acids (PUFA) than modern diets, shaped the genetics of human nutrition. Both n-6 and n-3 fatty acids (FA) are essential for humans, and our diet must contain balanced amounts (Gerster, 1998; Simopoulos, 2002b), however the availability of a PUFA rich diet meant that humans did not need the genetic capacity to synthesize them. After the agricultural revolution though, intake of cereals increased enormously. Cereals are rich in n-6 FA and low in n-3 and, as a consequence, the n-6/n-3 FA balance to which humans are adapted has changed dramatically over the last 10 000 years (Simopoulos, 2002a). Human genetics however cannot keep pace with so fast change, since the spontaneous mutation rate for nuclear DNA is estimated to be 0.5% per million years (Simopoulos, 2003). We are therefore adapted to much higher intake of n-3 FA in our diet than we actually consume today. One current potential food source that is lean and rich in n-3 PUFA is game meat (Sinclair & O'Dea, 1990; Mann, 2000), however different animal production systems can affect its natural FA composition.

This thesis deals with aspects of FA composition and antioxidants in reindeer and red deer muscle in relation to the animals' nutritional needs and meat quality traits. These species are managed in different rearing systems, which represent different approaches made worldwide in modern meat production systems to intensify the commercial use of game. While red deer farming is generally intensive production, reindeer husbandry is practised in a more extensive production form using semi-domesticated animals in a free ranging system.

Reindeer (*Rangifer tarandus tarandus* L.) husbandry in Sweden

According to Swedish law, the right to practice reindeer husbandry is a privilege accorded to the Sámi people (Reindeer Husbandry Act, 1971), and is also an important part of the life and culture of the indigenous population. Reindeer herding today is conducted by about 950 enterprises spread over about 40% of Sweden's geographical area, and meat production is the main source of income for these enterprises.

Reindeer are among the northernmost freely ranging ruminants in Scandinavia, and are well adapted to their habitat with a cold climate and snow during the majority of the year. Reindeer husbandry is based on utilization of natural pastures all year round, but because reindeer migrate from summer pastures on the mountain tundra to the valleys and forests in winter, their diet changes markedly during the year (Skjenneberg & Slagsvold, 1968). In summer, the diet is rich, consisting of fresh grass, sedges, shrubs and herbs, whereas in the autumn it changes gradually to include mostly lichens and dwarf shrubs (Eriksson *et al.*, 1981; Nieminen & Heiskari, 1989, Kumpula, 2001).

Traditionally the main slaughter period is in winter from November to the end of April (National Board of Agriculture, 2000), and during winter pasturing, reindeer

normally do not gain weight (Reimers, 1983). In recent years, the main reason for feeding reindeer commercial pellets has been to reduce levels of radioactive caesium in the meat (Åhman, 1999), however this supplementary feeding may also improve the nutritional status of the animals (Jacobsen *et al.*, 1977), since access to forage can be difficult during harsh winter conditions (Helle, 1984).

Because it is produced in small amounts (1300 tonnes in 2003/2004) (National Board of Agriculture, 2004b) reindeer meat is a very exclusive product, which is in high demand and features often on the menu in the more luxurious restaurants. The meat is mainly consumed fresh, but also as cold or hot-smoked and dried products. The average consumption per person per year of various types of meat in Sweden is: pork (36 kg); beef (24 kg); poultry (10 kg); game (moose (*Alces alces*) and roe deer (*Capreolus capreolus*)) (2 kg); lamb/sheep (0.9 kg) and reindeer (0.2 kg) (National Board of Agriculture, 2004a).

Red deer (*Cervus elaphus* L.) farming in New Zealand

New Zealand is the largest producer of farmed deer, with about 5 000 deer farms ranging in size from small hobby farms to extensive commercial operations. These farms are home to approximately 1.7 million deer, or half the world's farmed deer population. This figure includes an estimated 1 million female deer (hinds or cows) and 700 000 stags or bulls. Over the past 10 years the number of farms has doubled, and the number of slaughtered animals has increased four-fold (about
400 000 slaughtered in 2001) (http://www.nzgib.org.nz/n5.html, 04-mar-2005). Because they are easy to handle, red deer are the main species farmed in New Zealand (85%), but elk/wapiti (*Cervus elaphus nelsoni*), and small numbers of fallow deer (*Dama dama*) are also kept. The deer graze natural pasture, primarily rye grasses all year round. However, in winter when grass growth is limited, supplementary feeds such as cereal grains or preserved pasture (hay and silage) may be fed (http://www.nzgib.org.nz/n26.html, 04-mar-2005). All deer are transported to and slaughtered at licensed deer slaughter premises.

According to New Zealand Game Industry Board statistics, over 90% of the deer industry's products are exported. All meat for export is chilled at -1.5°C in vacuum packages, which gives a storage time as fresh meat for up to 14 weeks (http://www.nzgib.org.nz/n95.html, 04-mar-2005). The major market for New Zealand venison is Western Europe and Scandinavia, accounting for approximately 80% of total venison exports, with Germany the biggest importer. The amount of venison exported increased from 15 thousand tonnes in the year 2000 to 22 thousand tonnes in the year 2004
(https://www.deernz.org/export/exportreport.cfm?id=53, 2005).

Swedish deer farming

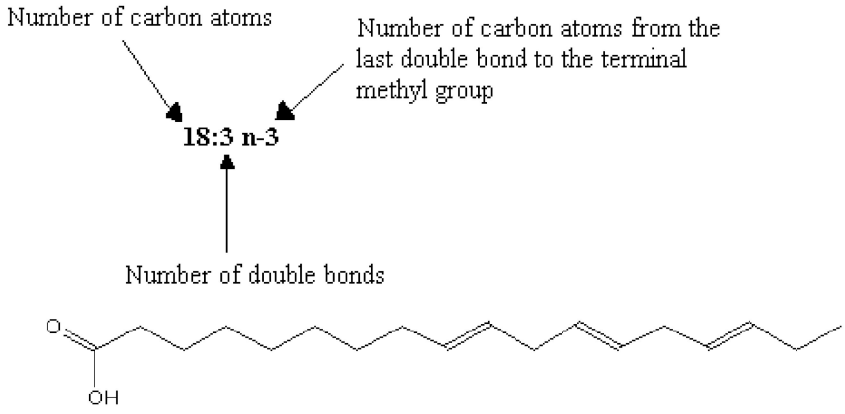
Compared with its New Zealand counterpart, the Swedish deer industry is small, consisting of mainly fallow deer and, to a lesser extend red deer farms. At the time of writing, the number of meat producing farms is 611, but this figure is increasing. In 2004, about 4 500 animals were slaughtered and total meat production was about 480 tonnes. The number of animals used for breeding in the

same year was 15 600 fallow deer and 4400 red deer. The animals are shot on the pasture and then transported in a cooling truck to a slaughter/processing facility. A few farms have their own small abattoir and sell the meat directly, but most animals are sold to vendors that handle the transport and slaughter (pers. comm.: Sigrid Ericsson, Svenska Djurhälsovården; Carl Nathanson, Svenska Hjortavelsförbundet)

Lipids, definitions and biochemistry

Lipids are usually divided into two main classes: polar lipids (PL) and neutral lipids (NL). NL consist mainly of triacylglycerols (TAG), and minor amounts of mono- and diacylglycerols, whereas PL include mainly phospholipids (Henderson & Tocher, 1987). TAG serve mainly as an energy source, whereas phospholipids are mostly constituents of biological membranes (Henderson & Tocher, 1987; Jakobsson *et al.*, 1990; Saleh *et al.*, 1999; Scollan *et al.*, 2001b). FA are involved in determining the physical and chemical properties and capacities of biological membranes (Wiseman, 1996; Gibbons, 2003), and also serve as precursors in the synthesis of several different chemical messengers and eicosanoid hormones, as well as other regulating factors (Kinsella, 1988; Horrobin, 1995).

Fatty acids consist of carbon chains with a methyl (CH_3) group at one end and a carboxyl (COOH) group at the other. The length of the carbon chain, and number of double bonds determine the properties of the FA (Fig. 1). Saturated FA (SFA) lack double bonds, whereas unsaturated FA can contain up to six. Those having one double bond are called monounsaturated FA (MUFA), whereas those with two or more double bonds are called PUFA. The main forms in which FA are present in lipids are: Free FA (FFA), acylglycerols (FA bound to a glycerol), phospholipids (diacylglycerols including a phosphatic acid derivate), glycolipids (diacylglycerols including a mono-, di-, tri or tetra saccharide) and sphingolipids (containing sphingosine) (Belitz & Grosch, 1999).



18:3 n-3

Figure 1. Principle nomenclature of fatty acids

Whereas terrestrial and aquatic plants contain the necessary desaturases and elongases to synthesize 18:2 n-6 and 18:3 n-3 and their longer derivatives of the n-3 and n-6 family, mammals lack Δ 12 and Δ 15 desaturases that are essential for insertion of double bonds at n-3 and n-6 (Innis, 1991) (Fig. 2), however it has been discussed whether mammals are able to elongate and desaturate 18:2 n-6 and 18:3 n-3 in significant amounts towards 20:4 n-6 and 20:5 n-3, 22:5 n-3 and 22:6 n-3 (Gerster, 1998; Arts *et al.*, 2001; Burdge *et al.*, 2002).

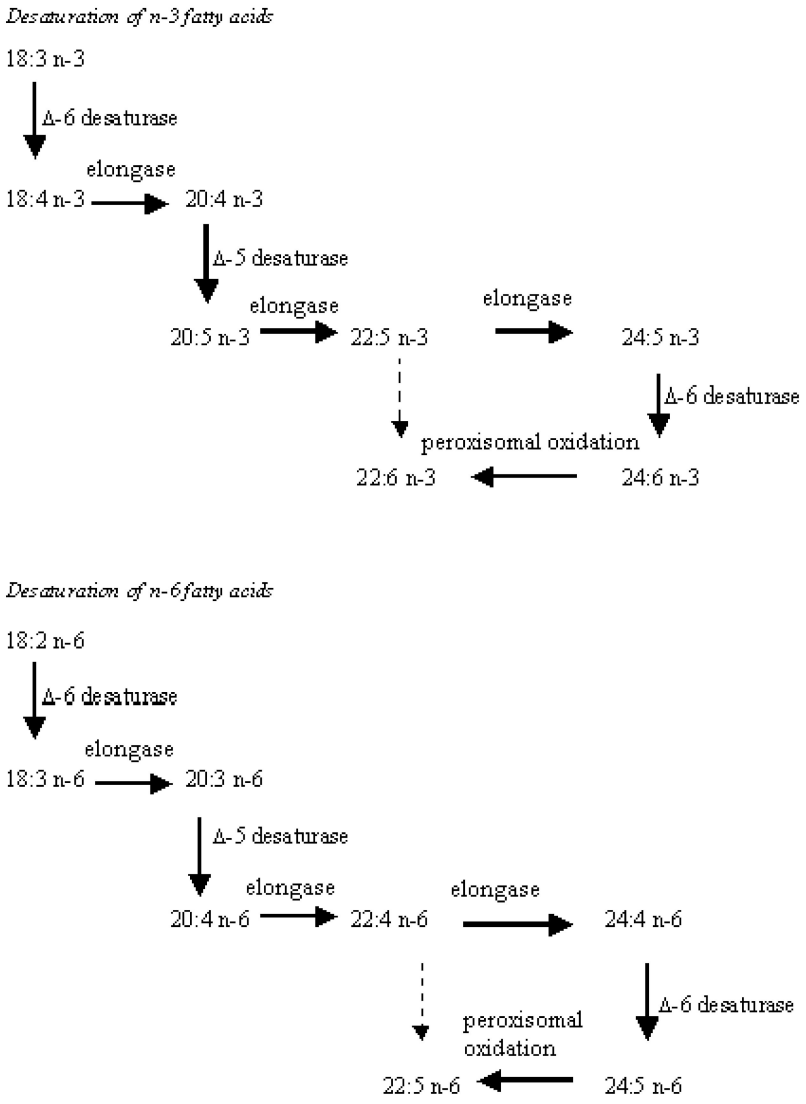


Figure 2. Schematic of the desaturation and elongation steps involved in the conversion of 18:3n-3 to 22:6n-3 and 18:2 n-6 to 22:5 n-6. Vertical and horizontal lines represent desaturation and elongation steps, respectively (Sprecher, 2000).

Oxidation

Oxidation in meat and meat products and its kinetics are described by Cosgrove *et al.* (1987) and Kanner (1994). Oxidation leads to the formation of lipid radicals ($L^{\bullet}$) that react further to lipid peroxides ($LOO^{\bullet}$).

In general lipid oxidation can be described in three steps:

Initiation $LH + \text{Initiator} \rightarrow L^{\bullet}$ (1)

Propagation $L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$ (2)
$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$ (3)

Termination $LOO^{\bullet} + LOO^{\bullet} \rightarrow$ non-radical products (4)
$LOO^{\bullet} + LO^{\bullet} \rightarrow$ non-radical products (5)
$LOO^{\bullet} + L^{\bullet} \rightarrow$ non-radical products (6)

Autooxidation in meat can be initiated by light, heat, presence of metal ions and radicals. Very low concentrations of radicals are needed to start the reaction. The reactivity of unsaturated fatty acids increases with their chain length and number of double bounds (Cosgrove *et al.*, 1987). Once initiated, oxidation propagates in a chain reaction (steps 2-6). In the termination reactions, lipid peroxides ($LOO^{\bullet}$) will react freely, forming a wide range of more stable products including aldehydes, alkanes and conjugated diens.

Antioxidants and squalene

Antioxidants delay or inhibit the process of oxidation, even when present in low concentrations (Halliwell & Cutteridge, 1988). Some antioxidants function as radical scavengers or peroxide decomposers, while others quench singlet oxygen, remove catalytic metal ions or oxygen, or inhibit enzymes. The cellular antioxidants can be classed as low molecular substances and enzymes that are either water-soluble or fat-soluble.

Vitamin C is a water-soluble antioxidant, whereas Vitamin E and carotenoids, the precursors of retinol (vitamin A), are fat-soluble.

Vitamin E is a generic name for all substances that have the biological function of α-tocopherol. These include tocopherols, with a saturated phytyl side-chain and tocotrienols, with an unsaturated isoprenoid side-chain substituted to a chroman head. The different forms of tocopherols and tocotrienols are specified by the use of the Greek letters α,β,γ, and δ to denote the number and position of methyl groups linked to the chroman head (Frank, 2004).

Carotenoids are hydrocarbons built from eight isoprene bodies (40 C atoms). Due to their structure and the conjugated double bonds, both vitamin E and the carotenoids, are radical scavengers that can build relatively stable radicals. In addition, carotenoids, tocopherols and tocotrienols are quenchers for singlet oxygen. Vitamin C and Vitamin E have been found to interact as antioxidants, tocopheroxy radicals are reduced back to tocopherols by ascorbic acid (Packer *et al.*, 1979).

Squalene is triterpene (30 C atoms) that is present in plants and animal tissues as a key intermediate in the biosynthetic pathway to steroids. Significant amounts of squalene in plant sources are detected in *e.g.* olive oil, wheat germ oil, bran oil and yeast (Bosku, 2000) as well as in *Amaranthus* grain and *Echium* plants (He *et al.*, 2002).

Squalene also acts as a quencher of singlet oxygen and has been found to protect α-tocopherol in oxidation processes (Psomiadou & Tsimidou, 2002).

Lipolysis

Lipolysis describes an enzymatic release of FFA from both TAG and phospholipids, and is thought to increase lipid oxidation, since FFA are very sensitive to oxidation (Nawar, 1996; Coutron-Gambotti & Gandemer, 1999). Since lipolysis is an enzymatic reaction, its intensity depends on enzyme activity, which in turn depends on a number of factors in the meat and processing, such as temperature, water activity, pH and salt content (Toldra & Flores, 1998; Vestergaard *et al.*, 2000; Andres *et al.*, 2005). In adipose tissue, lipoprotein lipase, hormone sensitive lipase (also: neutral lipase) and monoacylglycerol lipase are active at different pH, whereas in muscle lysosomal acid lipase hydrolyzes tri-, di-, and monoacylglycerols at acid pH (4.5-5.5), phospholipases A1 and A2 hydrolyze 1- and 2-acyl ester respectively of sn-3 phosphoglycerols, neutral lipase is active at pH 7.0-7.5 and acid and neutral esterases hydrolyze short chain FA from tri-, di-, and monoacylglycerols (reviewed by: Toldra & Flores, 1998). In adipose tissue, neutral lipases contribute greatly to hydrolysis and FFA are generated mainly from TAG (Toldra & Flores, 1998). In muscle, only minor lipolysis of TAG has been shown in dry cured ham (Buscailhon *et al.*, 1994), while other studies found that both phospholipids and TAG were affected by lipolysis in refrigerated meat (Alasnier *et al.*, 2000a). No differences in hydrolysation between phospholipid classes, FA chain length or unsaturation were found by Buscailhon *et al.* (1994).

Lipids in nutrition

Discussions on the FA composition of meat have intensified in recent years (Wood *et al.*, 2003). The advantages of different types of diets, such as Mediterranean or evolutionary diets, have been debated in the media and in scientific papers (Simopoulos, 1999; Simopoulos & Sidossis, 2000, Mann, 2000). A diet rich in PUFA, especially long chained (LC) n-3 FA (≥C20) has beneficial effects on human health (Williams, 2000). The LC n-3 FA are important from a health perspective, for example in the prevention of arteriosclerosis and autoimmune diseases (Kinsella, 1988). The amount of PUFA in the Western diets is decreasing (Simopoulos, 2001), as an increasing part of the diet is represented by fast food and convenience foods (Dumagan & Hackett, 1995). High intakes of TAG, containing high amounts of SFA and MUFA, in modern western diets are associated with adverse effects on human health, such as cardiovascular diseases, obesity and diabetes (Mann, 2000; Simopoulos, 2001). It is not only the amount of PUFA in the food that is important, but also the ratio between n-6 and n-3 PUFA,

for which values of 1 to 4 have been recommended (Simopoulos, 2002b). In today's Western diets this ratio is between 15 and 20, whereas during earlier stages of human evolution it was close to 1 (Simopoulos, 2001; Simopoulos, 2002b).

A daily intake of eicosapentaenoic acid (EPA = 20:5 n-3) and docosahexaenoic acid (DHA = 22:6 n-3) of at least 0.22g each has been suggested as adequate for adults (Simopoulos, 2002b), making it important to include sources rich in n-3 PUFA in daily diet. Oily fish contains high amounts of LC n-3 PUFA, but since fish consumption is low, meat is a significant source of n-3 PUFA for many people (Scollan *et al.*, 2001b). Therefore there is a need to produce healthier meat by improving its FA composition (Wood *et al.*, 2003). Evolutionary diets contained high amounts of lean meat from game, which was low in total fat and rich in phospholipids and PUFA (Sinclair & O'Dea, 1990; Simopoulos, 2001). Consumers today are interested in alternatives to conventional meat products (Rule *et al.*, 2002), and value game meat as an ecologically 'natural' and exclusive alternative (Rywotycki, 2003).

Reindeer meat has been found to contain higher amounts of n-3 PUFA compared to cattle (Wiklund *et al.*, 2001a), and can influence on the dietary n-6/n-3 ratio, with benefits to health (Näyhä, 1997). Due to its low consumption, the wider impact of reindeer meat on nutrition is negligible. However, it is important on a local scale, as the consumption of reindeer meat is traditionally relatively high among Sámi reindeer herders compared with people of Swedish, Finnish or Norwegian origin living in the same areas (Laitinen *et al.*, 1996; Nilsen *et al.*, 1999). Studies on health among reindeer herders (Näyhä, 1997) showed a significantly lower incidence of ischaemic heart disease in Finnish Sámi compared with Finns.

Red deer meat, in contrast, is produced in significant amounts all over the world (Rywotycki, 2003) *e.g.* the venison export from New Zealand was 22 thousand tones in 2004 (http://www.nzgib.org.nz/n3.html, 04-mar-2005). New Zealand has pioneered the development of farm-based production systems for deer. However, changes in the traditional reindeer herding practises, and in the feeding systems used in intensive deer farming have lead to lower amounts of n-3 PUFA in meat from both animals (Wiklund *et al.*, 2001a; Wiklund *et al.*, 2003b).

Meat quality aspects

Meat quality describes the attractiveness of meat to consumers (Wood *et al.*, 1999), and is a wide-ranging term, encompassing such diverse issues as technological, nutritional, hygienic and sensoric quality of the meat (Hofmann, 1994).

Consumer preferences give rise to attempts to decrease total fat content (Wood, 1990), while nutritional interests lead to increase levels of PUFA, especially n-3 PUFA (Wood *et al.*, 2003). Changes in fat content and FA composition influence various aspects of meat quality, particularly meat and fat firmness, shelf life and flavour (Wood *et al.*, 2003). Total fat content influences sensory aspects such as juiciness, for example too lean meat has been associated with decreased juiciness of pork (Wood *et al.*, 1986) as well as decreased tenderness and juiciness of lamb

(Priolo *et al.*, 2002). Bosi *et al.* (2000) found that Parma ham from pigs fed high oleic acid sunflower oil contained higher amounts of unsaturated FA, but had poorer texture properties (they were too soft). Shackelford *et al.* (1990) also found decreased firmness in pork after feeding with different plant oils.

FA composition also influences the taste, since the range of volatile flavour precursors deriving from different amounts unsaturated FA contributes to the taste of cooked meat (Wood & Enser, 1997; Mottram, 1998). For example, different feeding systems (pellets and pasture) have been shown to affect the taste of red deer meat (Wiklund *et al.*, 2003a).

In addition, unsaturated FA are more prone to oxidation (Morrissey *et al.*, 1998; Mottram, 1998) compared with saturated fats. This sensitivity to oxidation increases with increasing unsaturation, for example the oxidizability of 22:6 n-3 is much higher than that of 18:2 n-6 (Cosgrove *et al.*, 1987). So higher levels of PUFA lead to increased oxidation, which in turn can reduce sensory quality, but also colour stability and thereby shelf life, as a relation between lipid oxidation and myoglobin oxidation have been reported (Kanner, 1994; Juncher *et al.*, 2001; Wood *et al.*, 2003). This will also affect the quality of processed meat, since oxidation products are mainly formed during processing (Dobarganes & Marquez-Ruiz, 2003).

Levels of antioxidants, such as vitamin E, influence the rate and intensity of the oxidation process (Gray *et al.*, 1996), and thus lipid and colour oxidation in meat and meat products can be decreased by dietary supplementation with antioxidants (Buckley *et al.*, 1995; Liu *et al.*, 1995; Liu *et al.*, 1996; Bosi *et al.*, 2000; Coronado *et al.*, 2002), and addition of antioxidants during processing (Freybler *et al.*, 1993; Kanner, 1994).

Meat colour

Myoglobin is primarily responsible for the colour of meat. It is present in different chemical states, which have different colours depending on the oxidation state of the iron atom in the heme group (ferrous (Fe^{2+}) or ferric (Fe^{3+})) and the sixth ligand of the heme group. When ferrous heme lacks a sixth ligand it has a purple red colour and is called deoxymyoglobin, while with oxygen as sixth ligand it appears cherry red (colour of fresh meat) and is called oxymyoglobin (Faustman & Cassens, 1990) (Fig. 3). Ferric heme with water as sixth ligand is called metmyoglobin, has a brown colour, and is the dominant form in brownish meat (Faustman & Cassens, 1990).

Meat colour is an important quality attribute for the consumer (Gatellier *et al.*, 2001). The browning of meat, which determines the colour display life, is caused by oxidation of myoglobin and oxymyoglobin to metmyoglobin (Ledward, 1992). The reaction rate of the colour oxidation process depends on numerous factors, including pH, temperature, water activity, light, bacteria, lipid oxidation, antioxidant content, partial oxygen pressure, oxygen consumption and reducing enzyme activity in the meat (Faustman & Cassens, 1990; Ledward, 1992). Substances added during processing also affect colour stability, for example nitrite binds to the myoglobin and builds a stable complex of nitrosyl-myoglobin (dark red) as described by (Gray & Pearson, 1984; Morrissey & Tichivangana, 1985).

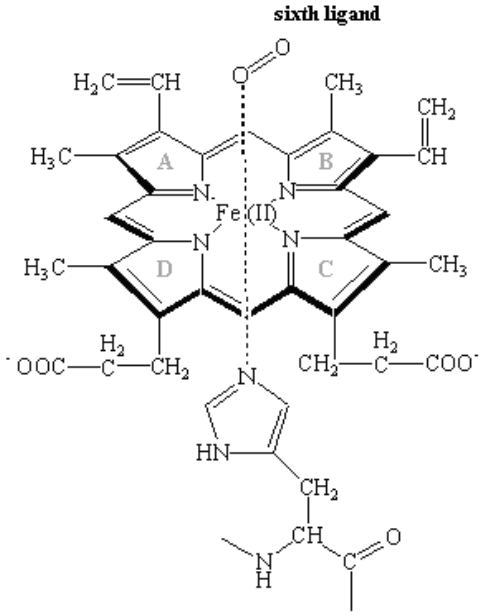


Figure 3. Chemical structure of oxymyoglobin.

Storage and processing of meat

Fresh meat is sold chilled to a temperature of ca +4 °C. Preservation of meat quality is a important criterion for its shelf life, since raw, chilled meat has traditionally been a perishable product (Gill, 1996; Jeremiah, 2001). However, international trading demands new techniques to provide longer storage times. Due to long transport distances and high export quantities, deer meat from New Zealand is stored in vacuum packages and deep chilled to –1.5 °C, and can be considered as fresh meat up to 14 weeks after slaughter (http://www.nzgib.org.nz/n95.html, 04-mar-2005). The low temperatures, and vacuum retard bacterial growth, lipid oxidation and colour deterioration, but it is unclear to what extent this method is superior to traditional storage methods (4°C, normal/vacuum package).

Frozen storage has been a method considered sufficient to preserve meat over longer time periods (Jeremiah, 1980), however freezing can also negatively influence structural and chemical properties of meat, *e.g.* increase content of FFA and lipid oxidation products (Miller *et al.*, 1980).

Processing is a primarily way to preserve meat, but also adds to its value. Some frequently used methods to preserve whole meat are dry curing (Buscailhon *et al.*, 1994), drying (Egbunike & Okubanjo, 1999; Gandemer, 2002; Cava *et al.*, 2003), curing and fermenting (Paleari *et al.*, 2002), and smoking (Pearson & Gillett, 1996). Traditional processes of preserving reindeer meat are smoking and drying, which often follows a curing and smoking step (Niinivaara & Petäjä, 1984).

Different processing steps can also negatively affect meat quality and change, for example, lipid quality traits. Heating of meat and meat products *e.g.* hot smoking, can disrupt the cell membranes and promote lipid oxidation (Gray & Pearson, 1987), which affects the nutritional and sensory properties of the meat product (St. Angelo *et al.*, 1987; Byrne *et al.*, 2002). Use of antioxidants during processing, or alternative processing methods can reduce these negative effects (Freybler *et al.*, 1993; Kanner, 1994; Coronado *et al.*, 2002; Nassu *et al.*, 2003).

The traditional method of drying reindeer meat has been used over many centuries and is described as similar to freeze-drying (Niinivaara & Petäjä, 1984). Earlier, pieces of meat were dried outdoors during winter, in temperatures sometimes below –20 °C, but in the modern drying process, smaller pieces of reindeer meat are salted, sometimes cold-smoked, and then dried in a well-ventilated room. Depending on the size of the pieces, the entire drying procedure takes about two weeks. Dried reindeer meat has a tougher texture than dried ham. Indeed, considering the high DM and the different processing, dried reindeer meat is more comparable to other traditional products from around the world, for example boucané from Réunion (Poligne *et al.*, 2001) or Kilishi from Nigeria (Egbunike & Okubanjo, 1999). Therefore it is difficult to compare dried reindeer meat with more commonly available dried meat products, such as parma ham, which usually comprise whole hams with bones and subcutaneous fat that are salted and then slowly dried and ripened for a long time (Zanardi *et al.*, 2000).

Dry curing and drying of meat also involve pro-oxidative factors. Salt is known to be a pro-oxidant, as are long exposure to air, dehydration and absence of nitrite (Vestergaard & Parolari, 1999). Enzymatic activity can lead to high amounts of FFA, which are more prone to oxidation than TAG (Gray & Pearson, 1984; Enser, 1987). In products such as dry-cured ham or dry-cured salami, a certain amount of volatiles, lipid oxidation compounds and lipolysis products is desired since they are responsible for the particular taste of these products (Gray *et al.*, 1996; Pastorelli *et al.*, 2003). On the other hand, exessive amounts result in off-flavours and rancid taste (Mottram, 1998), and some lipid oxidation products even adversely affect human health (Dobarganes & Marquez-Ruiz, 2003).

Effects of diet on fatty acid composition in meat of ruminants

FA composition of meat is mainly affected by the FA composition of the animal's diet. Monogastric animals, such as pigs, are more sensitive to dietary modification than are ruminants (Rule *et al.*, 1995). Meat from grazing ruminants is rich in n-3 FA due to the high content of these FA in grass and leaves (Elgersma *et al.*, 2003), however, intensive feeding with pellets leads to a higher n-6/n-3 ratio in the meat, as concentrates are often based on grains, containing high amounts of n-6 FA (Wood & Enser, 1997). In addition, pellets are often produced with oils instead of whole seeds. Unprotected C18 unsaturated FA are hydrogenated in the rumen by microorganisms, if they are not naturally protected by cell walls as in grass and seeds, or by synthetic coatings (Wood & Enser, 1997; Demeyer & Doreau, 1999; Scollan *et al.*, 2001a). Because of this hydrogenation, feeding of concentrates to ruminants usually results in a lower amount of PUFA in the tissues compared with monogastric animals.

Lipids and fatty acid metabolism in the animals

FA are not only important for human nutrition, but also for animal growth and reproduction (Innis, 1991; Leskanich, 1999; Mattos *et al.*, 2000). A deficiency of PUFA has been suggested to retard growth in young reindeer (Soppela, 2000). n-3 PUFA are involved in development of retina and brain and cellular growth (Innis, 1991). It is suggested that mammals are not able to elongate and desaturate the FA in a significant amount (Arts *et al.*, 2001; Rooke *et al.*, 2001), indicating that they need to obtain LC PUFA from the diet. However it has also been suggested that a higher intake of 18:3 n-3 could enhance *de novo* synthesis of LC n-3 PUFA, such as 20:5 n-3 and 22:5 n-3 (Scollan *et al.*, 2001b).

FA composition is also important for membrane functionality (Wiseman, 1996; Gibbons, 2003), and in cold climates, a higher amount of PUFA might be required to guarantee the proper fluidity. In pigs, environmental temperature influenced FA composition of back fat (Lefaucheur *et al.*, 1991). Studies on mammals that hibernate showed that animals with higher PUFA in their diet, and subsequently in their tissues, had longer hibernation periods and lower body temperatures during hibernation (reviewed by: Florant 1998). Observations in reindeer show that bone marrow is rich in PUFA, and the proportions of unsaturated FA are greater in distal parts of the animals, with a clear gradient of unsaturation in adipose tissue (Pond *et al.*, 1993; Soppela & Nieminen, 2001). This might indicate a connection between the FA composition of tissues and environmental temperature in mammals, and may even show the need for certain amounts of PUFA in the diet.

Parameters, other than diet, that influence FA composition and lipid storage in meat are breed, animal age and sex (Mersmann, 1990; Malau-Aduli *et al.*, 1998). Studies on a range of species show that age and sex can influence lipid metabolism, and thereby FA composition in the muscle (Wood & Enser, 1988; Huerta-Leidenz *et al.*, 1996; Matsuoka *et al.*, 1997; Högberg *et al.*, 2001). It has also been shown that sex hormone status influences not only lipid metabolism in adipose tissue (Prior, 1983), but also NL and phospholipid composition of the meat (Matsuoka *et al.*, 1997; Malau-Aduli *et al.*, 1998). Malau-Aduli *et al.* (1998) found higher levels of SFA and MUFA, and lower levels of PUFA in PL fraction of steers compared to heifers. Leat (1976) concluded that the higher amounts of unsaturated FA in female sheep and cattle are due to the effect of sex hormones on desaturase enzymes, a conclusion that Högberg *et al.* (2001) also suggested in pigs.

Objectives of the individual studies

- **Study I: Fatty acids, antioxidants and oxidation stability of processed reindeer meat**
  The overall aim was to study the relationship between lipid constituents in fresh and two traditionally processed reindeer meat products by investigating: i) changes in lipid class composition during processing, ii) FA composition in total lipids, iii) FA composition in separated polar and neutral lipids, and iv) vitamin A and E content and significant changes in lipid constituents in fresh, smoked and dried meat.

- **Study II: Effects of feeding regimen and chilled storage on water holding capacity, colour stability, pigment content and oxidation in red deer meat**
  The purpose was to compare the water holding capacity, colour stability, pigment content and levels of oxidation products in meat from a group of red deer that had grazed natural pastures, and a group fed a commercial pelleted feed mixture. In addition, a comparison of carcass parameters was included.

- **Study III: Fatty acid composition and vitamin E and A content in meat from reindeer of different age and sex, grazing and fed different diets**
  We investigated the possibility of improving the FA composition of meat from pellet-fed reindeer by adding linseed, a rich source 18:3 n-3, to pellets, mimicking the FA composition of the natural forage in terms of a lower n-6/n-3 ratio. We included animals of different age and sex in this study as those might influence lipid metabolism and thereby FA composition in the muscle as well as vitamin E and A content. Calves were not separated by sex, since earlier results showed no differences in meat FA composition between reindeer calves of different sex (Study I).

- **Study IV: Influence of production system, age and sex on carcass parameters and some biochemical meat quality characteristics of reindeer**
  The aim was to analyse lipid class composition and IMF in relation to trim fat, carcass weight and conformation, and to investigate how these parameters are affected by sex, age and different feed sources. As differences in fatness between the groups were shown in study III, analyses of lipid classes will add further information on the lipid fractions investigated earlier, since PL fraction contains most PUFA. In addition we also aimed to identify an unidentified substance, which was found during FA analyses in NL fraction of the reindeer meat (study III).

Material and Methods

Animals

In **study I**, a total of 16 reindeer calves (eight males and eight females, age about 10 months) were included. The animals had been fed a pelleted feed mixture (CPD) (Table 1) (Renfor Bas, Lantmännen, Holmsund, Sweden) for two months before slaughter. Slaughter and processing followed the usual abattoir practice (Arctic Deli AB, Harads, Sweden). Fresh samples were taken directly after slaughter (about 45 min *post mortem*) from the left *Musculus semimembranosus,* frozen in –20 °C for 6 days, and finally stored at –80 °C. The right *M. semimembranosus* was taken from the carcass at one day *post mortem,* and divided in two parts of about 500 g. One randomly chosen part was warm smoked, and the other was dried. The total weight of the muscle sample was on average 1.0 kg. After processing, samples were taken and frozen at –20 °C for six days and finally stored at –80 °C. The initial six days of storage was done to ensure all samples were treated equally, because samples could not be frozen at –80 °C at the abattoir.

In **study II**, a total of 16 male red deer (age 1 year) were included. Eight animals had grazed pasture and eight had been fed a pelleted feed mixture (Standard Deer Nuts, Reliance Stockfoods Ltd, Dunedin, New Zealand) (Table 1) for 10 weeks prior to slaughter. The feeding of the animals was carried out at AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand. The animals were exposed to normal pre-slaughter handling, including yarding at the farm, a short transport and subsequent overnight lairage at a deer slaughter premises. At slaughter, all animals were stunned with a captive bolt. The slaughter procedure included electrical stimulation of the carcasses using a MIRINZ low voltage stimulator (rectangular pulses, with 7.5 ms duration positive pulses only and an output of 90-95 V). Carcasses were stimulated with a battery clip attached to the upper lip of the jaw and a stainless steel hook contacting the anus. Current was applied for 55 s during bleeding out. Samples from the left side *Musculus longissimus dorsi* (at the last rib) were taken at 30 min *post mortem* and frozen in liquid nitrogen (-196 °C). One day *post mortem*, *M. longissimus dorsi* from the left side were excised and cut in five pieces that were randomly allocated to sampling at 1 day *post mortem*, 1, 3, 6 or 12 weeks of refrigerated storage at –1.5 °C. Finally all samples were stored at –80 °C until further analyses.

In **studies III** and **IV**, a total of 38 reindeer (seven males and seven females, aged 2 years and older, and 24 calves, aged about 10 months) were included. The older animals and a group of seven calves were previously grazing winter pasture in the forest. The other calves were allocated to two groups which were fed for two months before slaughter with either conventional pellets (CPD, $n = 7$) or pellets enriched with crushed linseed (LPD, $n = 10$) (Renfor Bas, Lantmännen, Holmsund, Sweden (Table 1)). All animals were slaughtered in early April, according to standard abattoir procedures (Grundnäs Kött AB and Arvidsjaur Renslakt AB, Sweden). Samples were taken directly after slaughter (about 45 min

post mortem) from the *M. longissimus dorsi*, frozen at –20°C for six days and finally stored at –80°C until analyses.

Table 1. *Industrial nutrient analyses for Renfor Bas, Renfor Bas enriched with crushed linseed (Svenska Lantmännen, 2003, Sweden) and Standard Deer Nuts, Reliance (Stockfoods Ltd, Dunedin, NZ)*

Nutrient content	Renfor Bas (CPD)	Renfor Bas plus linseed (LPD)	Standard Deer Nuts
Crude protein, %	10.0	10.4	13.6
Energy, MJ/kg	10.0	10.0	11.0
Fibre, %	15.0	14.6	7.4
Crude fat, %	3.0	3.05	2.8
Water, %	12.0	12.0	
Vitamin A, IU/kg	9000	9000	10 000
Vitamin D_3, IU/kg	3000	3000	2000
Vitamin E, mg/kg	60	60	5
Calcium, %	0.8	0.8	
Phosphate, %	0.4	0.4	
Magnesium, %	0.3	0.3	
Selenium, %	0.6	0.6	

Processing of the reindeer meat

Before smoking, a salt solution containing 13% salt (NaCl, containing 0.6% nitrite), 1.9% sugar and 0.25% ascorbate in water was injected into the meat, which was then cured for three days at 4 °C. The meat was then rinsed with water, matured for about 1 h at 40 °C, and dried at 45 °C for 45 min, before it was smoked over alder tree (*Alnus glutinosa*) chips at 80 °C until a core temperature of 65 °C was obtained. The meat was chilled overnight, and the next day samples were collected, frozen at –20 °C for six days and finally stored at –80 °C.

For drying, the meat was dry-salted for 4-5 days (without additional nitrite) at +4 °C, rinsed with water, cold-smoked over alder tree chips at 40 °C for 2½ hours, and then dried at 17-18 °C in a well-ventilated room for about 2 weeks. Finally samples were collected, frozen in –20 °C for six days and finally stored in –80 °C.

Carcass parameters, drip loss and dry matter content

Dressed carcass weights and grading scores were recorded at the abattoir. Temperature was measured with a digital thermometer (Ebro, TFX 392 SK-S, Germany) and pH values were measured with a portable pH meter (Orion, model 265, Germany) equipped with a Xerolyte electrode (InLab®427, Mettler Toledo, Switzerland). The pH meter was adjusted to muscle temperature at each measurement.

Drip loss (purge) was measured by the following procedure: (1) the combined weight of muscle and the vacuum pack was recorded before opening; (2) at opening, any surplus drip on the meat was removed using a paper towel, and the drip-free weight of the meat recorded. The combined dry bag (average weight of 25 empty vacuum bags) and drip-free meat weights were subtracted from

unopened package weight to derive the total drip weight. Drip weight was then expressed as a percentage of the original weight of packed meat.

Average dry matter was determined after the minced samples were blended with sea sand and dried at 105 °C for 16-18 h (Nordic Committee on Food Analysis, 1991).

Pigment content and colour

Pigment content of the meat samples was analysed using the Nit409 method (Trout, 1991) with minor modification. Sub-samples of 3 g of meat were taken and homogenized with 30 ml of 0.04 M phosphate buffer using a Sorbent blender for 30s (at a speed of 6 on a scale from 1 – 10). The samples were kept on ice and centrifuged at 6000 rpm and 4 °C for 15 min in an IEC PR7000 centrifuge. After centrifugation, 4 ml of each sample was mixed with 1.4 ml 0.15 M TritonX-100 solution and 100 µl 0.065 M sodium-nitrite solution, and the samples shaken and kept at room temperature (20 °C) for 1 hour. The amount oxidised pigment was measured as metmyoglobin, at a wavelength of 410 nm using a spectrophotometer Unicam Spectronic UV 300.

Triplicate colour measurements were made on each freshly cut steak 2 hours after opening the vacuum bag, then twice daily using a Minolta Chroma meter (CR-300, Japan), as found appropriate for venison (Stevenson *et al.*, 1989). Days of acceptable colour (display life) were calculated as the time taken to reach an a^* value of 12 using linear interpolation between consecutive samples, as has been used previously for venison (Stevenson *et al.*, 1989; Wiklund *et al.*, 2001b).

Fat content, fatty acids and lipid classes

Lipid extraction and total lipid content of the meat

Lipid extraction was performed according to Hara & Radin (1978), with minor modifications as described by Pickova *et al.* (1997). Connective tissues and visible fat were removed, the frozen samples were minced, and a sub-sample of ca 9 g of muscle tissue was taken for extraction. The samples were homogenised for 3x30 s in 100 ml of HIP (hexane : isopropanol (3:2), v/v) using an Ultra Turrax (T25, Janke & Kunkel, IKA Werke, Germany), and 43 ml of a Na_2SO_4 solution (6.67%) were added. The homogenate was centrifuged for 5 min at 4000 rpm (2103 rcf) (Sorvall Super T21, Sorvall Products L.P., Newton, Connecticut, USA) and the upper phase transferred to a new flask and evaporated. The lipid content of the meat was determined from this total extracted lipid (intramuscular fat, IMF), which was then dissolved in 4 ml chloroform. The samples were stored at –80 °C in normal atmosphere until further analyses.

Extraction of lipid from the feed

The different feed samples were extracted according to the method of Folch *et al.* (1957) with minor modifications. Pellets and dry lichens were milled, and a sub-sample of 2 g was wetted in 8 ml water. The samples were then homogenized for

3x30 s in 150 ml of chloroform : methanol (2:1, v/v) using an Ultra Turrax (T25, Janke & Kunkel, IKA Werke, Germany). The samples were transferred to a separatory funnel, and 40 ml KCl solution (0.8 %) added. The lower phase containing the lipids was transferred to a new flask and evaporated. The lipid content from the lichens and the pelleted feed was determined from this total extracted lipid.

Thin layer chromatography (TLC) analysis of lipid classes

The total lipids from meat were analysed with TLC to investigate lipid class composition. The analysis was done according to Olsen & Henderson (1989) with minor changes. As a stationary phase, glass plates pre-coated with silica gel high performance TLC plates (20x10 cm; Silicagel 60; 0.20 mm layer, Merck, Darmstadt, Germany) were used. Prior to use, the plates were pre-developed to full length with hexane : diethyl ether : acetic acid (85:15:1) as a mobile phase, and dried for 5 min at 110 °C. Then the upper 1 cm of silica gel was removed, and the plates were activated for 1 h at 110 °C and stored in a vacuum dessicator until further use. The samples were diluted to a concentration of 1µg/µl in hexane, and an amount of 5µl per sample was applied with a CAMAG TLC Sampler 4 (Camag Switzerland), 2 cm from the base edge of the TLC plates in 2-mm bands with an application speed of 250 nl/sec. Nitrogen was used as spray gas. All samples were applied in duplicate, and the distance between tracks was 10 mm.

The lipids were separated in a Twin Through Chamber 20x20 (Camag Switzerland) using 25 ml hexane : diethyl ether : acetic acid (85:15:1) as mobile phase. The chamber saturation was increased by placing a piece of filter paper in the chamber (Camag, Switzerland). Plates were removed from the chambers when they had developed to 6.9 cm from the origin, then air dried at room temperature, sprayed with a solution of 3% cupric acetate in 8% phosphoric acid and then charred for 20 min at 160 °C. Quantitative analysis of the separated lipid classes was done by scanning the plates with a CAMAG TLC Scanner 3 (Camag, Switzerland). The scanning was performed at a speed of 20 mm/sec, and a data resolution of 100 µm/step with a slit dimension of 6.00 x 0.45 mm at a wavelength of 350 nm. Identification of the lipid classes was performed by comparison with an external standard (TLC 18-4A, Nu-Chek Prep, Elysian, USA). For data filtering, the mode Savitsky-Golay 7 and manual baseline correction were used.

Separation of lipids

Total lipids were separated on prepacked 6 ml solid-phase-extraction (SPE) columns (Isolute SI 500 mg, IST, UK) into NL and PL fractions according to Prieto *et al.* (1992) with minor modifications. The samples were dissolved in 2 ml diethyl ether : acetic acid (100:0.2) and applied on the columns which were previously activated with 6 ml hexane. NL were eluted with 18 ml hexane : diethyl ether (200:3), and the PL fraction with 6 ml methanol : acetonitrile (65:35). After evaporating the solvents, the samples were dissolved in 0.5 ml hexane and stored under normal atmosphere at –80 °C until further analysis.

Preparation of FAME (fatty acid methylesters)

FA from the total lipids and NL and PL fractions were methylated according to Appelqvist (1968). Samples were methylated in 2 ml of a 0.01 M solution of NaOH in dry methanol, either with (total lipids and feed) or without (NL and PL) adding 3 ml BF_3 reagent (boron triflouoride-methanol complex) After methylation, the FAME were extracted with 2 ml hexane and the upper layer was transferred to a new tube and evaporated under nitrogen gas to dryness. The FAME were dissolved in 0.5 ml hexane and stored under normal atmosphere at –20 °C for a maximum of one week before gas chromatography analysis.

Capillary gas liquid chromatography (GC)

The completeness of FA methylation was checked by analytical TLC using hexane : diethyl ether : acetic acid (85:15:1) as solvent. The plates were developed by spraying with a mixture of 10% phosphomolybdic acid in ethanol : diethyl ether (1:1) and drying for 15 min in 100 °C. The FAME were analysed with a gas chromatograph (CP9001, Chrompack, Middelburg, Netherlands) equipped with a flame ionisation detector and split injector as described by Pickova *et al.* (1997). A BPX 70 column (SGE, Austin, Texas), length 50 m, id 0.22 mm, and film thickness 0.25 µm was used. The GC was programmed to start at 158 °C, with a rate of 2 °C/min until to 220 °C and a final constant time of 13 min. at 220 °C. The other GC-conditions were as described in Pickova *et al.* (1997). The peaks were identified by comparing their retention times with those of the standard mixture GLC-68A (Nu-Chek Prep, Elysian, USA) and other authentic standards. The response factors were also evaluated by comparing with the GLC-68A standard.

GC-MS analyses of the unknown substance

For the mass spectra analyses of the unknown substance, a BPX 70 column (SGE, Austin, Texas), length 50 m, id 0.22 mm, and film thickness 0.25 µm was used. GC conditions were as described for the FA analyses earlier, on a gas chromatograph (CE Instruments, Milano, Italy) connected to a mass spectrometer (Finnigan, Manchester, England). Helium was used as carrier gas and the injection mode was splitless. The electron energy was 70 eV, the ion source 200 ºC, and full-scan mass spectra were recorded (Johnsson & Dutta, 2003).

Vitamins

For the analysis of retinol, α- and γ- tocopherol, an HPLC method described by Högberg *et al.* (2002) was used, as follows: 2x1 g muscle (0.4 g for the dried meat) was cut into pieces and homogenised in two tubes together with 1.2 ml of 20% ascorbic acid solution, 0.6 ml methanol and 1.2 ml of KOH-water (1:1). After saponification and cooling, tocopherols and retinol were extracted with 2x4 ml of hexane. The hexane/vitamin solution was evaporated under nitrogen gas and diluted with mobile phase consisting of 95% methanol : acetonitrile (1:1) and 5% chloroform. Analyses were carried out with a Merck Hitachi L7100 pump, an

Fl L-7485 detector and an L-7200 auto sampler (Merck Hitachi, Eurolab, Darmstadt, Germany). The HPLC column was a 4.0x250 mm RP-18 LiChroCART (Merck KGaA, Darmstadt, Germany). Mobile phase was pumped at a flow rate of 1.2 ml/min. Identification and quantification were done by external standards. Retinol was detected with an excitation wavelength of 344 nm and emission wavelength 472 nm; γ-tocopherol and α-tocopherol were detected with excitation wavelengths of 290 and 260 nm respectively, and with an emission wavelength of 327 nm.

TBARS

For the analysis of thiobarbituric acid reactive substances (TBARS) in the reindeer meat, a modified method (method 4) described by Draper *et al.* (1993) was used to prepare samples for analysis by high performance liquid chromatography (HPLC). Approximately 1 g of each sample was homogenized with 5 ml of 5% trichloroacetic acid (TCA) solution and 0.5 ml of 0.5 mg/ml butylated hydroxytoluene in methanol (BHT). The solution was then centrifuged at 3500 rpm for 15 min (Centurion K_2R Series, Centurion Scientific LTD, West Sussex, UK). From the supernatant 2 ml was taken and heated together with 2 ml of a 0.2% solution of thiobarbituric acid (TBA) for 30 min at 85 °C. Samples were then cooled to room temperature, and the TBARS extracted from the solution with 2ml of butanol. The samples were then diluted 1:10 with the solvent phase (60% potassium phosphate 50 mM, pH 6.8; 40% methanol). HPLC analyses were carried out with a Merck Hitachi L7100 pump, an Fl L-7485 detector and an L-7200 auto sampler (Merck Hitachi, Eurolab, Darmstadt, Germany). The HPLC column was a 4.0x250 mm RP-18 LiChroCART (Merck KGaA, Darmstadt, Germany). The solvent was pumped at a flow rate of 0.7 ml/min. Identification and quantification were done by using malondialdehyde (MDA) as an external standard. TBARS were detected with an excitation wavelength of 532 nm and emission wavelength 553 nm.

For the analysis of TBARS in red deer, a slightly modified method described by Miller (1998) was used to prepare samples before analyses by spectrophotometer. Analyses were done in duplicates. The semi-frozen samples were minced, connective tissues and visible fat were removed, and a sub-sample of approximately 1 g of muscle tissue was taken for extraction. The samples were homogenized with 9.1 ml of 0.61 M TCA solution and 0.2 ml of 0.09 M BHT in methanol, using an Ultra Turrax (Janke & Kunkel, T25 IKA-Labortechnik,) for 2 x 20 s at a speed of approximately 17 000 rpm. The homogenate was then filtered through a filter paper 00k (Munktell Filter AB, Grycksbo, Sweden). Two times 1.5 ml of the filtrate were transferred to new tubes, and 1.5 ml of a 0.02 M solution of TBA was added to the first (test sample) and 1.5 ml water to the second (sample blank). After a reaction time at room temperature (20 °C) of 15 to 20 hours (over night) in darkness, the reaction complex was detected at a wavelength of 530 nm using a spectrophotometer (UV 2401 PC, Shimadzu, Japan). Quantification was made by using MDA as an external standard, and a blank was measured to detect background absorbance. Amounts of TBARS were

calculated as MDA equivalents by subtracting the blank from all standards and samples and by subtracting the sample blank from each sample.

Statistical analysis

In study I, III and IV, the statistical analyses were carried out with the Statistical Analysis System (SAS Institute, 1997) using the GLM procedure.
In study II statistical analyses were conducted with GenStat (Genstat 5 Committee, 2002)

Table 2. *Overview of the analyses and treatments of meat samples in the different studies included in this thesis. (X) refers to unpublished data*

Analyses and Treatments	Study I (reindeer)	Study II (red deer)	Study III +IV (reindeer)
Storage	(X)	X	(X)
Processing	X		
Production system		X	X
Carcass parameters		X	X
pH		X	
Dry matter	X		
Water holding capacity		X	
Pigment+ colour stability		X	(X)
Vitamin E and A	X	(X)	X
Fat content (IMF)	X	X	X
Lipid classes	X	(X)	X
Fatty acid composition	X	(X)	X
TBARS	X	X	

Summary of results

Study I: Processing of reindeer meat significantly increased FFA, whereas PL, cholesterol and TAG decreased. Minor changes in FA composition of the smoked meat were found, but the composition of the dried meat differed significantly from both smoked and fresh meat. Fresh and smoked meat had low levels of TBARS (0.11µg/g DM and 0.21µg/g DM respectively), whereas dried meat had higher levels (8.33µg/g DM). Retinol was found only in fresh meat and tocopherols decreased significantly as a result of the processing. We concluded that the smoking process slightly changed FA composition, lipid class composition and vitamin content, whereas drying resulted in major changes in all analysed parameters.

Study II: The pellet-fed deer had significantly higher carcass weights, dressing percentage and IMF than pasture-fed deer. Carcasses from the pellet-fed deer had higher temperatures during 0-10 h *post mortem,* and lower ultimate pH values than carcasses from the grazing animals. Meat from the grazing deer had significantly longer colour display life at all measured time points, and after 3, 6 and 12 weeks of refrigerated storage meat from the pellet-fed deer had significantly higher drip loss. TBARS increased during storage, but no significant differences between the treatment groups were found. Muscle pigment content was significantly higher in grazing than in the pellet-fed deer. We could not confirm a correlation between lipid and colour oxidation, but the pigment content of the meat did not seem to have an influence on colour stability or TBARS formation.

Study III: Differences between meat from male and female reindeer were found mainly in the NL fraction, and were therefore related to fatness. FA composition of meat from calves differed significantly from that of both males and females. Reindeer fed CPD had significantly higher ratios of n-6/n-3 FA compared with the grazing animals in both PL and NL fraction, whereas PL fraction of the animals fed LPD was slightly, but not significantly higher than that of grazing reindeer. In addition the amount of long chained polyunsaturated FA, namely 20:4 n-6, 22:5 n-3 and 22:6 n-3, in the PL fraction of animals fed LPD were lower compared with the grazing animals due to the content of these FA in the natural feed. Biohydrogenation of lipids was lower in grazing reindeer compared with pellet-fed reindeer. Reindeer seemed unable to elongate dietary FA in significant amounts. We conclude that it is possible to maintain the favourable FA composition of meat from reindeer grazing pasture in terms of the n-6/n-3 ratio but not the LC FA, by adding crushed linseed to the pellets.

Study IV: Feeding reindeer calves pellets resulted in higher slaughter weights, higher trim fat content and better carcass conformation scores compared with grazing calves. Adult female reindeer had the highest and grazing calves had the lowest slaughter weights, trim fat and IMF. However there was no significant difference in IMF between pellet-fed and grazing calves. There was no difference in lipid class composition of the meat from the two pellet-fed groups, whereas the grazing calves had a higher amount of phospholipids. In addition squalene was identified as a component of intramuscular lipids in reindeer meat and quantified.

Unpublished results: In addition to the results from study I-IV, vitamin content, fatty acid and lipid class composition were analysed on samples from study II, and will be published in a separate study.

Meat from grazing animals had a significantly higher content of α-tocopherol compared with meat from pellet fed red deer. Storage did not decrease levels of α-tocopherol. Grazing animals had significantly higher phospholipids and significantly lower TAG. A significant increase of FFA after 6 and 12 weeks of storage was shown in both groups. Meat from grazing red deer had significantly lower n-6/n-3 ratio compared to pellet-fed animals.

Meat colour stability was analysed on the samples from the pellet fed reindeer from study III in addition to the presented result on FA, lipid classes and vitamin content. Samples were measured at day 1 and after storage at 4 ºC for 7 and 19 days. Differences in colour stability could be measured after 19 days of storage. Meat from LPD-fed reindeer had lower colour stability compared to meat from CPD-fed ones.

General discussion

Methodological considerations

Lipid analyses

For lipid extraction a method according to Hara & Radin (1978) was chosen for the meat samples whereas the method according to Folch *et al.* (1957) was used for feed material. In addition to using less toxic solvents, the Hara & Radin (1978) method was shown to be sufficient for extracting lipids from easy extractable materials (Pickova, 1998). However for extraction of lipids from the feed Hara & Radin (1978) was found to be insufficient, which necessitated use of the more powerful method according to Folch *et al.* (1957).

Two different methods of methylation were used in the current studies. The PL and NL fractions were methylated without BF_3, while total lipids of meat and feed have been methylated with BF_3. Methylation without BF_3 is far milder and does not methylate FFA (Appelqvist, 1968). The lipids of the dried meat were analysed only as total lipids and methylated with BF_3. This was because lipid class analyses revealed a high FFA formation and significant changes in quantification of all separated lipid classes in the dried meat. Due to their polarity, FFA acids are eluted in SPE together with the NL fraction in the method used (Prieto *et al.*, 1992). This would lead to misinterpretation of data if the separate lipid classes were reported, since FFA originate both from NL and PL fractions and probably to a large extend from the PL, since phospholipids have shown to be more sensitive to lipolysis than TAG (Buscailhon *et al.*, 1994). Applying the sample containing unmethylated FFA however would have harmed the column.

Recent results show no significant differences between methylation of both, PL and NL with and without BF_3 on samples with no or low content of FFA (Sampels & Pickova, unpublished data). TLC has been proposed as a better method to separate lipid classes compared with SPE since, in the method described by Pickova (1998), FFA are separated from TAG and phospholipid fraction. However methods based on SPE separation are more rapid and efficient (Prieto *et al.*, 1992). Furthermore, losses of FA were found during TLC (probably due to oxidation during the developing time), giving different results in phospholipid fraction compared to PL when methylation without BF_3 was chosen, and FA were expressed in µg/g (Sampels & Pickova, unpublished data).

TBARS

(Studies I & II)

Zipser & Watts (1962) have reported that nitrite interacted in the analysis of TBARS, whereas Hernandez *et al.* (1999) have shown the opposite. We were unable to clarify whether such an interaction existed in the smoked reindeer material.

TBARS were analyzed by HPLC in study I (Draper *et al.*, 1993), whereas in study II a spectophotometric method was used (Miller, 1998). It should be noted that the use of a HPLC leads to a separation of MDA-TBA complex from other

colored complexes that TBA can build in meat extracts (Draper *et al.*, 1993), whereas in the spectophotometric method all TBA reacting compounds that absorb at the same wavelength are measured. The results obtained from the spectophotometric methods might therefore be higher at the same levels of oxidation compared to values obtained with the HPLC method, and can not be directly compared.

Effects of feeding regimen and processing on meat quality

Fatty acids

In the present studies, feeding regimens clearly influenced meat quality in both reindeer and red deer meat, and led to changes in lipid class and FA composition. In both animals, grazing led to higher amounts of n-3 PUFA and a lower ratio of n-6/n-3 compared to feeding with conventional pellets (Fig. 4 and 5) (study I, III, unpublished data study II, & Wiklund *et al.*, 2001a) and thereby to a better nutritional quality of the meat. Differences between species and production systems make a direct comparison difficult, but it can be noted that the reindeer had lower amounts of SFA even when pellet fed compared to both grazing and pellet-fed red deer (Fig. 4). This difference does not depend on fatness, since the IMF content was 3.47% for reindeer and 1.62% and 1.86% for grazing and pellet fed red deer respectively.

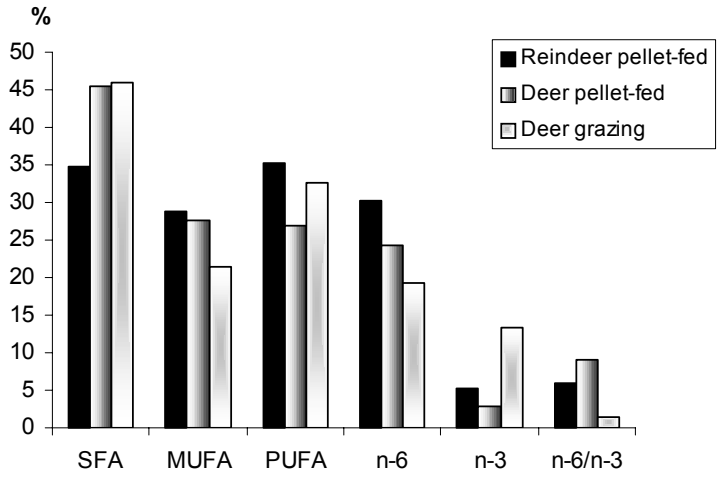


Figure 4. Fatty acid composition (averages, %) of total lipids from reindeer fed pellets (study I) compared to red deer grazing and fed pellets (study II); (significant differences, $P<0.05$, between grazing and pellet-fed red deer except for SFA).

FA composition in deer was only analysed as total lipids, while in the studies on reindeer, lipids were analysed separately as PL and NL lipid fraction, since dietary FA influence the composition of the two fractions differently due to their different function as mentioned before (Scollan *et al.*, 2001b).

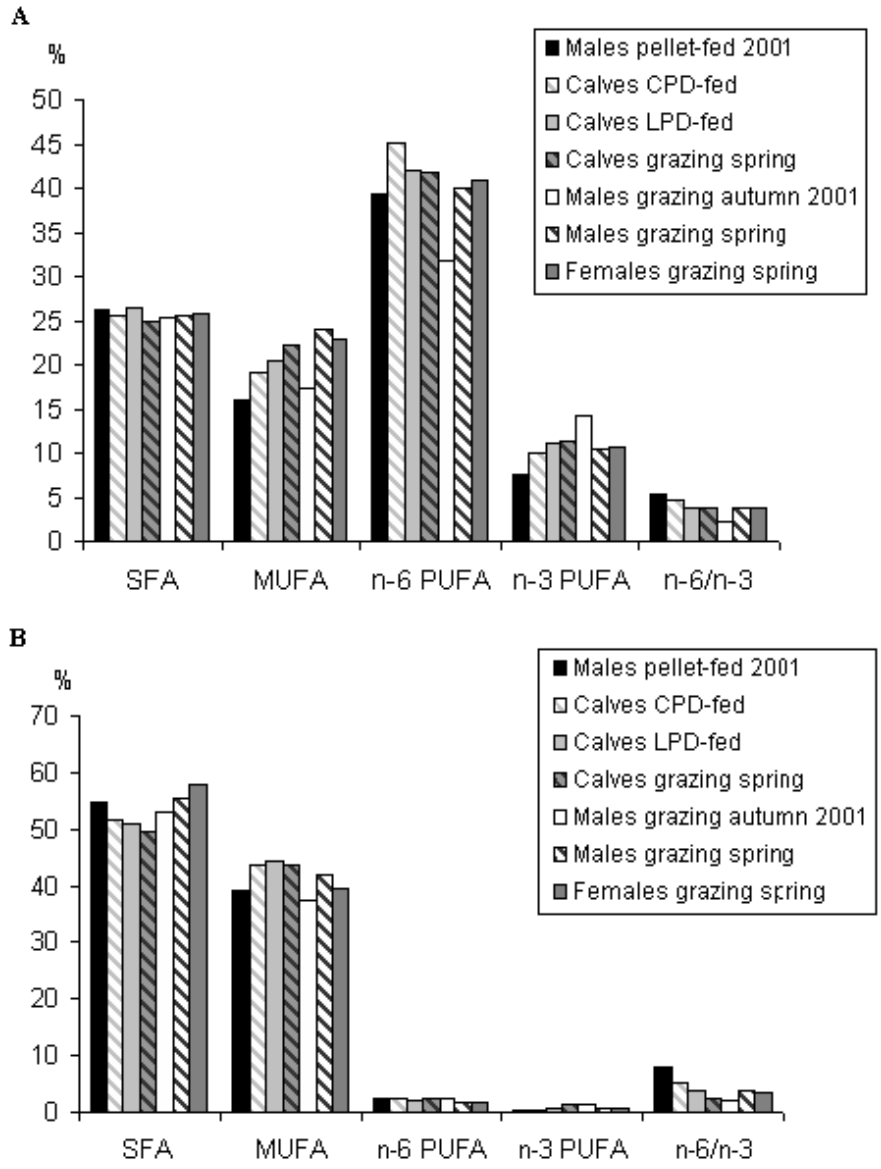


Figure 5. Fatty acid composition in meat (averages, %) from reindeer of different age from different feeding regimen at different times of the year. A= polar lipid fraction; B= neutral lipid fraction (Studies I, III & Wiklund *et al.*, 2001a).

In the processed reindeer meat, and in red deer meat after storage, significant differences were seen in FA composition of total lipids (Study I & unpublished data study II) (Fig. 6 and 7). In general, amounts of PUFA decreased whereas SFA and MUFA increased. As phospholipids are rich in PUFA (Sinclair & O'Dea, 1990; Scollan *et al.*, 2001b), these results are in line with earlier results showing that phospholipids are hydrolyzed to FFA to a greater extent than TAG

(Buscailhon *et al.*, 1994), and that PUFA are more prone to oxidation compared with SFA (Cosgrove *et al.*, 1987; Mottram, 1998). In the reindeer study, when analyzing separated lipid classes after smoking, changes in FA composition were more pronounced in PL fraction than in NL, indicating that oxidation is greater in PL fraction compared to NL. However, the actual differences between fresh and smoked meat in terms of percentage FA in both the analyzed lipid classes (NL and PL) were quite small. This could indicate, that despite these differences, direct oxidation is not a very important factor during this type of smoking, and that lipid degradation due to lipolysis is more important. Similar conclusions have been made by Buscailhon *et al.* (1994).

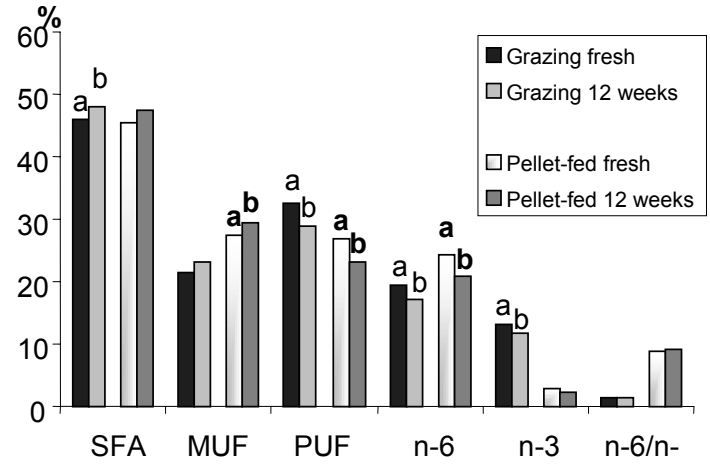


Figure 6. FA composition (averages, %) of total lipids from pellet fed fresh, smoked and dried reindeer meat (Study I). Bars with different superscripts differ significantly (P<0.05).

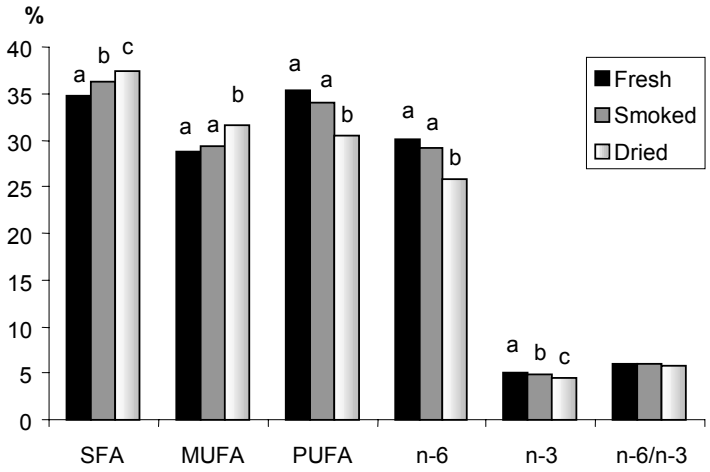


Figure 7. Fatty acid composition of total lipids (averages, %) of fresh and stored meat from red deer grazing and pellet-fed (unpublished data, study II). Bars with different superscripts differ significantly (P<0.05) (plain letters for grazing red deer and bold for pellet-fed).

Lipid oxidation, colour stability and α-tocopherol

(Studies I, II & III)

Differences in FA composition, and thus sensitivity to oxidation, can influence colour stability (Liu *et al.*, 1996; Renerre *et al.*, 1996). More unsaturated FA are more prone to oxidation (Cosgrove *et al.*, 1987), and the relationship between higher content of PUFA and lower colour stability have been discussed (Yang *et al.*, 2002b). In the study quoted, meat from pasture-raised cattle had reduced red meat colour compared with grain fed animals at the day of slaughter, although this difference disappeared after ageing of the beef. After ageing though, the grazing increased lipid oxidation in the meat samples (Yang *et al.*, 2002b).

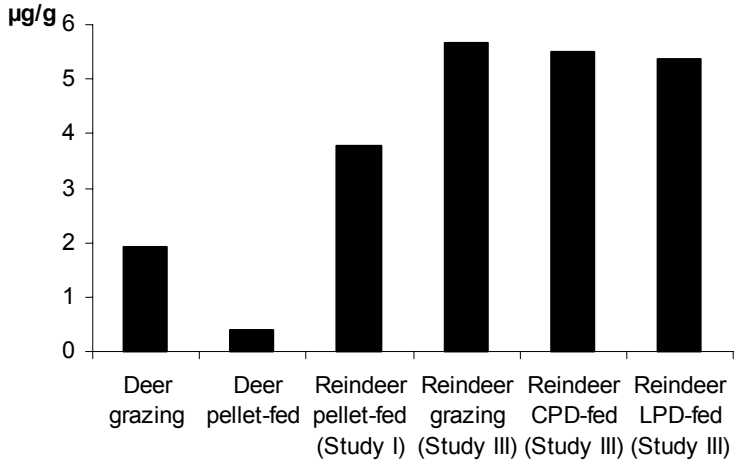


Figure 8. α-tocopherol content (µg/g) in meat from red deer and reindeer fed different diets (Study I, III, unpublished data study II)
Abbreviations: CPD= conventional pellets; LPD linseed pellets.

In the study II, lipid oxidation and colour oxidation were higher in the meat from pellet fed deer that had a lower content of PUFA. The grazing red deer had significantly higher amounts of α-tocopherol in their muscle compared with the pellet fed animals (unpublished data from study II), whereas in the reindeer the vitamin E content of pellet-fed and grazing animals was similar (studies I & III) (Fig. 8). Even if the two production systems including feed formulas differ from each other, and use of different species does not allow a direct comparison, it is interesting to note that the reindeer had a higher content of α-tocopherol in the meat in general.

It is suggested that the differences in α-tocopherol content in meat from pellet fed and grazing red deer were mainly responsible for the faster lipid oxidation and shorter shelf life (faster browning) in the meat from pellet fed deer during storage and display (study II) (Fig. 9). Therefore it is hypothesized that the α-tocopherol content had a more important role compared to FA composition. It has been shown earlier that α-tocopherol has a protecting effect against colour oxidation (Monahan *et al.*, 1994; Faustman *et al.*, 1998). Gatellier *et al.* (2004) and Gatellier *et al.*

(2005) showed similar effects in beef, where grazing led to higher amounts of PUFA but also higher vitamin E content and better oxidation stability. In the same studies a slightly better colour stability of the meat from grazing animals was also found, indicating that negative effects of FA composition on colour stability can be reduced by efficient supplementation with antioxidants. In general naturally growing plants contain sufficient amounts of antioxidant substances (also discussed above).

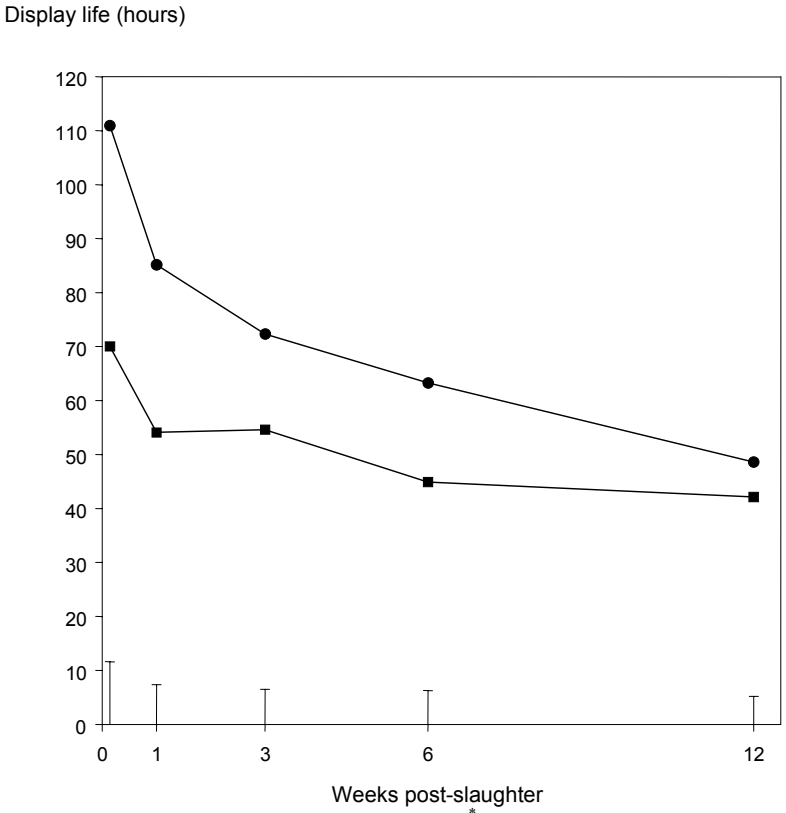


Figure 9. Mean display life (hours of Minolta a^* value ≥ 12) in *M. longissimus* from the red deer from two treatments (● pasture grazing and ■ pellet fed), measured at 1 day, 1, 3, 6 and 12 weeks of refrigerated storage (-1.5° C) in vacuum bags, with error bars indicating standard error of difference (S.E.D) (Study II).

Because significant differences in colour stability were already apparent in fresh red deer meat but differences in lipid oxidation were not, our results suggest that meat pigment oxidation occurs faster than lipid oxidation, and can reveal effects of diet and storage earlier than measurements of lipid oxidation (study II). A similar conclusion was reached by Monahan *et al.* (1994), who found that myoglobin oxidation in pork stored at +4 ºC preceded lipid oxidation.

However, in the reindeer study the α-tocopherol was similar in both CPD- and LPD-fed animals and we found slightly lower colour stability in the meat from LPD-fed animals with a higher content of PUFA (Fig. 10) (unpublished data, study III).

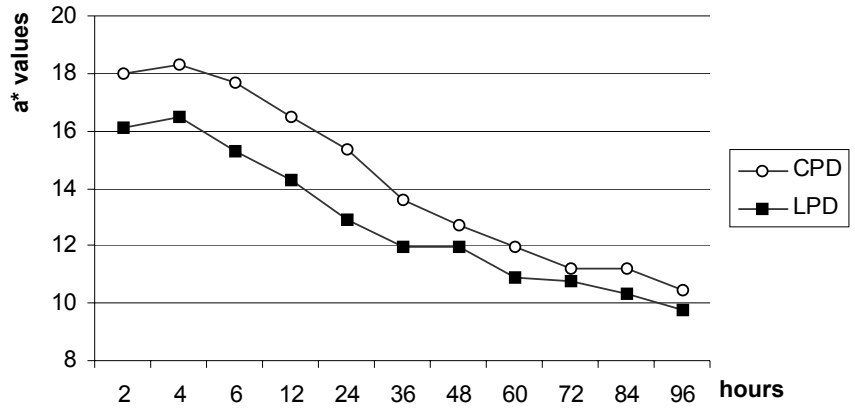


Figure 10. Colour stability of meat from reindeer calves fed two different diets (CPD= conventional pellets; LPD= linseed pellets), measured after 19 days of refrigerated storage (+4 ºC) in vacuum bags (unpublished data study III).

Feeding n-3 FA enriched pellets (LPD) to reindeer led to a nutritionally better FA composition in the meat compared to feeding conventional pellets, without affecting the levels of FFA or α-tocopherol content (Fig. 5, 8 and 14)(study III). In pellet fed reindeer, no difference in colour stability was found in fresh meat and after storage at +4 °C in vacuum package for 7 days (unpublished data study III). However, after storage for 19 days colour stability of the meat from the animals fed n-3 enriched pellets differed from that of meat from reindeer fed conventional pellets (Fig. 10). Comparison of these results with those from the red deer study (study II) confirms that negative effects on colour oxidation stability, caused by altered FA composition are less pronounced than those caused by low antioxidant levels. Whether the differences in FA composition also affects processing stability needs to be examined. Difference of α-tocopherol between the pellets (which contained 56.5 and 48.4 µg/g α-tocopherol in CDP and LDP respectively) and the lichens (which contained 9.4-15.8 µg/g) were not reflected in the meat (Study III) (Fig. 8).

It can be argued that it might be impossible to increase α-tocopherol further in reindeer muscle due to a possible saturation level, which has been shown to exist in meat from cattle (Yang *et al.*, 2002a). Jensen *et al.* (1998) present data from several trials on pigs and poultry in which vitamin E increases only slightly with high supplementation. In particular, it should be noted that reindeer meat seems to have a relatively high α-tocopherol content, compared with the red deer from our study and meat from other species (Högberg *et al.*, 2002; Yang *et al.*, 2002a;

Gatellier *et al.*, 2004). Nevertheless, dietary compounds, such as carotenoids, squalene or phenolic compounds, may be able to increase antioxidant capacity and thereby preserve colour and oxidation stability even in meat with higher amounts PUFA.

Lipid oxidation during processing and storage

(Studies I & II)

In study I, no significant increasing of TBARS could be found in smoked meat compared with fresh meat (Fig 11). It can be concluded that the processing method, which involves addition of ascorbate to the salt solution during curing, might also contribute to these results, since ascorbate may act as an antioxidant together with tocopherol, as suggested by Packer *et al.* (1979) and Gille & Sigler (1995). Furthermore, the added nitrite acts as antioxidant, and the phenolic compounds from the smoke have been suggested to have an antioxidative potential (Tóth & Potthast, 1984; Pearson & Gillett, 1996). These factors together are suggested to have contributed to the small changes in tocopherol content and FA composition and low oxidation rate in the smoked meat.

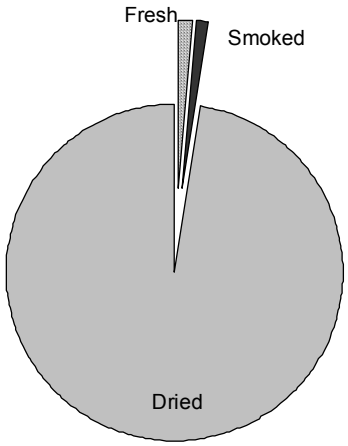


Figure 11. TBARS (µg/g DM) in fresh, smoked and dried reindeer meat (Study I).

As opposed to fresh and smoked reindeer meat, traditional drying led to significant changes in FA and lipid class composition due to oxidation and enzymatic lipolysis (study I). In the dried meat, TBARS levels were as high as 8.33 µg/g DM, which is equivalent to 5.5µg/g fresh weight. This gives the meat a clear off-taste, since Younathan & Watts (1959) have shown that amounts equivalent to 2 µg/g TBARS (spectophotometric measurements) are noticed by consumers. Lanari, *et al.* (1995) discussed even lower levels of 0.5 µg/g as critical.

In products such as dry-cured ham or dry-cured salami, a certain amount of volatiles originating from lipid oxidation compounds and lipolysis products is desired since they are responsible for the particular taste of these products (Gray *et al.*, 1996; Pastorelli *et al.*, 2003). On the other hand, too high amounts result in

off-flavours and rancid taste (Mottram, 1998). Dried reindeer meat traditionally has a relatively strong taste, and the samples used in the study were taken from normal processing and no reclamations of products from the same batch were reported. It is not clear however what oxidation values are considered 'normal' for this product. From a nutritional point of view it should be considered that some lipid oxidation products are toxic and adversely affect health (Kubow, 1992; Dobarganes & Marquez-Ruiz, 2003).

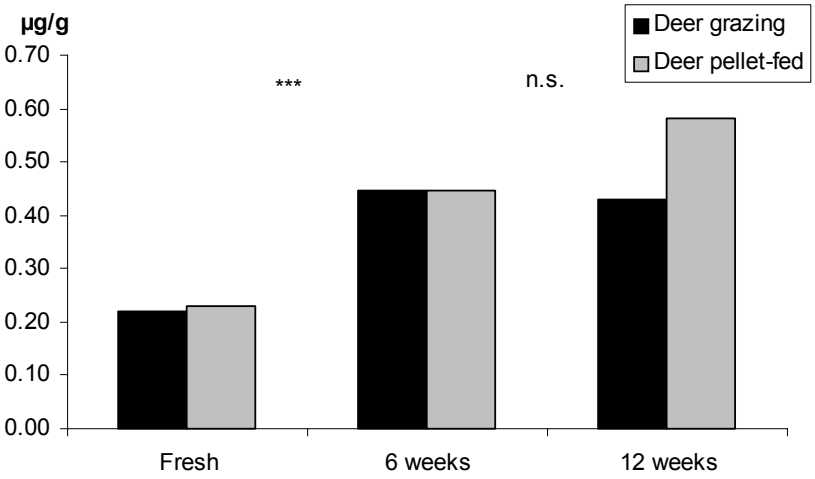


Figure 12. TBARS (µg/g) in meat from red deer grazing or fed pellets. Fresh and after 6 and 12 weeks of storage in vacuum package in –1.5 ºC (Study II). The degree of significance between treatments (values from grazing and pellet-fed deer combined) is given above the bars. n.s. = non significant; *** = $P \leq 0.001$.

Storage of red deer meat resulted in significant oxidation (study II) (Fig 12). However the TBARS values did not reach the critical value of 2 mg MDA equivalent/kg meat. The deer meat had levels of 0.22 µg/g at day 1, and 0.51 µg/g after 12 weeks of storage at –1.5 ºC (P <0.001) (study II), whereas the reindeer meat had TBARS values equivalent to about 0.03 µg/g in fresh meat and 0.07 µg/g after smoking (P = 0.950) (study I). Even if the values cannot be compared directly due to the use of different methods, it seems that the storage process (study II) did not harm the meat quality in terms of oxidation more then the smoking process (study I). We did not carry out a sensory evaluation, but we speculated that even if a significant increase of oxidation was measured in study II this might not have any significant effects on red deer meat taste, since Younathan & Watts (1959) showed that consumers did not notice changes in taste due to oxidation until much higher amounts equivalent to 2 µg/g TBARS were reached. Because of its stronger taste due to curing and smoking, these critical values might be even higher in the smoked meat.

Lipolysis and lipid oxidation

(Studies I & II)

In both the reindeer and the deer study the changes in FFA concentrations after smoking/storage were more significant than the oxidation. In study I, the FFA concentration was significantly higher after smoking (Fig 13), whereas the TBARS did not differ significantly (Fig. 11). In study II, FFA content in stored deer meat was significantly higher after both 6 weeks and 12 weeks of storage (unpublished data study II), whereas TBARS values did not increase significantly between week 6 and 12 of storage (Fig. 12 and 14). The changes in the meat from grazing red deer were more pronounced than in the pellet fed animals.

Similar results, namely significant increase of FFA but only a tendency to increased TBARS, were found in chill-stored pork (Monin *et al.*, 2003). Buscailhon *et al.* (1994) concluded that, in dry cured ham, oxidation mainly occurs in FFA, and that direct oxidation of phospholipids was only a minor problem. In a study of chicken, Alasnier *et al.* (2000b) suggested that there was no relation between lipolysis and oxidation, however lipolysis was increasing faster in the first days of storage, whereas oxidation increased linearly (Alasnier *et al.*, 2000b). We suggest that lipolysis occurs faster than oxidation, and is thereby a better early indicator of quality deterioration.

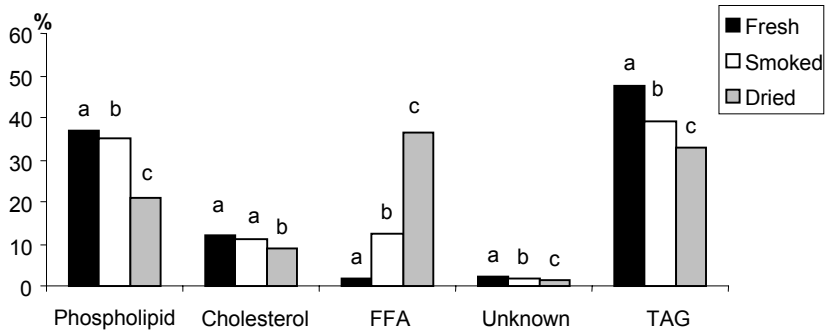


Figure 13. Lipid classes' composition (averages (%)) of fresh, smoked and dried reindeer meat (Study I). Bars with different superscripts differ significantly ($P<0.05$).

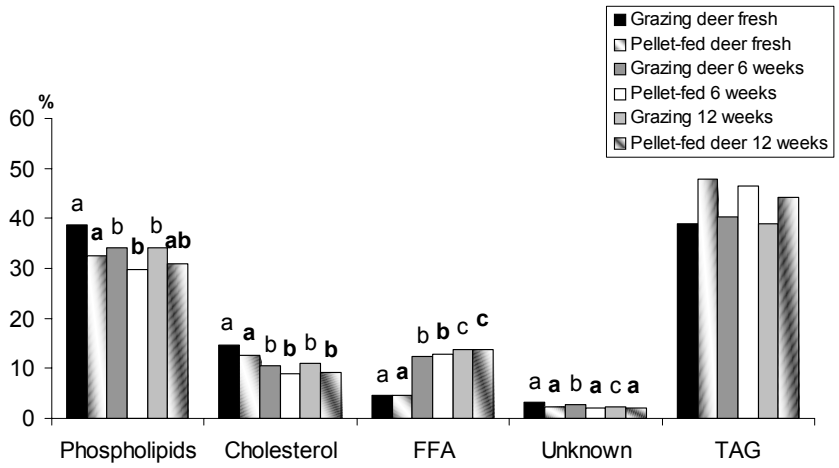


Figure 14. Lipid class composition (averages(%)) in meat from red deer fresh and after storage (unpublished data study II). Bars with different superscripts differ significantly ($P<0.05$) (plain letters for grazing red deer and bold for pellet-fed).

We were not able to quantify to what extent loss of FA depends on oxidation or lipolysis, since we did not analyze the composition of FFA or the total amounts of TAG, phospholipids and FFA but only percentages. However, FFA in processed reindeer meat (study I) seemed to originate both from TAG and phospholipids, whereas in chilled stored red deer meat (study II) TAG did not change significantly, and FFA arose mainly from phospholipids (Fig. 12 and 13). These differences in lipolysis activity might depend on differences in FA composition as well as differences in processing as suggested by (Gandemer, 2002). Alasnier *et al.* (2000a) demonstrated that FFA in rabbit meat during storage at 4 ºC increased and arose from both phospholipids and TAG. In a study on ham Buscailhon *et al.* (1994) concluded that phospholipids were most sensitive to lipolysis but that there was no specification of lipases to phospholipid class, FA chain length or unsaturation.

Since dried reindeer meat (study I) has higher DM and much shorter drying period (two weeks) than dried ham, enzymes involved in lipolysis can therefore be assumed to be less active in our samples, due to time and water activity, compared to long time ripening hams. Vestergaard *et al.* (2000) showed that strong dehydration after 10 months of aging decreased lipolytic activity, whereas only mild dehydration and salt diffusion increased lipolysis in ham during the first 6 months of ripening. However, compared with Spanish ham (Antequera *et al.*, 1992), drying of reindeer meat led to a threefold increase of FFA, suggesting that lipolysis occurs before or during drying in spite of the short drying time, as suggested by Vestergaard *et al.* (2000). Another possibility is that the enzymes are still active in the dried reindeer meat; however, this was not investigated in our samples.

Squalene

It is known that minor components of plants as phytosterols or phenolic compounds can have effects on consumers health (Parr & Bolwell, 2000; Piironen *et al.*, 2000; Ostlund *et al.*, 2002). In study IV we could detect squalene in reindeer meat. Levels of squalene were four to 10 times higher than those of in pork (Slover *et al.*, 1987), corresponding to 0.5-1% squalene in total fat, when calculated as percentage of total intramuscular fat. These values are relatively high compared with values suggested for common human dietary fats and oils (0.002-0.3% squalene/total fat) (Rao *et al.*, 1998). Squalene has been reported to have positive health effects, for example lowering serum cholesterol in hamsters (Khor & Chieng, 1997), chemopreventive effects on colon cancer in rats, and anti tumorigenic activity (Rao *et al.*, 1998). In the study by (Khor & Chieng, 1997) it was demonstrated that the presence of α-tocopherol enhanced the serum-cholesterol lowering effects of squalene. The levels of squalene in reindeer meat together with the high levels of α-tocopherol (as discussed above) might have positive effects on consumer health.

Effects of animal factors

α-Tocopherol: metabolism and bioavailability

The differences in α-tocopherol content between red deer and reindeer depended partly on the α-tocopherol content in the pelleted feed for the red deer, which was much lower than that of the reindeer pellets (Table 1 and Fig. 8) (studies I, II & III). However, we suggest that these differences also arose from differences in metabolism between species since the analysed lichens, which account for a significant part of the reindeer winter diet, had a much lower content of α-tocopherol compared to the pellets, which was not reflected in the meat (study III) (Table 3). These differences may also partially depend on the presence of squalene in reindeer meat, which has been shown to protect α-tocopherol against oxidation (Psomiadou & Tsimidou, 2002). Other components from the reindeer diet, *e.g.* the lichens could also have antioxidant capacity, or increase tocopherol bioavailability, and thereby led to the high content of α-tocopherol, since the results of Frank (2004) indicate that phenolic compounds affect tocopherol levels in tissues. A vast number of secondary metabolites with different biological functions have been analysed in lichens, *e.g.* quinones and usnic acid (Stepanenko *et al.*, 1997; Ingolfsdottir, 2002), and aqueous extracts of lichens have shown a higher antioxidant effect in a linoleic acid emulsion than α-tocopherol (Gülcin *et al.*, 2002). However, to our knowledge, lichen compounds and their effects on reindeer have been studied only in relation to animal nutrition, since reindeer, caribou (*Rangifer tarandus*) and musk deer (*Moschus moschiferus*) are the only species that include lichens in their diet to substantial amounts (Storeheier *et al.*, 2002) and seem well adapted to digest them, in contrast to other herbivores as reviewed by Palo (1993).

Table 3. *Fat content (%), α-tocopherol (µg/g) and fatty acid composition of pellets and various lichens (% of total identified FA) analysed in duplicates, mean values (Study III)*

	Conventional pellets (CPD)	Linseed pellets (LPD)	*Cladina mitis*	*Cladina stellaris*	*Cretaria islandica*	*Cladina arbuscula*
Fat content %	3.75	3.93	2.39	2.31	4.67	3.16
α-tocopherol µg/g	56.5	48.4	9.38	15.8	15.7	10.7
12:0	7.05	6.08	0.64	0.14	0.47	0.55
14:0	2.75	2.42	0.69	0.28	0.66	0.58
16:0	17.4	19.3	9.51	8.62	9.38	9.19
16:1	0.21	0.16	0.59	0.74	0.63	0.88
18:0	2.27	2.59	4.66	3.19	2.83	4.13
18:1 n-9	21.8	20.1	22.3	29.2	30.5	29.7
18:2 n-6	37.0	33.8	44.6	44.3	35.6	37.5
18:3 n-3	8.75	13.00	8.01	6.18	12.5	5.70
20:2 n-6	0.08	0.07	0.35	0.58	0.27	0.36
20:3 n-6	n.d.	n.d.	0.07	0.05	0.07	0.30
20:4 n-6	n.d.	n.d.	0.81	0.52	0.77	0.67
20:3 n-3	n.d.	n.d.	0.11	1.33	0.26	1.25
20:5 n-3	n.d.	n.d.	0.33	0.45	0.81	0.29
22:4 n-6	0.24	0.23	n.d.	n.d.	n.d.	n.d.
22:5 n-6	n.d.	n.d.	0.10	0.00	0.06	0.10
22:5 n-3	n.d.	n.d.	0.12	0.02	0.03	0.19
22:6 n-3	n.d.	n.d.	n.d.	n.d.	0.03	0.04
SFA	30.6	31.3	19.5	13.6	15.6	20.1
MUFA	23.7	21.9	25.5	32.6	33.5	33.2
n-6 PUFA	37.3	34.2	46.1	45.5	36.8	39.1
n-3 PUFA	8.75	13.0	8.57	7.97	13.7	7.47
n-6/n-3	4.26	2.64	5.38	5.78	2.70	5.26

Effects of production system on fatty acid metabolism

(Studies III & IV)

The present studies (studies III & IV) confirm that different production systems with different diets affect FA composition in reindeer meat (Wiklund *et al.*, 2001a; Wiklund *et al.*, 2003b). But even on natural pasture, FA composition of the diet varies greatly between pastures and time of the year, and depends on the plants consumed by the animals. In reindeer this effect might be intensified due to their migration between summer and winter pastures. Increased levels of MUFA and PUFA have been found in meat from lambs grazing in Norwegian mountains compared to the lowlands (Adnoy *et al.,* in press).

When comparing reindeer slaughtered in autumn, after grazing summer pasture (Wiklund *et al.*, 2001a), with the animals slaughtered in spring (studies III & IV) differences were found (Fig. 5). In the grazing animals from summer pasture lower amounts of n-6 PUFA and higher amounts of n-3 PUFA and thereby a lower n-

6/n-3 ratio were found compared to the animals from winter pasture. These differences between the grazing animals are probably due to the different amounts of these FA in the summer pasture compared to the winter pasture. During late summer the reindeer graze mainly graminoids in addition to woody plants and mushrooms (Nieminen & Heiskari, 1989; Mathiesen *et al.*, 2000). Green feed, such as grass, is rich in 18:3 n-3, whereas the lichens, which are consumed in increasing amounts during winter (Storeheier *et al.*, 2003), are richer in 18:2 n-6 than is grass (study III; Elgersma *et al.*, 2003). However, in study III FA composition of the feed was not completely reflected by that of the analysed muscles (Table 3 and 4). We could not detect 12:0, which was present in the pelleted feed, in the muscle samples analysed, and 18:2 n-6 was lower in meat from grazing than from pellet-fed reindeer, in spite of the higher amounts in the lichens compared with the pellets. This indicates that the reindeer use 12:0 as an energy source and do not store it in the muscles, whereas the lowered amount of 18:2 n-6 can be explained by the fact that, during winter, reindeer graze green plants containing higher amounts of n-3 FA (Elgersma *et al.*, 2003), other than lichens (Nieminen & Heiskari, 1989; Storeheier *et al.*, 2003).

Because phospholipid composition is relatively stable due to their functionality, they are generally less affected, variation in meat is relatively small and changes in n-3 FA are balanced by other similar FA, usually from the n-6 family (Ratnayake *et al.*, 1989; Scollan *et al.*, 2001b). In a study on beef fed a linseed-enriched diet, the increase of 18:3 n-3 led to a decrease of 18:1 n-9, 20:3 n-6 and 20:4 n-6 in the meat (Scollan *et al.*, 2001b). In study III, the significantly higher amount of 18:3 n-3 in the LPD is reflected in the PL fraction in addition to a significantly higher amount of 18:2 n-6, which are probably balanced by lower contents of 20:4 n-6 and 22:5 n-3 (Table 4). The lower values of 20:4 n-6 in LPD-fed compared to CPD-fed reindeer could be a sign of reduced elongation from 18:2 n-6 due to interference of synthesis of 20:5 n-3 from 18:3 n-3 as suggested by Ratnayake *et al.* (1989). In line with their intake, grazing reindeer had the highest amounts of 20:4 n-6 (study III) (Table 3 and 4).

Table 4. *Fatty acid composition of meat from reindeer calves fed different types of pellets compared to free ranging calves (% of total identified fatty acids) (mean values and standard deviation) (Study III)*

Polar lipids	CPD-fed (*n=7*)	S.D.	LPD-fed (*n=10*)	S.D.	Grazing (*n=7*)	S.D.	*P*
16:0	10.9^{ab}	0.67	11.1^{b}	0.91	10.1^{a}	0.72	0.035
16:1 trans	0.35^{a}	0.05	0.46^{b}	0.06	0.32^{a}	0.04	<0.001
16:1	1.11^{a}	0.26	1.20^{ab}	0.12	1.33^{b}	0.15	0.077
18:0	14.3	0.66	14.9	0.60	14.3	0.74	0.122
18:1 trans	0.50^{a}	0.14	0.41^{a}	0.14	0.11^{b}	0.04	<0.001
18:1 n-9	14.9^{a}	2.20	16.2^{a}	1.85	18.1^{b}	1.14	0.010
18:2 n-6	29.0^{a}	2.85	27.2^{a}	1.95	22.6^{b}	1.01	<0.001
18:3 n-3	1.63^{a}	0.43	2.31^{b}	0.32	1.25^{c}	0.22	<0.001
20:3 n-6	1.06	0.12	1.02	0.10	1.05	0.09	0.728
20:4 n-6	14.2^{a}	0.74	12.9^{b}	1.19	17.0^{c}	1.44	<0.001
20:3 n-3	0.27^{a}	0.04	0.25^{ab}	0.04	0.32^{b}	0.06	0.046
20:5 n-3	2.63^{a}	0.59	2.89^{ab}	0.27	3.22^{b}	0.54	0.077
22:4 n-6	0.74	0.13	0.70	0.08	0.80	0.18	0.252
22:5 n-6	0.02^{a}	0.02	0.03^{a}	0.03	0.10^{b}	0.02	<0.001
22:5 n-3	5.27^{a}	0.45	5.31^{a}	0.29	6.03^{b}	0.42	0.001
22:6 n-3	0.38^{a}	0.05	0.37^{a}	0.05	0.51^{b}	0.03	<0.001
SFA	25.6^{a}	0.44	26.4^{b}	0.63	24.8^{c}	0.93	0.001
MUFA	19.1^{a}	2.42	20.5^{ab}	1.99	22.2^{b}	1.18	0.026
n-6	45.2^{a}	2.91	42.1^{b}	1.78	41.7^{b}	2.07	0.013
n-3	10.1^{a}	1.49	11.1^{ab}	0.69	11.3^{b}	0.86	0.077
n-6/n-3	4.58^{a}	0.72	3.82^{b}	0.24	3.72^{b}	0.38	0.003
Neutral lipids							
16:0	26.7	1.73	26.0	2.14	26.2	2.02	0.770
16:1 trans	0.51^{a}	0.05	0.51^{a}	0.08	0.84^{b}	0.21	<0.001
16:1	1.50	0.17	1.50	0.20	1.56	0.17	0.760
18:0	22.2	1.55	22.8	1.51	21.7	1.58	0.370
18:1 trans	1.43^{a}	0.34	1.18^{a}	0.34	0.48^{b}	0.06	<0.001
18:1 n-9	39.3	1.58	40.0	2.08	39.2	1.91	0.659
18:2 n-6	2.09	0.37	1.88	0.21	1.99	0.46	0.491
18:3 n-3	0.26^{ab}	0.12	0.32^{a}	0.09	0.18^{b}	0.04	0.017
20:4 n-6	0.20^{a}	0.03	0.19^{a}	0.05	0.46^{b}	0.20	<0.001
22:4 n-6	0.11^{a}	0.06	0.07^{b}	0.03	0.11^{a}	0.04	0.043
22:5 n-3	0.20^{a}	0.05	0.29^{a}	0.10	1.05^{b}	0.78	0.002
SFA	51.8	1.94	51.6	1.63	50.9	2.35	0.649
MUFA	45.3	1.65	45.6	1.83	45.2	1.97	0.921
n-6	2.45	0.38	2.19	0.25	2.63	0.74	0.183
n-3	0.47^{a}	0.12	0.62^{a}	0.13	1.28^{b}	0.87	0.009
n-6/n-3	5.34^{a}	0.80	3.67^{b}	0.60	2.53^{c}	0.98	<0.001

Means with different superscripts within a row differ significantly (P<0.05).

Levels of 18:1 n-9 in tissues have been suggested to increase not only due to dietary intake, but also as a result from biohydrogenation of 18:2 n-6 (Scollan *et al.*, 2001a). In our study, 18:1 n-9 was higher in meat from grazing reindeer compared to meat from both pellet-fed groups (Table 4). We suggest that this may be due to the higher amounts of 18:1 n-9 in the lichens than in both types of pellets, which had similar amounts of 18:1 n-9 (Study III) (Table 3), rather than increased biohydrogenation. Biohydrogenation in the rumen also produces 18:1 *trans* (Scollan *et al.*, 2001a). The lower amounts of 18:1 *trans* in both NL and PL fraction of grazing reindeer compared to pellet-fed reindeer indicates a lower hydrogenation of the ingested lipids in the grazing animals. No difference between the two different pellet types was found.

Although feeding reindeer pellets with linseed decreased the n-6/n-3 ratio, only 18:3 n-3 was significantly increased (Study III) (Table 4). The slightly increased amount of 20:5 n-3 in the meat of the LPD-fed animals compared to the CPD-fed reindeer group indicates an enhanced synthesis from its precursor 18:3 n-3, but no significant synthesis of 22:5 n-3 was found. This suggests that reindeer cannot elongate and desaturate the FA in significant amounts and need to ingest long chain PUFA via the diet. A similar lack of elongation and desaturation was found in pigs (Rooke *et al.*, 2001) and rats (Arts *et al.*, 2001).

Effects of animal age and sex on lipid metabolism

(Studies III & IV)

In general, the present study showed no important effects of sex and age on the FA composition in the meat; indeed, we suggest the main differences depend on fatness (studies III & IV). The lipid class composition accurately reflected the intramuscular fat (IMF) content in the different reindeer groups (study IV) (Table 5, Fig.15). With an increasing deposition of fat, the percentage of phospholipids usually decreases because they are involved in the cell membrane structure, and are therefore present in relatively stable quantities (Sinclair & O'Dea, 1990; Scollan *et al.*, 2001b).

Table 5. *Carcass conformation, trim fat content and dressed carcass weight of free ranging reindeer of different age and sex (least-square means and standard errors) (Study IV)*

	Males (*n* = 7)	S.E.	Females (*n* = 7)	S.E.	Calves (*n* = 7)	S.E.	*P*
EUROP carcass confirmation[1]	5.0	0.41	4.6	0.41	4.7	0.41	0.756
Trim fat [2]	4.1[a]	0.33	5.6[b]	0.33	3.9[a]	0.33	0.003
IMF, %	2.6[a]	0.33	4.2[b]	0.33	2.1[a]	0.33	<0.001
Carcass weight	31.2[a]	1.21	32.2[a]	1.21	21.0[b]	1.21	<0.001

Means with different superscripts within a row differ significantly (P<0.05).
[1]EUROP carcass confirmation: 15 = well developed …. 1= emaciated.
[2] EUROP fat classification: 15 = very fat …1= very lean.

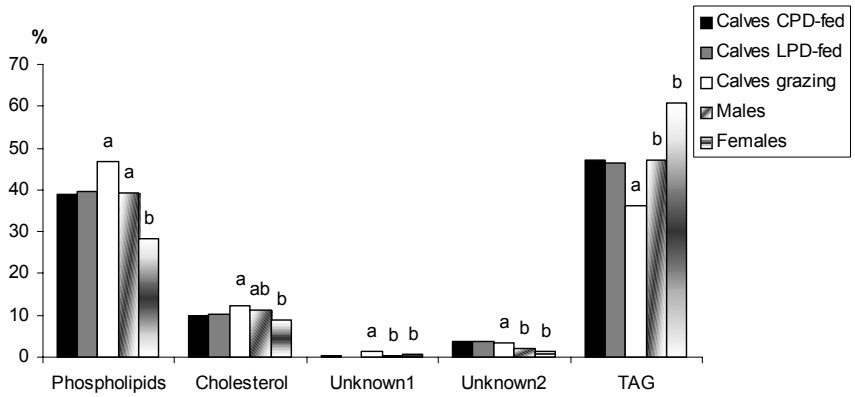


Figure 15. Lipid class composition (averages, %) in meat from reindeer of different age and feeding regimen (Study IV). Pellet fed calves differ significant from grazing calves except for unkown 2. Bars of grazing reindeer with different superscripts differ significantly.

Female reindeer had much higher IMF content and subcutaneous fat compared to calves and males (Table 5). This was partly due to metabolic differences. Calves are still growing, and therefore unable to deposit fat to the same extent as adults (Adamczewski *et al.*, 1987; Mersmann, 1990). In young animals, energy is mainly used for development of muscle mass whereas, after maturation, surplus energy is mainly stored as adipose tissue (Mersmann, 1990). Adult male mammals are generally more muscular and less fatty than females (Mersmann, 1990). Studies on other game animals have also shown similar results, with males having higher carcass weights than females (Hoffman *et al.*, 2005).

Females need to build up more reserves, in order to take care of the calves. Adamczewski *et al.* (1987) showed that poor maternal condition reduces production of viable calves, and that muscle weight reached a plateau whereas fat gain continued to increase, suggesting that female reindeer have targets for levels of body fat and protein rather than for total body weight.

Another reason male reindeer have less subcutaneous fat are the physiological changes that occur in males during the rutting season. Male reindeer are known to build up a subcutaneous fat layer in summer and autumn and Kiessling *et al.* (1987) showed that male reindeer increase their neck muscle size up to 500%. After the rutting season, the muscles decrease to their usual size. Kiessling *et al.* (1987) suggested that male reindeer build up a strong neck musculature, mainly to be able to carry the heavy antlers, and to fight competitors for females, but also for storage of energy. During rutting season males spend a lot of time fighting and usually decrease their feed intake, as has also been described for impala and farmed red deer (Stevenson *et al.*, 1992; Hoffman *et al.*, 2005). Both the subcutaneous fat layer and the energy and protein stored as muscle tissue are consumed to a great extent during this season. Since rutting takes place in October, at the beginning of winter, it is suggested that male reindeer cannot build up a new subcutaneous fat layer after the rutting season, and therefore start winter in a poorer physical condition than females.

Differences in fatness between females and males could also partly depend on different grazing behaviour of reindeer due to sexual segregation, as has been

shown for reindeer on the Seward Peninsula (Chetkiewicz *et al.*, 1991), and for other ungulates (Pérez-Barbería & Gordon, 1999; Barboza & Bowyer, 2000). Barboza & Bowyer (2000) showed that small female deer (*Cervidae*) often graze on higher quality pasture due to their smaller rumen, resulting in a decreased ability to digest material with high fibre content. Reindeer in general are limited in digesting material high in fibre (reviewed by: Mathiesen, 1999), however as rumen size in reindeer is related to body size, no differences in rumen size can be expected between male and female in the reindeer used in our investigation (study IV).

In addition to the sexual differences in fat deposition, reindeer that were fed pellets and have surplus energy intake built a subcutaneous fat layer, indicating its importance for energy storage (Wiklund *et al.*, 2001a; study IV). From the present study we conclude that a positive energy balance in reindeer calves leads to an increased subcutaneous fat layer rather than to higher IMF (study IV). As subcutaneous fat is commonly used as insulation in mammals (Prestrud, 1991), this might be one of several strategies to protect against cold.

Conclusions

Meat quality

- Reindeer meat is suggested to be a nutritionally beneficial meat product due to its low fat content, favourable FA composition and high content of α-tocopherol.
- The substantial increase of FFA in studies I and II showed that lipolysis plays a significant role in the processing of reindeer meat and stored red deer meat. Lipolysis can be detected earlier than FA oxidation during processing/storage, and can serve as an early indication of quality deterioration.
- The high antioxidant content in the meat, and the smoking procedure used, protected lipid quality in the reindeer meat during smoking. The drying process however significantly influenced oxidation and lipolysis, and decreased the content of vitamins E and A.
- Meat from grazing red deer (with higher vitamin E content) had superior colour stability. Shelf life or colour stability was closely related to feeding regimen. Differences in vitamin E intake had greater influence on meat colour than did FA composition.
- Meat pigment oxidation occurs faster than lipid oxidation and thereby reveals effects of diet and storage earlier than measurements of lipid oxidation. The relatively small differences in pigment content of the meat samples did not seem to affect colour stability or lipid oxidation.
- The discovery of squalene in reindeer meat adds significantly to its proposed nutritional value for human nutrition.

Effects of diet

- Effects on FA composition of reindeer and red deer meat were found to be mainly diet dependent.
- Changes in FA composition in the different types of pellets had no influence on tissue lipid class composition.
- Feeding reindeer with pelleted feed containing crushed linseed beneficially affected the FA composition, and decreased the n-6/n-3 ratio, but the animals were still unable to elongate and desaturate towards 22:5 n-3 and 22:6 n-3, indicating that reindeer need to ingest these FA via the diet.

Future research

Based upon the research in this thesis, the following future investigations are suggested:

- The impact of different dietary FA composition on oxidation, lipolysis and sensory aspects in reindeer meat during storage and processing from animals grazing and fed conventional pellets should be investigated.
- Development of lipolysis and oxidation during long-term deep chilled storage, and their importance for meat quality (as in study II) should be studied more thoroughly in order to evaluate and better utilize the potential of this type of storage.
- Minor components in the meat originating from the feed *e.g*. antioxidants and squalene should be studied in order to better understand their metabolism and the synergistic effects between these substances in the meat, and to provide information on their possible effects on consumer health. Also the general effect of reindeer meat consumption on the health of the reindeer herders needs to be further explored.

- Lipid metabolism in reindeer, and their strategies for survival in the arctic climate are not yet fully understood, but are subjects of great interest for future research. We hypothesize that long chain PUFA play an important role in maintaining tissue functionality, based on membrane fluidity, in terrestrial non-hibernating mammals, particularly in species that use peripheral cooling to save energy in the cold.
- The type of feeding investigated is used not only prior to slaughter, but also both as an emergency feed source during harsh winter conditions and to increase maternal fitness and calf survival in the spring. These animals might have special requirements for dietary essential FA content and thereby justify a tailored diet that is specific not only for species but also for the different stages and seasons of their life cycle.

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