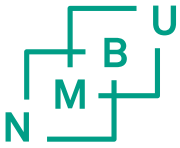
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Preface

This thesis is written at the Department of Chemistry, Biotechnology and Food Science (IKBM) at the Norwegian University of Life Sciences in Ås municipality in the spring and summer of 2014. The thesis is part of the project "Sunnere storfekjøtt" with project leader Bjørg Egelandsdal.

During my studies in Food Science I discovered my interest for Meat technology as well as health aspects concerning foods, thus the choice to write my Master project as a part of the project "Healthier beef meat" was a simple one. My task was to take part in the collection process of the first animal sample set of the project and to make first assessment of how much can standard minced beef meat may vary. It was motivating to know that some of the values that were obtained during my thesis may end up as reference values in an updated Norwegian Food Composition Database and possibly form a basis for declarations of millions of future minced meat packages.

I want to give a big thanks to everyone involved in this thesis. Thanks to Nortura for helping me in the recruiting process of farmers. Thanks to Animalia for the database: "*Statistisk oversikt over klassifiseringen av storfe i Norge i 2012".* Thanks to everyone that helped me in the laberatory. I also want to give special thanks my supervisors: professor Bjørg Egelandsdal, Ellen-Margrethe Hovland, professor Anna Haug, and Ellen Skuterud for valuable guidance and input.

Lastly, I want to thank all the farmers and producers of Norwegian Red Cattle that participated in this research – without your voluntary participation this research would not have been possible.

Ås, 11 August 2014

Marion Haugen

Abstract

Research conducted in other countries than Norway has shown an association between meat intake and colorectal cancer risk. The Norwegian Directorate of Health recommends limiting the intake of red and processed meat to 500 g/week based on this research. The nutritional value of Norwegian meat may differ in nutrients to meat from other countries because of breed and feed differences. In order to understand better the link to colon cancer observed in other countries the typical composition of Norwegian red meat should be better understood. The nutritional value of products is registered in the Norwegian Food Composition table. Calculating intake of various nutrients, declaration of food, research, teaching and nutrition politics are all based on the numbers of these tables. The values on minced meat have not been updated since 2005 and it is important to get updated numbers that can be used in e.g. health research. Updated numbers for the nutritional value and oxidation indicators (heme, DPPH, TBARS and total PV) of standardized 14% minced beef meat, measured 10 days post-slaughter, can be found in this thesis. Eighteen animals of the breed Norwegian Red Cattle were chosen based on the assumption that these animals were representative for the Norwegian meat intake. The variation in the data was identified and the average nutrition values were compared to values reported from other countries in food composition tables. The results showed a variation in following fatty acids, vitamins and minerals: C14:0, C16:0, C18:0, C16:1, C18:1trans, C18:1n-7, C18:1, n-9, C18:2trans, C18:2, CLA, C20:4, C18:3, cholesterol, iron, zinc, sodium, calcium, magnesium, phosphorous, potassium, selenium, iodine, retinol, β–carotene, α-tocopherol, γ-tocopherol, vitamin $K_{1,,}$ vitamin K_2, thiamin, riboflavin, vitamin B_6, pyridoxal, pyridoxine, pyridoxamine, niacin and vitamin $B_{12.}$.

Norwegian minced meat fulfills the EU criteria for the following nutrient claims: ”a source of”: iron, phosphorus, potassium, niacin and vitamin B_6 as well as the claim ”rich source of”: protein, zinc and vitamin B_{12}. Compared with other countries the Norwegian minced meat has room for improvement regarding SFA content, n-6:n-3 ratio, calcium, magnesium, phosphorous, potassium, selenium, iodine, thiamin, riboflavin, vitamin B_6, niacin and vitamin $B_{12.}$ Variation was also identified in all oxidation indicators. Norwegian minced meat contains on average 13.2 mg/100g hemin, has a TBARS level of 0.194 mg/kg, a DPPH value of 71.9% and a total peroxide value of 0.740 mmol/kg.

Sammendrag

Utenlandsk forskning har vist en mulig sammenheng mellom tykktarmskreft og kjøttinntak. Basert på denne forskningen anbefaler helsedirektoratet å redusere inntaket av rødt og bearbeidet kjøtt til 500 g i uken. Næringsverdien til Norsk storfekjøtt kan være forskjellig fra andre land grunnet forskjeller i storferaser og fôr. For å bedre kunne forstå sammenhengen mellom tarmkreft og kjøtt observert i andre land burde den typiske sammensetningene til norsk rødt kjøtt bli bedre forstått. Næringsverdien til norske råvarer er beskrevet i Matvaretabellen. Deklarasjon av matvarer, forskning, undervisning og ernæringspolitikk tar utgangspunkt i disse tallene. Verdiene for kjøttdeig har ikke blitt oppdatert siden 2005 og det er viktig å skaffe oppdaterte tall som kan brukes i ernæringsforskning. Oppdaterte tall for ernæringsverdi og oksidasjonsindikatorer (heme, DPPH, TBARS og total PV) til 14 % standardisert kjøttdeig, målt 10 dager etter slakting, ble fremskaffet i denne oppgaven. Datagrunnlaget besto av 18 dyr av rasen Norsk Rødt Fe som antas å være representative for norsk kjøttinntak. Gjennomsnittet og variasjonen i datasettet ble identifisert og den gjennomsnittlige ernæringsverdien ble sammenlignet med tall fra utenlandske matvaretabeller. Resultatene viste at det var variasjon mellom følgende fettsyrer, vitaminer og mineraler: C14:0, C16:0, C18:0, C16:1, C18:1trans, C18:1n-7, C18:1, n-9, C18:2trans, C18:2, CLA, C20:4, C18:3, ukjente fettsyrer, kolesterol, jern, sink, natrium, kalsium, magnesium, fosfor, kalium, selen, jod, retinol, β–karoten, α-tokoferol, γ-tokoferol, vitamin K_1, vitamin K_2, tiamin, riboflavin, vitamin B_6, pyridoxal, pydridoxine, pyridoxamine, niacin og vitamin B_{12}.

Norsk kjøttdeig oppfyller EU sine krav til å benytte næringsstoffpåstandene: ”kilde til”: jern, fosfor, kalium, niacin og vitamin B_6 samt ”rik kilde til” protein, sink og vitamin B_{12} basert på gjennomsnittlig næringsinnhold. Sammenlignet med andre land har norsk kjøttdeig forbedringspotensialer når det kommer til SFA-innhold, n-6:n-3 ratio, kalsium, magnesium, fosfor, kalium, selen, jod, tiamin, riboflavin, vitamin B_6, niacin og vitamin B_{12}. Det ble også påvist variasjon i alle oksidasjonsindikatorene. Norsk kjøttdeig inneholder i gjennomsnitt 13,2 mg/100 hemin, har en TBARS på 0,194 mg/kg, en DPPH verdi på 71,9% og har en total peroxide verdi 0,740 mmol/kg.

Abbreviations

BP – Blood pressure

CHD – Coronary heart disease

CRC – Colorectal cancer

CVD – Cardiovascular disease

GI - Glycemic index

LAB - Lacto acid bacteria

MUFA – Monounsaturated fatty acids

NOCs – N-nitroso compounds

NRC – Norwegian Red Cattle

NRV – Nutrient Reference Value

PLP - Pyridoxal 5' –phosphate

PMP - Pyridoxamine 5' –phosphate

PNP - Pyridoxine 5' –phosphate

P:S ratio – Polyunsaturated fatty acids : saturated fatty acids ratio

PUFA – Polyunsaturated fatty acids

ROS – Reactive oxygen spices

SFA – Saturated fatty acids

TFA – Trans fatty acids

Table of content

1 Introduction

Meat and meat products is a natural part of the Norwegian diet, and the Norwegian man consumes 181 g/day on average, while women consume 116 g/day on average during the year 2010-2011 (Helsedirektoratet 2012). Animal foods are also an important contributor to different nutrients in our diet such as proteins, and vitamin and minerals, especially vitamin B_6, vitamin B_{12}, iron, zinc and selenium (Norden 2012). In addition, meat also contain a lot of monounsaturated fatty acids (MUFA) and are a nutrient dense food, with low glycemic index (GI) (Helsedirektoratet 2012).

In later years beef meat has received criticism especially from a health perspective. Epidemiological evidence has suggested a link between high consumption of processed meat and the increased risk of colorectal cancer (CRC) (World Cancer Research Fund 2007), type-2 diabetes, obesity, and coronary heart disease (CHD). Weaker associations have been observed for red meat (Norden 2012). Based on these reports, the Norwegian Directorate of Health recommends limiting the amounts of processed- and red meat plus choose leaner meat and meat products. The amount of red and processed meat should be limited to 500 g per week (Helsedirektoratet 2014).

Red meat is generally, defined as meat from beef, pork, mutton and game, while white meat includes chicken and turkey. Processed meat is defined as "meat preserved by smoking, curing or salting, or by the addition of preservatives like nitrites" *i.e* products like ham, bacon, salami, sausage and smoked meat. The World Cancer Research Fund (2007) concluded in their report that there were convincing evidence that red meat (cattle, sheep, pig and goat) and processed meat increases the risk for developing CRC. Still, there are some uncertainties about the causality between consumption of red meat and CRC (Alexander et al. 2010; Norden 2012; Oostindjer et al. 2014). According to the report "Dietary advice for promoting public health and prevent chronic diseases: 2011" (Helsedirektoratet 2011 b) they suggest that the causality should be classified as probable. This mostly because of the lacking evidence in research (Alexander et al. 2010; Helsedirektoratet 2011 b) and because the mechanisms to explain this link is lacking. Two of the main hypotheses in the link between processed meat and CRC involves heme iron found in red meat, including formation of reactive oxygen spices (ROS) and N-nitroso compounds (NOCs) (Oostindjer et al. 2014).

However, all the research behind the Norwegian advice to reduce meat intake, are based on a research done outside Norway and not on Norwegian meat and within the frame of Norwegian dietary habits. More knowledge about Norwegian meat and diet should be obtained to see if the same association between CRC and red meat is found in Norway as well. The Norwegian Food Composition table is providing the basis for calculating intake of various nutrients, and is also an important tool in food safety and nutrition policy, declaration and education and research (*Norwegian Food Composition Database* 2013). Because of this the Norwegian Food Composition table should be updated frequently and include mean values for a specific food in Norway. However, as in the case for the processed minced meat (14% fat), updates have not been made since 2004-2005 (*Norwegian Food Composition Database* 2013), since analysis are both expensive and time demanding.

Therefore the research questions in this thesis are:

"What characterizes the composition of Norwegian beef meat with regards to nutritional value and oxidation indicators?

And how are Norwegian nutritional values compared to other countries?"

2 Literature/Theory

2.1 Cattle production in Norway

Milk, meat, eggs and wool are the major farm animal products in Norwegian agriculture (Knudsen 2007). According to Knudsen (2007) the Norwegian cattle production has been relatively stable with around 1 million cattle from year 1980 to 2000, as seen in Figure 1. The declining cattle production in recent years is mainly due to cutbacks in milk production and in 2006 the total cattle population were around 920 000 animals (Knudsen 2007). Updated numbers from Animalia (2013) show that the total cattle production was 850 666 animals in 2013.

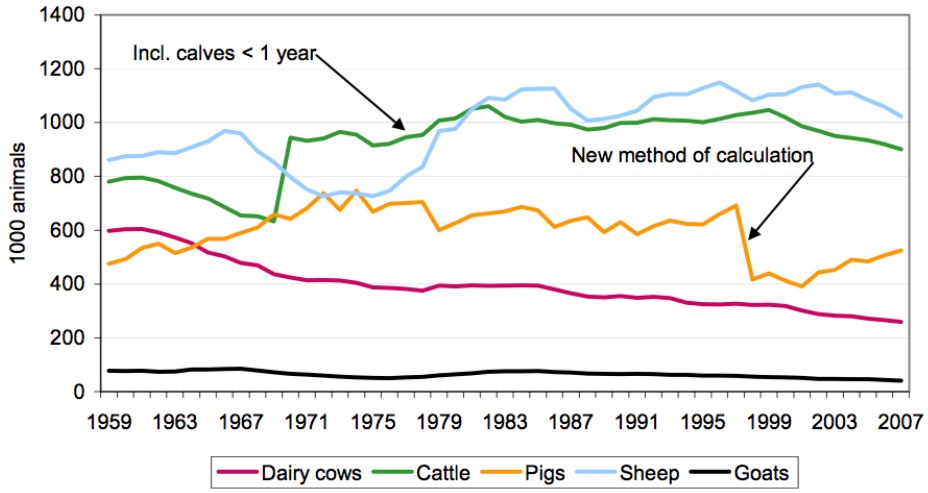


Figure 1: The development of livestock populations in Norway from year 1959–2007 (Knudsen 2007).

According to Helsedirektoratet (2013) the collective production of livestock in 2011 was around 323.4 mill kg, while the production was 326.2 mill kg in 2012. The growth in production is mostly due to a large increase of poultry meat production (up 6.4 mill kg) and a 0.7 mill kg increase in pork meat production. The production of beef, however, decreased with 3.6 mill kg (Helsedirektoratet 2013).

2.2 Meat consumption in Norway

Based on the wholesale estimations of Helsedirektoratet (2013) the consumption of meat has steadily increased over the years. Table 1 shows the consumption of meat and entrails from year 1953 to 2012. However, the wholesale estimation is based on amount of available meat in Norway and includes the whole carcass with bones. This number does not give a particularly good overview of the meat we actually consume.

Table 1: Consumption of meat (kg per capita per year) at wholesale level. The numbers are rounded. (Helsedirektoratet 2013).

Year	1953-55	1979	1989	1999	2011	2012*
Meat and entrails (Kg)	36	54	53	63	75	75

*Preliminary numbers.

Animalia (2013) estimated total kg meat, and total kg beef, consumed per capita per year. The calculation takes into account wastage through the whole production line and at consumers. Animalia's calculated actual consumption can be seen in Table 2 below.

Table 2: Calculated raw weight consumption of meat and cattle in kg per capita per year (Animalia 2013).

Year	2008	2009	2010	2011	2012
Total meat (Kg per capita)	52.4	50.4	50.2	50.7	51.3
Cattle (Kg per capita)	14.3	13.5	12.9	13.2	13.5

This calculation shows that the total consumption of meat in 2012 was 51.3 kg per capita, and that the cattle consumption accounted for 13.5 kg per capita. This consumption corresponds to 140 g meat per capita per day (Animalia 2013). This corresponds quite good to the Norkost 3 dietary survey of Helsedirektoratet (2012) where people report what they have been eating , showed that the total consumption of meat in 2011-2012 was 147 g per day, whereas women consumed 116 g and men 181 g.

2.2.1 Imported meat

In 2012 the total import of meat and meat products to Norway was 27300 tons. Cattle contribute to the largest amount of this import, with 17700 tons in 2012, followed by import of pig, at an amount of 3600 tons (Animalia 2013) As seen from Table 3 import of cattle has increased a lot since 2010. According to Animalia (2013) import increased from 7.4 mill kg to 17.6 mill kg in 2012, because of a decrease in production of cattle.

Table 3: Total amount of import in Norway, and total amount of cattle import, numbers are given in tons. Total import numbers also include white meat. Table is modified and obtained from Animalia (2013).

Year	2008	2009	2010	2011	2012
Total import*	20 000	13 500	12 000	18 000	27 300
Import of cattle	11 000	7 500	5 400	10 300	17 700

*Numbers are rounded off to nearest thousand, because of insecurity in the data.

Norway import beef meat mostly from 18 different countries as seen in Figure 2 (Totalmarked: kjøtt og egg 2014). In addition, import of small amount is from France, Australia, Spain, Ireland, Italy, Netherlands, Turkey and USA. The import of beef in 2012 came mainly from Germany (8055 tons) followed by Namibia (1697 tons) and Botswana (1594 tons). Botswana, Namibia and Swaziland are part of the Southern African Customs Union, which from 2008 have a free trade agreement with Norway to promote trade and economic cooperation between the countries (Dåsnes 2013), which can explain the large amount of imported cattle from these countries.

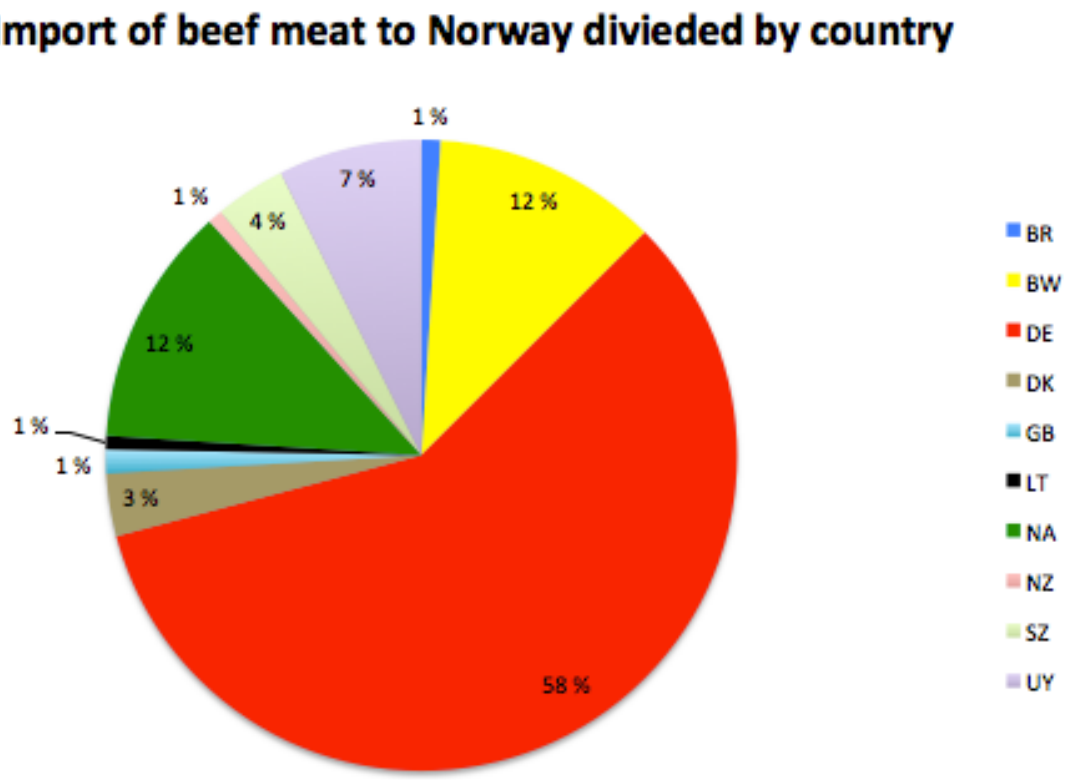


Figure 2: Relative share of countries Norway imports beef from. Main country Norway import beef from is Germany, followed by Namibia, Botswana and Uruguay. (Br = Brazil, BW = Botswana, DE = Germany, DK = Denmark, GB = South Georgia and the South Sandwich Islands, LT = Lithuania, NA = Namibia, NZ = New Zealand, SZ = Swaziland and UY = Uruguay). Figure is made based on numbers from Totalmarked: kjøtt og egg (2014).

2.3 Different types of feed for cattle

The feed given to cattle forms the basis of product quality of the meat. Not only can the feed influence appearance, smell and taste of the meat, but the nutritional value of the meat is highly influenced by feed. In Norway the most common feeds for cattle are grass and pasture, conserved roughage, concentrate feed or a combination of all (Gjefsen 1996).

2.3.1 Grass and pastures

According to Gjefsen (1996) grass and grass products are an important part of the ruminant feed in Norway. Half of the lands total agricultural area is pasture of surface cultivated land; additionally large parts of non-cultivated areas are used as pasture. Grass crops available where meadows are cleared, fertilized and groomed, are called cultivated pastures, while uncultivated pastures often are mountains and forests areas. Even though most of the grass crops are harvested and stored for use, pasture on fresh grass is still used, especially for milk cows (Gjefsen 1996).

Which plants the pasture constitutes, is called botanical composition. This means a lot for the nutritional value of feed. The most common grass species in cultivated pasture and meadow in Norway are timothy (*Phleum pretense*), meadow fescue (*Festuca pratensis*), smooth brome grass *(Bromopsis inermis),* smooth meadow grass *(Poa pratensis),* orchard grass *(Dactylis glomerata),* common bent *(Agrostis capillaris)* and different types of ryegrasses *(Lolium sp.).* Pasture legumes, especially red clover *(Trifolium pretense)* is often a valuable supplement to grass in pasture and meadow. The nutritional value of different types of pasture grass is given in Table 4 *(Gjefsen 1996).*

Table 4: Nutritional value of pasture and grass to ruminants (K, K. Heje, 1995) as stated in (Gjefsen 1996). AAT proteins are explained as amount of amino acids absorbed in the intestine of cattle.

Type	Dry matter (%)	Kg feed per feed unit	Protein, AAT	g/kg dry matter (digestible raw protein)
Mixed meadow, under 10% clover	17.9	6.0	80	172
Mixed meadow, 10-50% meadow	19.7	4.9	80	207
Timothy	18.1	6.3	80	164
Rye grass, early summer	18.1	6.3	80	164
Rye grass, late summer	18.6	7.0	70	69
Meadow fescue	16.5	6.3	80	184
Orchard grass	16.7	6.2	80	237
Smooth brome grass	15.1	7.1	80	199

May and June are the two months with the largest growth of plants in the pastures. Plants grow fast in spring and early summer, blossom and set seeds late summer, and leaf and stems wither at the end of the growth season. Accordingly, the nutrient content of the plants also changes during the season's growth stage.

Harvest point of crop

The meadow groups are very nutritious when the plants are in an early development stage, but the nutritional value decreases when the plants are further in their developments and starts to blossom. This is important for the assessments of harvest time when we should make roughage like silage or hay, but it is also important for pasture quality (Gjefsen 1996).

Towards blooming indigestible compounds such as lignin are formed. This affects the content and availability of easily digestible nutrients of the plant. So, even though the yield (crop) of dry matter of each acres increase towards blooming, the amount of feed units will increase and be at maximum a week after shooting as seen in Figure 3. The amount of protein, measured as kg AAT, increases a little until two weeks after shooting (Gjefsen 1996).

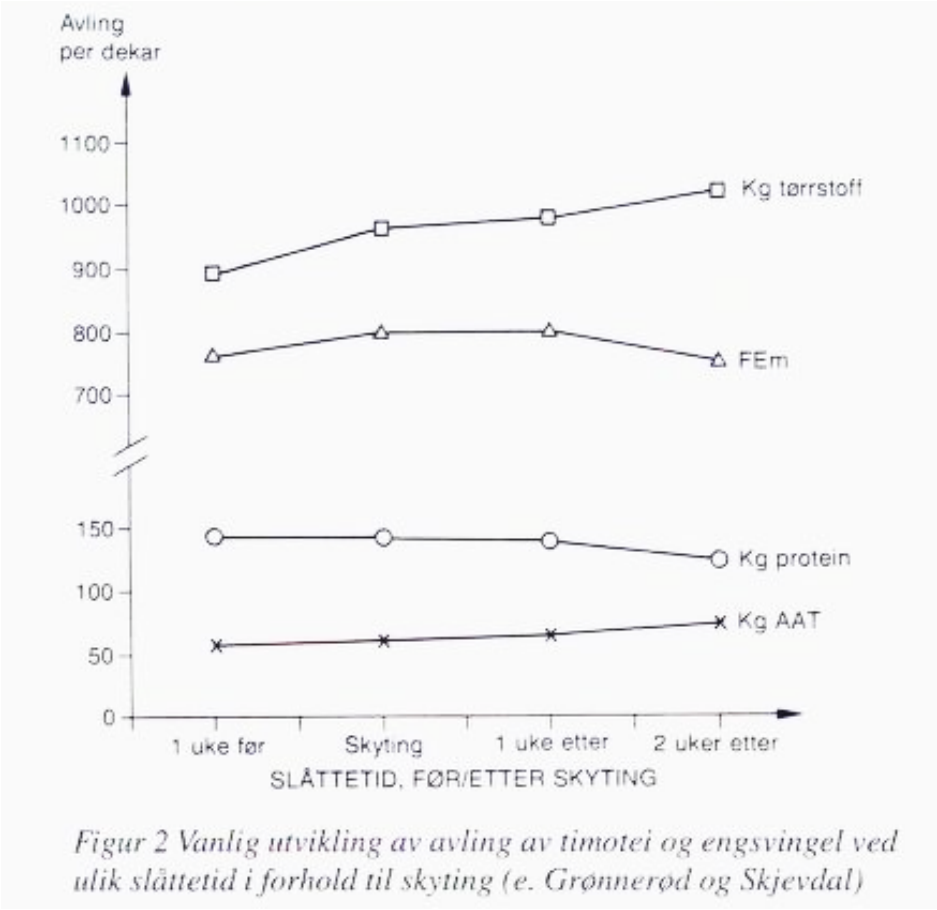


Figure 3: Common development of timothy and fescues crop, at different harvest time in relation to shooting. FEm is the shortening for feed units, and AAT is amount of amino acids absorbed in the intestine (Gjefsen 1996).

The process shown in Figure 3 applies for the first harvest in the lowland of eastern-Norway. Research in areas higher in altitude and further north in the country has shown that the development of feed value does not decrease as fast after shooting as on the lowland in eastern Norway (Gjefsen 1996).

The use of pasture must be adjusted to the development of meadow crops. Many harvest the pastures to hay or silage early in the summer, simultaneously as they use other part of the meadow for pasture. Later in the summer a bigger area for pasture must be used because the uptake of feed from pasture are reduced accordingly as the plants gets too far in their development (Gjefsen 1996).

Conservation and storage of the pasture crops

Conserved feed constitutes a large part of the feed ration to ruminants in periods where fresh feed is not available. In Norway the limited growth season due to winter, and the climate leads to relatively long indoor feeding periods for cattle, ranging from 5-9 months depending on location (Mo 2005). The climate also affects what can be cultivated in Norway's farmland, and according to Knudsen (2007) the production of roughage is more or less the alternative crop in many parts of Norway, which makes the grass-based livestock production the backbone of Norwegian agriculture.

Storage of pasture crop requires that we are able to conserve it, so spoilage bacteria and molds that attack the crop do not ruin it. In addition, it is desirable to maintain the feed value at the same level as when harvested, this is however difficult when losses often occur (Gjefsen 1996).

Harvesting and conservation of pasture crop can be done with many different methods and different types of technical equipment. Drying the grass to hay was the most common method in the early days, while ensilage and storage in siloes or round bails is the most common method today (Gjefsen 1996).

2.3.2 Hay

Hay is dried grass with water content below 17%. There will always be a loss of nutrients when grass is dried to hay because of plants cellular respiration, which uses easy digestible nutrients to maintain their life processes. Around 5-15% loss can be observed, depending on drying time. When the water content has been reduced to 30-

40%, the respiration stops, but the grass needs to be below 17% water to inhibit rotten hay or growth of molds (Gjefsen 1996).

2.3.3 Silage

The crop being conserved by ensilaging is called silage and silage is stored grass with high water content and a pH below 4.2. To produce silage all oxygen is removed to reduce loss trough plant respiration, inhibit growth of molds and other unwanted microorganisms, and to create better living conditions for lacto acid bacteria (LAB). The inherent LAB in the grass produces lactic acid that reduces the pH of the ensilage mass thus conserving it. When the pH is below 4.2-4.0 neither LAB nor any other bacteria can grow or multiply and the silage is stable (Gjefsen 1996).

If the air is completely eliminated, and availability of easily digestible carbohydrates is high enough, LAB will produce enough acid to lower the pH sufficiently. However, it is common to promote the effect of LAB by including additives like additional acid, additional LAB or easily digestible carbohydrates in the mass (Gjefsen 1996).

Ensilaged crops can either be stored as siloes or in round bales. In round bales the principle for storage is the same as in silo but the harvest equipment roles the crop into big balls that are wrapped in plastic. The use of silage preservation is widely used in Norway, probably due to the fact that it can, compared to hay, be harvested wet. Loss of nutrients by ensilage is mostly around 10-15% depending on conservation conditions (Gjefsen 1996).

2.3.4 Concentrate feed

Concentrated feed is mixtures produced by a large share of grain and grain products, which has a high content of protein or energy per kg. The mixtures sold in Norway are often adjusted to special animal species or productions and often include carbohydrate, protein, fat, minerals, vitamins and other additives (Gjefsen 1996).

The carbohydrate fraction of concentrates is often from oats or barley, but in some years where food grains like wheat and rye are not qualified for human consumption, they will also be used as feed. The protein often comes from soy flour and rapeseed flour. Fat is important to add to increase the energy level of the concentrate and to prevent rancidity

in concentrate feed, saturated fat is often used. Minerals and vitamins are often added in mixes specially adjusted to animal race (Gjefsen 1996).

To produce concentrate the raw materials are often grinned and then mixed according to recipe. After mixing of dry components fluid materials like molasses or fat are sometimes added. The feed is often extruded to pellets, by using high temperature and pressure (Gjefsen 1996).

2.3.5 Different feeds affect nutritional composition of cattle

The different feeding regimes of grass/forage or grain finishing cattle have been shown to affect the total fat content of cattle (Duckett et al. 2009; Leheska et al. 2008). The studies suggested that grass and forage feeding reduce the total fat content. This effects is mostly caused by the high availability of energy and glucose content for fat synthesis in grain finishing regimes (Van Elswyk & McNeill 2014).

Many papers have also studied the effect of feeding regime on fatty acid profile (Duckett et al. 2009; French et al. 2000; Leheska et al. 2008; Warren et al. 2008). Duckett et al. (2009) and Leheska et al. (2008) found an increased amount of total saturated fatty acid (SFA) in grass fed beef compared to grain fed. However, Van Elswyk and McNeill (2014) point out in their review that the amount is given as percentage of total fatty acids, and since total amount of fat was lower in grass fed beef in both studies, the increase in percentage does not translate to an actual increase in SFA intake. Van Elswyk and McNeill (2014) calculated the data to g/100g beef and found that the grass fed beef contained less total SFA per 100g than grain fed. Further the studies showed that grass-fed beef contained less MUFA than grain fed beef, both as percent of total fatty acids (Duckett et al. 2009; Leheska et al. 2008) and as g/100g beef (Van Elswyk & McNeill 2014).

When it comes to the feeds effect on PUFA, only a small tendency of increased amount of EPA (C20:5, n-3), DPA (C22:5, n-3) and DHA (C22:6, n-3) in grass/forage fed beef was noted (Duckett et al. 2009; Leheska et al. 2008). These results are caused by the well-established fact that unsaturated fatty acids ingested by ruminants are hydrogenated, to SFA. The amount of linolenic acid, however, was larger in grass/forage fed beef than grain fed in both studies. An increase in total PUFA was also observed in both studies

(Duckett et al. 2009; Leheska et al. 2008) with grass/forage fed beef, but according to Van Elswyk and McNeill (2014) calculated amount (g/100g beef) the results show that the amount actually where lower in grass/forage fed beef compared to grain fed beef. The percentage of conjugated linoleic acid (CLA) increased significantly in grass/forage fed beef compared to grain fed (Duckett et al. 2009), however, after calculations made by Van Elswyk and McNeill (2014) the amount in g/100g beef seems to be equal in grain fed and grass/forage fed beef.

The study by French et al. (2000) tried to avoid the problem of fat deposition, by using carcasses with similar weight and weight gains for all feed rations. According to the authors the changes in fatness due to differences in energy intake would not affect the type of fatty acid composition, since all were similar. The feed was either grass, grass silage, or concentrates in different amounts. Fifty steers were included in the study and divided into 10 blocks based on body weight, and in each block animals were randomly assigned different diets. The concentration of PUFA in intramuscular fat was highest ($P < .05$) for steers fed only grazed grass, then any of the other diets including concentrates or roughage in different amounts. Also, a decreasing proportion of concentrate in diets, and an increase in grass intake caused a linear decrease in the concentration of SFA and in the n-6:n-3 PUFA ratio, and a linear increase in the PUFA:SFA ratio (French et al. 2000).

In addition to the effect of environmental factors such as feeding system, genetic effects also influence the fatty acid composition. Studies has shown that the composition differs between breeds, but the effect of genetics can be difficult to measure because other effects like fat level, live weight or age at slaughter in addition to production systems can also effect the composition (De Smet et al. 2004).

Another effect from finishing cattle on pasture feed were observed in the study by Mercier et al. (2004) where animals finished on pasture had a significantly higher protection of lipid oxidation in the meat. The vitamin E concentration in the meat from pasture feed animals were higher (but not significant), and the pasture diet also affected the antioxidant protection of the body. The results from the study showed that the superoxide dismutase activity was significantly higher in pasture fed animals compared

to mixed fed animals. Additionally the catalase activity was also higher. So, even though pasture fed animals had a higher PUFA value, and thus, higher potential for lipid peroxidation, the pasture fed cows had lower oxidation in the meat (measured as TBARS) (Mercier et al. 2004).

2.4 Meat as a source of nutrients

Meat and meat products are nutrient dense food, which means they have a high amount of nutrients relative to the calorie content. Figure 4 (Helsedirektoratet 2012) demonstrates that meat only contributed to 12% of the daily total calorie intake, but contributed to 27% of the daily total protein intake, and a substantial proportion of various vitamins and minerals. Meats are usually good sources of vitamin B_6, vitamin B_{12}, iron, zinc, and selenium (Norden 2012) and provide a high amount of highly bioavailable vitamin A in the diet. In addition, meat can increase the intake of vitamin E. However, processed meats also contribute to a high salt intake, which is regarded as bad for the health. Whereas unprocessed meats are naturally low in salt (sodium). Meat may also contribute to increased fat intake, but 25% of the fat comes from MUFA and the amount of polyunsaturated fatty acid (PUFA) is significant(Helsedirektoratet 2012).

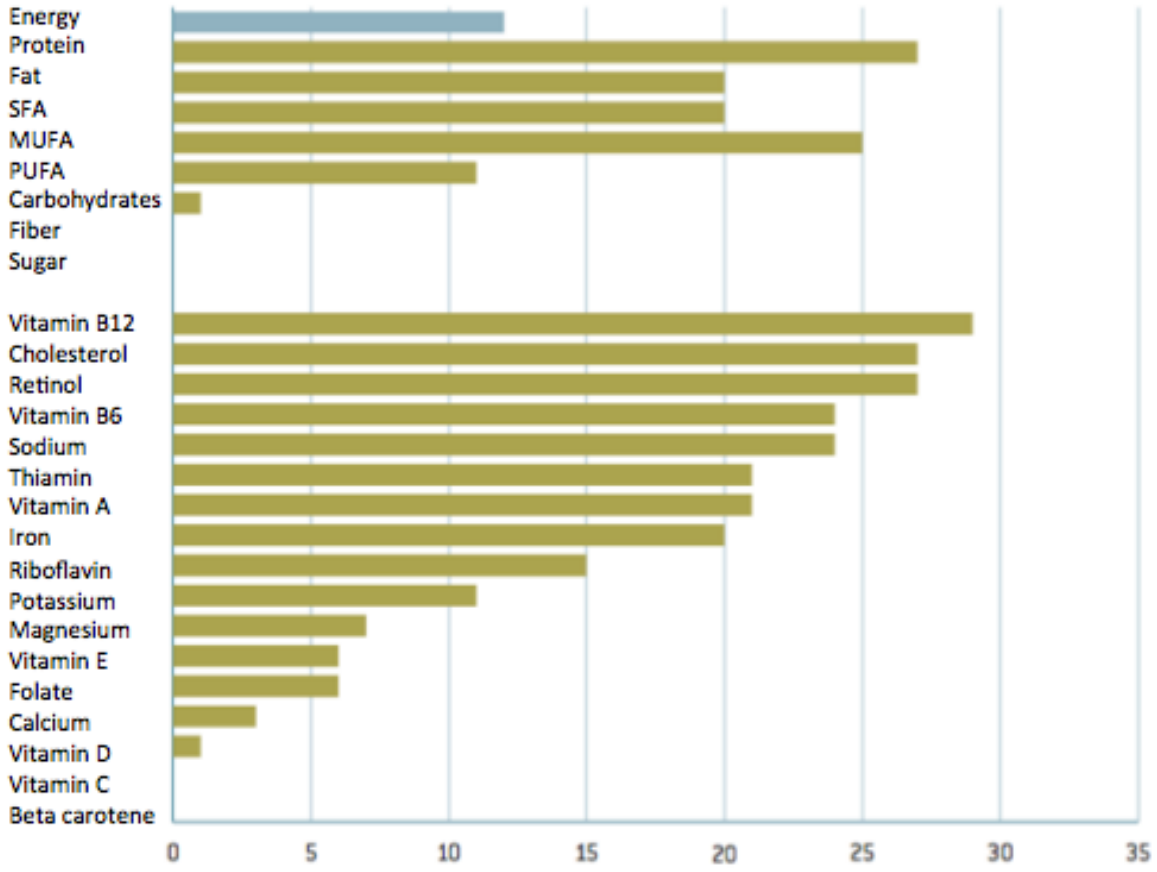


Figure 4: Meat and meat products contribution to different nutrients, vitamins and minerals in the Norwegian diet. Values are given as the percentage of the total daily intake. Modified figure from (Helsedirektoratet 2012).

It is different methods to evaluate whether a food is a good source of certain nutrients. One is to assess what proportion a given food contributes of the normal intake, like Figure 4 from Norkost 3 showed. Another method is to look at the content of the nutrient(s) compared to recommended daily intake or official references values for declaration of content. Nutrient reference values (NRV) are one of these official reference values commonly used in Europe. Since March 2010 nutrition claims can be used in marketing of a food if the content meets certain criterias (relative to NRVs) as described in the EU and Norwegian regulation on "nutrition and health claims made on foods" (Helse- og omsorgsdepartementet 2012) and in Livsmedelsföretagen et al. (2012), and food can be labeled as a "source of" or as a "good source of", depending on how much a food contains of a certain nutrient relatively to the NRV.

In the following theory chapter nutrients discuessed are chosed by the chriteria that meat contribute to over 5% of this nutrient based on total daily intake as shown in

Norkost 3 (Helsedirektoratet 2012) and Figure 4, or that meat is described as a good source, as in the case of zinc and selenium in the Nordic Nutrient Recommendations (Norden 2012).

2.5 Fat in meat

In many countries, including Norway, fat is an unpopular constituent of meat for the consumers. In addition to provide the body with energy in a concentrated form, dietary fat and fatty acid composition have been linked to increased risk of cardiovascular disease (CVD), certain types of cancer and obesity (Norden 2012). Especially SFA and trans fatty (TFA) acids are seen as bad for the health. On the other hand fats provide essential fatty acids and fat-soluble vitamins, has a low GI (Norden 2012), and the potential harmful effects of saturated fat has been questioned by many (Chowdhury et al. 2014). In beef meat the percentage of SFA ranges from 45-49%, MUFA from 43-50% and PUFA from 2-10% (Kerry & Ledward 2009).

2.5.1 Chemical structure of fat

Fat can be in the form of triglycerides, phospholipids, cholesterol and sterols. In meat products, fat are usually stored in adipose tissue in the form of triglycerides, while phospholipids and cholesterol are included in the cell membranes (Norden 2012).

Triglycerides are composed of one molecule of glycerol esterified with three fatty acid molecules and fatty acids in meat are often in the range of 16-18 carbon atoms. The fatty acid determines the fats physical and physiological properties and varies in: length of the carbon chain, degree of saturation, the number, position and structure of double bonds and where they are esterified (the position) in the triglyceride molecule. In saturated fatty acids (SFA) only single bonds exist between the carbon atoms of the fatty acid. The unsaturated fatty acids are either monounsaturated (MUFA), with one double bond, or polyunsaturated (PUFA) with two or more double bonds. The different fatty acids have different names depending on chain length and degree of saturation. The position of the double bonds can either be named from the methyl end (ω or n-) or from the carboxyl end (Δ or α) (Norden 2012).

The human body synthetizes SFA and MUFA in the n-7 and n-9 series from acetate (Norden 2012), but not all fatty acids can be synthesized in the body. The body lacks the enzymes Δ12- and Δ15-desaturase that are capable of introducing double bonds at the

n-6 and n-3 position. Because of this, linoleic acid (C18:2 n-6) and linolenic acid (C18:3n-3) are essential fatty acids, and required from the diet. However, the body can elongate and desaturate these fatty acids, and make other fatty acids in the n-3 and n-6 family (Pedersen et al. 2009).

Unsaturated fatty acids can either be in cis- or trans conformation, but naturally occurring unsaturated fatty acids are mainly in cis- conformation. Trans-fatty acids (TFA) are formed chemically by partial hydrogenation of oils (Norden 2012). TFA are not synthesized by the human body, and is not required in the diet (EFSA 2010). In cattle however, they are formed naturally by the biohydrogenation of unsaturated fatty acids in the rumen, and are therefore present in beef in quantities of 3-6% TFA as percent weight of total fatty acids (Norden 2012). Conjugated linoleic acids (CLA) is a special group of TFA and the most common isomer is cis-9, trans-11 CLA. Beneficial effects have been suggested for this fatty acid, in development of cancer and the balance between fat mass and muscles, but these properties are not well documented (McGuire & M.K 2000). Ruminant fat are the richest natural source of this fatty acid, which arises from hydrogenation of linoleic acid in the rumen (French et al. 2000).

2.5.2 Recommendation and intake

The Norwegian Directorate of Health recommends that the total content of fat in the diet should contribute to between 25-40 E%. The intake of SFA and TFA should be limited to 10 E%, while TFA should not exceed 1 E%. Two thirds of the diets total fatty acids should come from cis-monounsaturated (10-20 E%) and cis-polyunsaturated fatty acids (5-10 E%), whereas 1 E% should originate from n-3 fatty acids. In addition, linoleic acid (n-6) and linolenic acid (n-3) should provide at least 3 E%, including a minimum of 0.5 E% linolenic acid (Helsedirektoratet 2014). According to the most recent national dietary research study Norkost 3 (Helsedirektoratet 2012) the average dietary intake of total fat is 34E%. Further, SFA contribute to 13 E%, while the TFA intake is below 1E%. MUFA intake is 12 E%, while PUFA is 6.2 E%. According to the Norwegian Directory of Health, all fatty acids are within the recommendations, except from SFA, which is too high (Helsedirektoratet 2014).

In the Nordic countries meat and meat products are the third most important sources of fat, after spreads, butter and oils, and milk and milk products. These products are also a

main source of SFA, while meat and dairy products are the main source of TFA (Norden 2012). The dietary survey of Helsedirektoratet (2012) showed that meat and meat products contributed 20% of the fat intake per day, whereas 20% was SFA, 25% was MUFA and 11% PUFA.

2.5.3 Cardiovascular disease (CVD)

In 2012 CVD was the cause of 13 010 deaths in Norway, and is together with cancer the cause of eight out of ten deaths in Norway (Statistisk sentralbyrå 2012). Risk factors associated with CVD through population surveys are high cholesterol levels, high blood pressure (BP), smoking, age, sex and heritage. The level of cholesterol in the blood is assumed to be affected by the amount and type of fat in the diet. Sugar, fiber and some trace elements have also been discussed as risk factors (Pedersen et al. 2009). The Norwegian Directorate of Health's recommendation is to limit total fat intake, especially SFA, due to the reduced CVD-risk (Helsedirektoratet 2014).

Regarding associations between fat intake and CVD both the effect of total fat and type of fat have been studied. In a systematic review of Hooper et al. (2001) an assessment on the effect of reduction or modification of dietary fat intake on total and cardiovascular mortality and cardiovascular morbidity was conducted. Twenty-seven randomized control trials were included, and they found that an alteration of dietary fat intake reduced cardiovascular events by 16%. The review concluded that there could be a small but important reduction of CVD with reduction of total fat. On the other hand a review of Schwab et al. (2014) concluded that an association between total fat intake and risk of any CVD outcomes was unlikely. The review included 29 publications regarding association between dietary fat and fatty acids and cardiovascular disease and the mean intake of fat in these studies varied from 35 to 45 E%.

According to Pedersen et al. (2009) a number of surveys have suggested that SFAs increase the total- and LDL-cholesterol in plasma. However, in 2009 Skeaff & Miller examined the effect of SFA on coronary heart disease (CHD) in a meta-analysis, that included 28 cohort studies, and no association between intake and risk of CHD was found. The systematic review and meta-analysis of Chowdhury et al. (2014) also found null associations between SFA and CVD. The status report of Astrup et al. (2011) concluded that the data from individual cohort studies are too inconsistent, but that

mostly no associations are found between SFA and CHD. In addition, substituting SFA with carbohydrate is associated with a higher risk of CHD (Astrup et al. 2011). However, the effect seems to be dependent on type of carbohydrate. Carbohydrate with high GI values is associated with higher risk of myocardial infarction, while carbohydrates with low GI where associated with decreased risk of myocardial infarction when substituting SFA with carbohydrate (Jakobsen et al. 2010).

Regarding unsaturated fatty acids evidence from controlled clinical studies suggest that MUFA has positive effects on different risk factors for CHD (Kris-Etherton & Nutrition 1999). No significant association was however found in a systematic review by Schwab et al. (2014) between MUFA intake and CVD risk, where four different prospective cohort studies was included. The same systematic review also found convincing evidence for replacement of SFA with PUFA on a decreased risk of CVD, especially in men. TFA on the other hand was strongly related to CHD (Skeaff & Miller 2009).

Consumption of trans-MUFA increases the blood total and LDL cholesterol concentration in a dose-dependent matter, as well as reduced the blood HDL cholesterol. Prospective cohort studies show a consistent relationship between higher intake of TFA and increased risk of CHD. Still, whether there is a difference between ruminant and industrial when they are consumed in equal amounts on the risk of CHD is not known, because the available evidences is insufficient (EFSA 2010). A lot of new research are now being done on CHD with relation to fat and some authors (Mozaffarian 2014) feels that the predictions of their health effect are oversimplified. In the case of type-2 diabetes, authors like Forouhi et al. (2014) have found that the effect of fat on diabetes type-2 are different from odd-chain and even-number chain.

Since meat is a good source of fat, some think it potentially contribute to a high level of serum cholesterol in the population, which is a risk factor for CVD (Pedersen et al. 2009). However, meat also contains high amounts of MUFA that is believed to reduce LDL and increase HDL cholesterol. In addition, animal fat has a large proportion of palmitic acid, myristic acid and stearic acid, while stearic- and myristic acid are believed not to affect the plasma cholesterol (Astrup et al. 2011; Pedersen et al. 2009).

Research suggest that red meat intake is not associated with CHD, while processed meat is associated with an increased risk. However, potential mechanism of effect is not known (Micha et al. 2010). A Nordic Nutrition Recommendations working group examined papers published from 2000-2010 to evaluate the scientific basis of dietary guidelines in relation to red and processed meat. They concluded that there were still too few studies to draw a conclusion regarding red meat and processed meat intake and CVD risk, since the endpoint diversity in the reviewed studies gave insufficient evidence (Norden 2012).

2.5.4 Nutritional value of fat

The fatty acid composition of meat is important for the nutritional value of beef (Warren et al. 2008). The ratio between PUFA and SFA (P:S), and the ratio between n-6 and n-3 fatty acids (n-6:n-3) are measures that primarily determines the nutritional value. In general, the P:S ratio is suggested to be above 0.46 and the ratio of n-6:n-3 below 4.0 to have a positive impact to different lifestyle diseases, such as CHD and cancer (Warren et al. 2008). In general, the ruminant muscle has a low P:S ratio, since it does contain various C20 and C22 PUFA of the n-6 and n-3 series which are nutritional valuable (Lawrie & Ledward 2006). The content of fat and fatty acids vary considerably between different types of meat, and between different animals of the same breed. In addition, the type of fatty acid also varies with both feed and species. Thus there is many ways of improving the nutritional value of beef (Norden 2012).

Sterols are mostly found in vegetable sources, but can also be present in small quantities in meat (*Composition of Foods integrated dataset* 2002; *Fineli* 2013). Research have suggested that an intake of 2 grams plant sterols a day leads to a 10% reduction of LDL-cholesterol (Katan et al. 2003).

2.6 Protein in meat

Red meat contains high biological value protein. It is a great source of high digestible proteins and essential amino acids, and contribute to satiety and low caloric intake per gram (Pereira & Vicente 2013).

2.6.1 Biological function of proteins

All amino acids are made up of 21 primary amino acids, where 8-10 are essential (Damodaran et al. 2008). Proteins play a central role in all biological systems. Proteins are important in many different biological reactions, functioning as thousands of different enzymes. In addition, they function as structural components in cells and complex organisms. Categorized by function proteins can be: enzyme catalysts, structural proteins, contractile proteins, hormones, transfer proteins, antibodies, storage proteins and protective proteins (Damodaran et al. 2008).

2.6.2 Recommendations and intake

Protein is a macronutrient found in almost all foods of animal and plant origin. The sources differ, however, in protein quality, as discussed later in this chapter. Sources like meat, fish, milk and eggs have high quantities of proteins, as well as high quality proteins. Sources like pulses, nuts and seeds also have a high protein content, but the quality is lower (Norden 2012).

The recommended daily intake indicated by the Norwegian Directory of Health is that protein should contribute 10-20% of the energy intake from the age of two. From 65 years of age, the energy intake from protein should be increased to 15-20%. Another way to calculate protein requirements is that 1.1 g protein should be included in the diet per kg of body weight. The reasoning behind this level of recommendation is that this level of proteins will cover the need of essential amino acids for the general population (Helsedirektoratet 2014). In Norway 18% of the total energy per day comes from protein, where men consume more protein than women, 112 g and 81 g, respectively (Helsedirektoratet 2012).

Meat is high in proteins, for example, minced meat contains 18.8 g protein/100g meat and a raw beef strip loin contain 22.2 g/100g (*Norwegian Food Composition Database* 2013). According to Norkost 3 (Helsedirektoratet 2012) meat and meat products are the most important single source of proteins in Norway, contributing to 27% of the daily recommended intake (Helsedirektoratet 2012).

2.6.3 Protein quality

The protein content of a food is not the only measure of how valuable a protein source is. Proteins differ in their nutritional value or “protein quality”. The protein quality is

dependent on two factors: the essential amino acid composition and the digestibility of the protein (Damodaran et al. 2008).

When a protein has high quality it contains all the essential amino acids above a certain reference level and in "right proportions", the latter meaning that the proportion of essential amino acids should produce optimum rates of growth or maintenance capability (Damodaran et al. 2008). These factors are measured as a chemical score, as seen in Table 5 whereas 100% describes proteins with high quality. Animal protein is classified as being of high quality, containing all the essential amino acids. Proteins of major cereals and legumes often have a limiting amino acid: one essential amino acid that is below the level of reference (often compared with a high quality protein). In cereals, the limiting amino acid often is lysine (Damodaran et al. 2008).

Table 5: Protein content, chemical score and biological value of proteins from different sources. Modified table by (Damodaran et al. 2008).

	Protein source			
Property	Egg	Beef	Wheat	Rice
Protein content (%)	12	18	12	7,5
Chemical score (%)	100	100	40	59
Biological value (in rats)	94	74	65	73

The digestibility is also important, or how much of the protein is utilized in the body (bioavailability). According to Damodaran et al. (2008) three main factors affects digestibility: protein conformation, antinutritional factors and processing. The protein conformation affects how much of the protein in cleaved into polypeptides by proteases. Antinutritional factors include trypsin, chymotrypsin, tannins and phytate, which inhibit the complete hydrolysis of the protein. Antinutritional factors are often found in plant proteins. Lastly, processing can reduce the rate of hydrolysis, especially extrusion where high temperature and pressure is applied to the protein. Egg has the highest digestibility of 97%, meat has 94% while wheat has 86% (Damodaran et al. 2008).

2.7 Vitamin A

Vitamin A refers to a large group of nutritionally active retinoids, and certain carotenoids possessing the biological activity of retinol (Blomhoff & Blomhoff 2006).

Retinoids with vitamin A activity in animal tissue are mostly retinol, retinal and retinoic acid. Also some synthetic variations of retinoids are used in food fortification: retinyl palmitate and retinyl acetate. The carotenoids include over 600 known compounds, whereas around 50 exhibits provitamin A activity. The best known are β-carotene, which exhibits the greatest provitamin A activity (Damodaran et al. 2008).

2.7.1 Retinoids and carotenoids

The retinoids is mostly found in animal sources and are absorbed efficiently trough the diet (Damodaran et al. 2008). Animals cannot synthesize vitamin A, but carotenoids ingested can be converted to vitamin A in animals, mostly in the form of retinol esterified with a fatty acid, yielding retinyl ester, primarily as retinyl palmitate (Blomhoff & Blomhoff 2006). Carotenoids are synthesized by plants and originate mainly from plant sources. They are large group of pigments (Blomhoff & Blomhoff 2006), which are considered a provitamins (Pedersen et al. 2009).

When the carotenoid β-carotene enters the body, it gets cleaved enzymatically yielding two molecules of retinal. Although two molecules of vitamin A are made, β-carotene yields a lower vitamin activity compared to retinol because the process of cleaving is inefficient. The difference in vitamin activity between all the retinoids and carotenoid compounds has been researched for many years. The unit RAE "retinol activity equivalents" is a concept, which converts all sources of retinol and provitamin A into one single unit. The intestinal retinol-to β-carotene-to- carotenoid- conversion ratio is suggested to be 1:12:24 (Damodaran et al. 2008). According to WHO (2009) this difference in vitamin activity can lead to deficiency of vitamin A, if the sole source of vitamin A comes from a modest intake of vegetables and fruits.

In addition, carotenoids in many foods are absorbed poorly in the intestine. However, even though carotenoids have lower or no vitamin activity, they may have important antioxidant functions (Damodaran et al. 2008).

2.7.2 Vitamin A function

According to Blomhoff and Blomhoff (2006) important functions of vitamin A include: role in night vision, maintenance of epithelial surfaces, immune competence, reproduction, and embryonic growth and development. Pedersen et al. (2009) states that retinol is the most physiological active form in the body, but that retinoic acid is

also an active metabolite and plays an important role in reproduction and fetal development as well as in night vision (Pedersen et al. 2009). The body is able to oxidize retinol to retinal, and retinal to retinoic acid (Blomhoff & Blomhoff 2006).

2.7.3 Vitamin A deficiency

According to WHO (2009) around 45 countries is assumed to have significant vitamin A deficiencies. However, Norway is assumed to be free of vitamin A deficiency. The main cause of vitamin A deficiency is a diet insufficient in vitamin A because the body cannot synthesize essential nutrients. Deficiency of vitamin A can cause disorders like xerophthalmia (dryness in the eye), which is the leading cause of childhood blindness, and disorders like anemia and decreased resistance to infection (WHO 2009). However, according to Blomhoff and Blomhoff (2006) vitamin A is also termed a "double-edged sword" because intake above recommended daily intake are suggested to be associated with reduced bone mineral density, increased risk for hip fracture and embryonic malformation.

2.7.4 Recommendations and intake

Daily recommended intake of vitamin A is 900 RAE for men and 700 RAE for women. One RAE equals 1 µg retinol which equals 12µg β-carotene (*Norwegian Food Composition Database* 2013). According to the dietary survey by Helsedirektoratet (2012) men consume 1011 RAE per day, while women consume 769 RAE per day.

Meat and meat products as well as butter, margarine and oils are the main contributor of vitamin A (RAE) in the diet, whereas both sources contribute 21% of the total vitamin intake. Vegetables are the third largest group contributing 20%. However, all of the vitamin A ingested from meat comes from the more bioavailable retinol compound, thus meat and meat products are the best source of retinol in the diet, contributing 27% of the intake. The retinol content is also high in butter, margarine and oil, contributing to 26% in the daily diet, and also 2% of β-carotene. Vegetables does not contribute to retinol intake, but is the largest contributor to β-carotene, contributing to 85% of the intake (Helsedirektoratet 2012).

2.8 Vitamin B1 – thiamin

Thiamin functions as a coenzyme in vivo and participates in the glucose and energy turnover. The active form of the vitamin is thiamine diphosphate (TPP). Bioavailability of the vitamin appears to be complete in all foods; however, it is not fully evaluated

(Damodaran et al. 2008; Pedersen et al. 2009).

2.8.1 Recommendations and intake

Helsedirektoratet (2014) recommend a daily intake of 1.4 mg for men and 1.1 mg for women of thiamin. Clinical signs of deficiency have been observed at intakes below 0.5 mg/d, and no upper intake level has been established (Norden 2013). Dietary surveys from Helsedirektoratet (2012) showed that actually daily intake was 1.4 mg for men and 1.1 mg for women. According to Norden (2012) cereals, meat and meat products and dairy products are main food source for thiamin in the diet. That was also true for the dietary survey, where bread contributed to 30%, meat and meat products contributed to 21% and milk and milk products contributed to 10% of the daily recommended intake (Helsedirektoratet 2012).

2.9 Vitamin B_2 – riboflavin

Riboflavin is a generic term for a large group of biological active riboflavin compounds. Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are versions of riboflavin that functions as a coenzyme in multiple enzymes. Little is known about the bioavailability (Damodaran et al. 2008; Pedersen et al. 2009).

2.9.1 Recommendations and intake

Helsedirektoratet (2014) recommend a daily intake of 1.6 mg for men and 1.3 mg for women of riboflavin. Lower intake levels are set to 0.8 mg and no upper intake level is established, and the major sources in Nordic diets are milk and dairy products as well as meat and meat products (Norden 2013). Dietary surveys from Helsedirektoratet (2012) showed that actually daily intake was 2.1 mg for men and 1.6 mg for women. The dietary survey by (Helsedirektoratet 2012) found that milk and yoghurt, meat and meat products and cheese and bread are main food source for riboflavin in the diet, contributing to 25, 15 and 9% of total recommended daily intake levels.

2.10 Vitamin B_6

2.10.1 Structure and properties

Vitamin B_6 is a collective term for a group compounds also called pyridoxines. Pyridoxine is the part of the vitamin, which has vitamin activity. Vitamin B_6 can occur in many different forms, all dependent of which substituent the pyridoxine has at the fourth position as seen in Figure 5 (Damodaran et al. 2008).

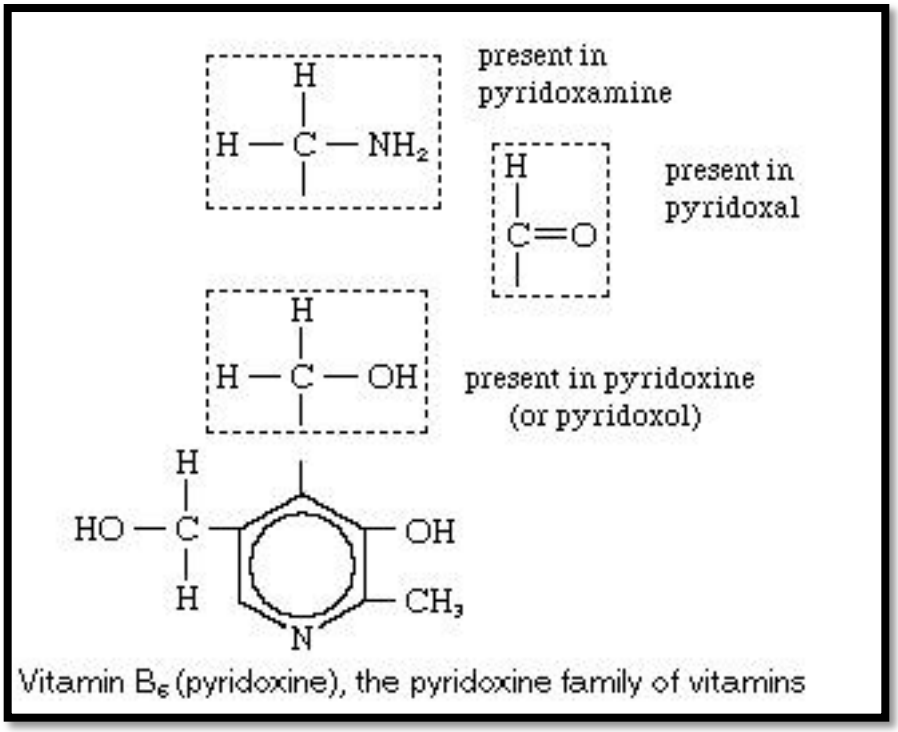


Figure 5: Different structures of vitamin B_6. The three different forms of vitamin B_6 are pyridoxamine, pyridoxal and pyridoxine. In pyridoxamine an amine is substituted to the 4th position, while pyridoxal and pyridoxol are substituted with an aldehyde and alcohol respectively (*Vitamin B6* 2014).

The three basic forms: Pyridoxine, pyridoxal and pyridoxamine can further be phosphorylated to compounds called pyridoxine 5' –phosphate (PNP), pyridoxal 5' –phosphate (PLP) and pyridoxamine 5' –phosphate (PMP). PLP and PMP are the two forms of vitamin B_6 that functions as a coenzyme, and thus catalyze enzymatic reactions in the body. Metabolism of amino acids, carbohydrates neurotransmitters and lipids, are some of the functions of these enzymes (Damodaran et al. 2008). Vitamin B_6 also interacts with vitamin B_{12} and folate to control the levels of homocysteine in the blood (McAfee et al. 2010). Since the body can convert all the forms of vitamin B_6 in vivo to PLP or PMP all forms of vitamin B_6 have vitamin activity. The vitamin B_6 can also be glycosylated, generally as pyridoxine-5'- β-D-glucoside (Damodaran et al. 2008).

All the chemical variations of B_6 are found naturally in food, but not all are found in every food. Plant products are the only food containing pyridoxine-glucoside, however, plant also contains all other forms of the vitamin. In sources like muscle meat and organs more than 80% of the vitamin are in PLP or PMP form, in addition to small amounts of non-phosphorylated variations.

2.10.2 Recommendations and intake

Helsedirektoratet (2014) recommend the daily intake of vitamin B_6 to be 1.5 mg for men and 1.2 mg for women, and according to Norden (2012) meat, potatoes, fish and dairy products are the major sources of vitamin B_6 in the diet. The most resent dietary surveys from Helsedirektoratet (2012) showed that actually daily intake was 1.9 mg for men and 1.5 mg for women. The survey also showed that meat and meat products is the biggest contributor to B_6 in the diet, contributing to 24% of the daily intake.

2.10.3 Difference in bioavailability

The high amount of B_6 in meat is not the only reason for meat being a good source of the vitamin. Meat contains mostly the PLP and PMP form of the vitamin, which together with pyridoxal, pyridoxine, pyridoxamine and PNP is believed to be more efficiently absorbed in the body than the glycosylated forms of B_6. The plant derived glycosylated forms are only partially utilized in humans, with a bioavailability of 50-60% relative to pyridoxine. Still, the glycosylated form can be an effective source of B_6 if the quantity of ingestion is high, because of its partial bioavailability (Damodaran et al. 2008).

2.11 Folate

Folate is an important water-soluble vitamin B, which has many different biological functions. The term folate is generic and includes a large group of compounds, like the naturally occurring folate in foods, and folic acid, which is the synthetic form of the vitamin (Norden 2012).

2.11.1 Structure and biological function

Folate refers to various components of folic acid (pterolyl-L-glutamic acid) with similar nutritional activity. This compound only exists in trace quantitates in nature, while tetrahydrofolic acid (H_4 folates) and dihydofolates (H_2 folates) are the compounds found in plants and animal sources. These compounds often contain a glutamate residue, which maybe can affect its bioavailability (Damodaran et al. 2008).

H_4 folate acts as a coenzyme and participates in the transfer of one-carbon units in vivo. These reactions are necessary in the metabolism of amino acids, and in the formation of the nuclei acids: purines and pyrimidine, and thus for the formation of DNA. Hence, folate is necessary for normal cell-division (Pedersen et al. 2009). A central folate-depending reaction is the re-methylation of homocysteine to methionine (Norden 2012).

2.11.2 Folic acid and cardiovascular disease

A probable cause-affect correlation between folate intake and reduced risk of cardiovascular disease (Helsedirektoratet 2011 b) has been found. Around 80 clinical and epidemiological studies have shown that an elevated level of total homocysteine in the blood is a strong risk factor for cardiovascular disease as sited in Refsum et al. (1998). This condition is denoted hyperhomocysteinemia. People with subclinical deficiencies of folate, vitamin B_6 and vitamin B_{12} show an elevated homocysteine level, and by eating food rich in these vitamins or taking supplements, the homocysteine levels are shown to decrease to low-normal range (Dinesh & Kalra 2004). So, it is clear that eating foods rich in folate reduce the homocysteine levels, however whether the increase in folic acid intake results in decreased risk of cardiovascular disease is still unclear. Multiple intervention studies including the study of Liem et al. (2003) have not confirmed that supplementing folate reduces the risk of cardiovascular disease, despite a reduction of homocysteine. Thus, others intervention studies like those of Schnyder et al. (2002) and the meta- study by Wald et al. (2002) indicate that supplements of folic acid can have a beneficial effect on CHD.

2.11.3 Recommendations and intake

Folate is one of few vitamins where supplements are recommended for some population groups in Norway (Helsedirektoratet 2014) and where the intake has been shown to be lower than the recommendations of 400µg for women and 300µg for men (Helsedirektoratet 2013). The recommendations is set to maintain a low level of homocysteine in the serum (Pedersen et al. 2009) and to reduce the risk of neural tube defects (NTD) (Norden 2012). According to the dietary survey of Helsedirektoratet (2012) the folate intake per day in Norway was 279 µg for men and 231 µg for women. 25% of the folate intake per person per day comes from bread, 17% from vegetables, 10% from fruit and berries and 6% from meat and meat products (Helsedirektoratet 2012). In minced raw meat, the level of folate is 3 µg per 100 g edible sample according to the Norwegian food composition database (*Norwegian Food Composition Database* 2013).

2.11.4 Bioavailability of folate

The folate content in food might be underestimated in food composition databases because common methods of analysis does not open up the food matrix and liberate all of the folate (Norden 2013). In addition, the degree of absorption varies from one food

to another, highly dependent on the chemical form of the vitamin and the presence of absorption inhibitors or enhancers in the meal (Norden 2012). The absorption of folate is, however, estimated to vary from 40-70% dependent on its source (Pedersen et al. 2009). Folic acid seems to have a better absorption than the naturally occurring folate. The NNR on the other hand conclude that there is no possible way to predict the overall bioavailability of folates from the composition of the diet, and that there are too few studies on absorption of folate from composite meals (Norden 2012).

2.12 Vitamin B12

Vitamin B_{12} is an important micronutrient only occurring naturally in food from animal origin (Damodaran et al. 2008). However, there exist some vitamin B_{12} producing bacteria's (Martens et al. 2002). LAB is such a bacteria and it is sometimes used in vegetable food sources to increase the vitamin B_{12} levels (Burgess et al. 2009) Multiple studies have shown that individuals consuming a vegetarian diet have low vitamin B_{12} levels (Alexander et al. 1994; Larsson & Johansson 2002) and the Nordic Nutrient Recommendation recommend all vegetarians to use B_{12} supplements (Norden 2012).

2.12.1 Structure and general properties

Vitamin B_{12} is a generic term for a group of vitamin active cobalamins. Cobalamin is found in six different forms that differ depending on the ligand attached to it. The synthetic form of B_{12} is called cyanocobalamin, and is used in fortified foods (Damodaran et al. 2008).

Vitamin B_{12} is important in enzymatic reactions acting as the coenzyme methylcobalamin or 5'-deoxyadenosylcobalamin. The first transfer one-units from one molecule to another while the latter is included in rearrangement reactions (Damodaran et al. 2008). B_{12} also often work in synergy with folate in the formation of active methyl (CH_3) (Pedersen et al. 2009). B_{12} is also needed together with folate and B_6 to lower homocysteine levels (Buttriss et al. 2005).

2.12.2 Recommendations and intake

Current recommendations for vitamin B_{12} intake in Norway are 2 µg/d for both women and men (Helsedirektoratet 2014). Recent dietary surveys show that men consume 8.9 µg/d and women 6.0 µg/d (Helsedirektoratet 2012). No upper intake level is established for vitamin B_{12} intake, and no risk seems to be present when ingesting up to 100 µg/d (Norden 2012).

Meat and meat products contributes to 29% of the daily recommended intake of $B_{12,}$ with only fish and fish products being a bigger source of the nutrient with 34%. Table 6 (*Norwegian Food Composition Database* 2013) shows some values of B_{12} per 100g edible meat cuts.

Table 6: Vitamin B_{12} content per 100g of beef, minced, tenderloin and striploin in Norway (*Norwegian Food Composition Database* 2013)

Product	Vitamin B_{12} content (μ g)
Beef, minced meat, 14% fat, raw	1
Beef, tenderloin, raw	1.6
Beef, striploin, raw	1.1

Small losses of B_{12} occur during processing, preserving or storage of food (Damodaran et al. 2008), and the amount of B_{12} in beef tenderloin indicate that consuming 100 g almost covers the daily recommended intake of vitamin $B_{12.}$

2.12.3 Bioavailability

Plants do not synthesize cobalamins, hence, they are not a source of vitamin $B_{12.}$ According to Dagnelie et al. (1991) some algea's do contain high amounts of B_{12}, but the bioavailability seems to be quite low. In animal tissue the B_{12} occurs mainly in the coenzyme form, but little is known on the bioavailability in foods (Damodaran et al. 2008). According to Hordaland homocysteine study milk provided the most bioavailable vitamin B12 (Vogiatzoglou et al. 2009).

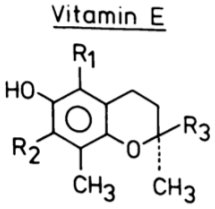
2.13 Vitamin E

Vitamin E is an essential fat soluble lipid for humans (Norden 2012), and an essential nutrient for growth and health of all animals (Liu et al. 1995). The main biological function of vitamin E in humans is proposed to be its antioxidant activity, where it might prevent propagation of free radicals in membranes and in plasma lipoproteins (Traber & Atkinson 2007).

2.13.1 Chemical structure

Vitamin E is the common term for two different groups of substances: tocopherols and tocotrienols. They are both synthesized in plants and occur in four different forms: α, β, ϒ and δ. As illustrated in Figure 6, the tocopherol has a chromane ring and a saturated

side chain (R_3). The number and position of methyl groups in the ring differentiate the tocopherols different forms. The tocotrienols have the same basic structure but the R_3 group is an unsaturated side chain (Pedersen et al. 2009).



	R_1	R_2	R_3
α-Tocopherol	CH_3	CH_3	$C_{16}H_{33}$
β-Tocopherol	CH_3	H	$C_{16}H_{33}$
γ-Tocopherol	H	CH_3	$C_{16}H_{33}$
δ-Tocopherol	H	H	$C_{16}H_{33}$

Figure 6: Chemical structure of the four different tocopherols, and listing of R-groups in the different forms of the vitamin (Sies & Stahl 1995).

α-tocopherol occurs in the highest amount in nature, and has the highest biologic activity, thus is the only form recognized to meet human requirements. α-tocopherol has three asymmetric carbon atoms, thus eight isomeric forms can exist. RRR-α-tocopherol is the naturally occurring form, while synthetic forms can contain all the isomers (Pedersen et al. 2009). All of these stereoisomers have equal antioxidant activity, but only those with the 2R configuration (RRR-, RSR-, RRS-, and SRR) have biological relevant activities, because the 2R-forms have much higher affinity to the α-tocopherol-binding protein: α-TTP in the liver (Norden 2012). The tocotrienols also have low affinity for this protein (Pedersen et al. 2009).

2.13.2 Recommendations and intake

Current daily recommendations for vitamin E intake in Norway is 10 µ-TE for men, and 8 µ-TE for women, whereas 1 α -TE is equal to 1 mg RRR- α-tocopherol (Helsedirektoratet 2014). According to NNR good sources of vitamin E are vegetable oils, vegetable oil-based spreads, nuts, seeds, and egg yolk (Norden 2012). The dietary survey Norkost 3 by Helsedirektoratet (2012) indicates that men's intake is 12 mg α-tocopherol per day, and women 10mg/d. The survey further showed that butter, margarine and oils was the biggest contributor of vitamin E in the daily diet, adding to 19% per person per day. Meat and meat products contribute to 6% of the daily intake of vitamin E. The amount of vitamin E in different meat products do vary, and raw minced

meat is indicated to contain 0.5 α-TE, while raw beef with trimmed fat contain 1.3 α-TE (*Norwegian Food Composition Database* 2013).

2.14 Iron

For all living organisms, iron is essential. Iron is included in hemoglobin in blood and myoglobin in muscle, where it has a function as an oxygen carrier (from lung to tissue), and as oxygen storage respectively. It is also included in many metabolic processes. Iron can be stored in the body by different proteins. In the tissue ferritin is the main storage protein. Small amounts of iron can also be found in plasma bound to serum ferritin. The amount of serum ferritin is suggested to reflect the size of iron body stores (Norden 2012). Iron is found in two different forms in food: haem iron and non-haem iron.

2.14.1 Heme-iron and non-heme iron

Non-heme iron is a ferric iron (Fe^{3+}) while heme iron is ferrous (Fe^{2+}). Non-heme iron is found in vegetable sources, while animal sources contain mostly heme iron in addition to some non-heme iron. Studies have shown that the heme-iron has a higher bioavailability than non-heme iron and according to Hurrell and Egli (2010) the estimated bioavailability of iron is in the range of 14-18% in mixed diets, and 5-12% in vegetarian diets. The heme iron is more available for absorption from foodstuffs than non-heme iron, partly because non-heme iron is insoluble in solutions with a pH greater than 3. Since the duodenum where iron is absorbed have an alkaline pH, it needs to be solubilized and chelated before absorption (Conrad & Umbreit 2000).

A variety of compounds are believed to inhibit and enhance the iron absorption. However, heme-iron is not affected by dietary constitutes, and is readily absorbed (Conrad & Umbreit 2000). According to Conrad and Umbreit (2000) dietary constituents like phytates, carbonates, phosphates, oxalates and tannates can form macromolecules with non-heme iron causing it to precipitate. Phytate is the main inhibitor of iron absorption (Hurrell & Egli 2010), but also calcium and proteins can negatively affect iron absorption. Calcium is different from other inhibitors because it affects the absorption of both heme and non-heme iron, while proteins like milk- and egg proteins affects only non-heme iron (Hurrell & Egli 2010).

Other components can enhance the absorption by reducing ferric iron to ferrous iron, which is soluble at neutral pH. To stay solubilized, the ferrous iron needs to be in that redox state, which is one of the reasons why ascorbic acid enhances absorption: it is a reducing agent that is continuously reducing the iron (Conrad & Umbreit 2000). In addition, there seems to be a component in meat and fish, referred to as the "meat factor" which enhance the absorption on non-haem iron from plant foods (Buttriss et al. 2005). Hurrell et al. (2006) found an increased absorption of non-haem iron, however, the mechanism underlying is not understood, but hypothesis include that meat stimulates gastric acid secretion that is important for iron absorption, and that products from protein digestion of muscle tissue could enhance absorption. The latter hypothesis involves a formation of cysteine-containing peptides that can reduce ferric iron to ferrous iron (Hurrell et al. 2006). As Hurrell and Egli (2010) points out in their article, iron absorption is studied mostly in single-meal isotope studies, where the dietary factors have been seen to influence the absorption of iron. In multi-meal studies however, a more modest effect has been seen (Hurrell & Egli 2010).

However, the most important factor affecting the absorption of iron is the adaptive regulation done by the body. Since the body does not have a mechanism for iron excretion, the absorption is strictly regulated by the human body (Hurrell & Egli 2010).

2.14.2 Iron deficiency

Iron deficiency can vary in manifestation from those related to anemia and those who have tissue iron deficiency, but manifestation of anemia and tissue iron depletion often overlaps and coexists (Anderson & McLaren 2012). According to WHO (1993-2005), anemia is a global public health problem, which affects both developing and developed countries. The primary cause of anemia is iron deficiency, while malaria, parasitic infection, nutritional deficiencies, and haemoglobinopathies (genetic defect leading to structural abnormalities in the globin proteins) also can contribute to anemia (WHO 1993-2005).

Iron deficiency anemia (IDA) is the term used for anemia caused solely from iron deficiency. The risk factors for IDA include low intake of iron, poor absorption of iron, and periods in life when iron requirements are especially high. The latter is the case for pregnant women and young children who are at higher risk, but anemia can occur in all

life stages. Heavy blood loss (e.g. menstruation or parasite infection), acute and chronic infections (malaria, cancer, HIV) can also lower blood hemoglobin concentrations. In addition micronutrient deficiencies of vitamin A, B_{12}, folate, riboflavin and copper can also increase the risk of anemia (WHO 1993-2005).

According to the database on anemia prevalence based on hemoglobin concentration made by the WHO (2007), five studies on woman's iron status was performed at a state level in 2007 in Norway. It was found that 7.9%, 8.5%, 9.4%, 7.7% and 5.3% of the test subjects had a hemoglobin level below 120 g/L. WHO (2011) have set limit values for development of anemia and a level below 120 g/L can imply that subjects suffer from "mild" anemia. In addition the WHO (2011) states that anemia is of mild public health significance if 5.0-19.9% of the population has anemia. According to the limits set by WHO anemia is a mild public health problem in Norway.

Anemia can have negative effects on work capacity and endurance as well as low birth weight and preterm delivery of children, and it can affect the motor and mental development in infants, children, and adolescents (Anderson & McLaren 2012).

2.14.3 Recommendations and dietary sources of iron

Recommendations for iron intake in Norway is 9 mg/d for men, 15 mg/d for women in fertile age and 9 mg for women after menopause (Helsedirektoratet 2014). There are also other recommendations for additional age groups; recommended intake can be seen in Table 2 of Helsedirektoratet (2014). According to Helsedirektoratet (2012) dietary survey iron intake per day is 13 mg/d for men, and 9.9 mg/d for women (average of women in fertile and non-fertile age). Meat consumption is a very important contributor to iron intake in Norway, and 20% of the total intake of iron comes from meat and meat sources (Helsedirektoratet 2012). Most of the iron from meat is in the heme form, which is better absorbed and utilized by the body, than non-haem iron which is found in vegetable sources and in animal foods (Buttriss et al. 2005).

2.14.4 Iron as a promoter for lipid peroxidation

Iron is also a metal that promotes lipid peroxidation in foods (Damodaran et al. 2008; Monahan et al. 1993). According to Damodaran et al. (2008) iron catalyzes both the initiation and propagation stages of lipid peroxidation. It is also believed that heme iron

is more catalytic than non-heme iron, but the mechanism for lipid oxidation is not completely understood (Damodaran et al. 2008; Monahan et al. 1993). The study done by Monahan et al. (1993) showed that when the concentration of iron in the muscle increased, the rate of lipid oxidation also increased. When muscle lipids are exposed to oxidation and breakdown compounds are formed, the quality of meat can be affected: both with regards to flavor, odor, loss of color and also with relevance to health issues like the link between iron and CRC (Damodaran et al. 2008; Monahan et al. 1993; Sesink et al. 1999).

2.15 Magnesium

Magnesium is an ion involved in different biochemical reactions in the body, however, the metabolism and requirement of magnesium is poorly understood.

2.15.1 Recommendations and intake

Helsedirektoratet (2014) recommend a daily intake of 350 mg/d for men and 280 mg/d women. No lower or upper intake levels for magnesium from natural sources are established. According to (Norden 2012) magnesium can be found in green, leafy vegetables, legumes, and whole grain cereals. Dietary surveys from Helsedirektoratet (2012) showed that actually daily intake was 439 mg/d for men and 346 mg/d for women. The dietary survey by Helsedirektoratet (2012) found that bread and dairy products (milk, yoghurt) are main food source for magnesium in the diet, contributing to 24 and 10% of total recommended daily intake levels. Meat and meat products contributed to 7% of the intake.

2.16 Potassium

Potassium is an essential nutrient naturally existing as a salt. The main use of potassium is for fertilizing, and deficiencies are rare (Damodaran et al. 2008). In the body most of potassium (98%) are intracellular, and it is an important cation. The 2% of potassium which are extracellular regulates membrane potentials (Norden 2012).

2.16.1 Recommendations and intake

Helsedirektoratet (2014) recommend a daily intake of 3.5 g/d for men and 3.1 g/d women. Lower intake levels are set to 1.6 g/d and upper intake levels are not established. According to (Norden 2012) potassium can be found in potatoes, fruits and berries, vegetables, and milk and dairy products. Dietary surveys from Helsedirektoratet

(2012) showed that actually daily intake was 4.2 g/d for men and 3.4 mg/d for women. The dietary survey by Helsedirektoratet (2012) suggests that milk and yoghurt are main food sources for potassium in the diet (13% of daily total intake) followed by bread and meat and meat products which both contributed to 11% each.

2.17 Salt – sodium

One of the key nutrition advices given in the report "recommendations on diet, nutrition and physical activity" from Helsedirektoratet (2014) is to "chose foods low in salt and limit the use of salt in cooking". These advices are based on a observed relationship between salt intake and high BP and increased risk of CVD (Helsedirektoratet 2011 a). Salt consists of sodium and chloride, and it is the sodium component of the salt, which is associated with increased risk of elevated BP and CVD. One gram of NaCl contains 0.4 g of sodium (Helsedirektoratet 2011 a).

2.17.1 Salt and blood pressure

According to Appel et al. (2006) elevated BP is an common and important risk factor for CVD, and both environmental factors and genetics affect increased BP. Environmental factors include diet, physical inactivity, toxins and psychosocial factors. Studies have indicated that dietary modifications which lowers BP levels are weight loss, reduced salt intake, increased potassium intake and moderation of alcohol consumption (Appel et al. 2006).

Sodium also serves many important functions in the body. It is a cation (Na^+), and regulates the extracellular fluid volume together with chloride (Cl^-). Additionally, it helps transport nutrients into cells, and regulates BP (Damodaran et al. 2008).

2.17.2 Intake and recommendations

The Norwegian Directory of Health recommends an intake of 2.3 g sodium per day, this corresponds to 6 g of salt. In long term an intake of 5 g per day is desired (Helsedirektoratet 2014). The survey assessed by Helsedirektoratet (2012) revealed that 81% of the men, and 49% of the women consumed more sodium than the recommended value. A mean value for men was 3.6 g sodium per day, while women consumed on average of 2.5 g/d. These numbers do, however, not include salt added to the food during cooking or during the meal, so the numbers can be underestimated (Helsedirektoratet 2012).

Meat and meat products is according to Helsedirektoratet (2012) the largest contributor of dietary sodium, contributing to 24% of the daily intake. However, this food group includes both processed and unprocessed meat, and not all types of meat contains high levels of salt as seen in Table 7 (*Norwegian Food Composition Database* 2013). The variation in sodium content from unprocessed meat, such as striploin, to highly processed meat as dry fermented sausage varies.

Table 7: Sodium content in processed and unprocessed meat products in mg/100g edible food (*Norwegian Food Composition Database* 2013)

Product	Sodium (mg)
Dry fermented sausage	2200
Beef, minced meat*	360
Beef, striploin, raw	48

* Norwegian minced meat is added salt

Salt is often added for a number of reasons: it have beneficial functions in enhancing flavor, preservative effects, it enhances color and improves the water holding capacity (Damodaran et al. 2008). In unprocessed meat, however, sodium concentrations are normally low (Norden 2012).

2.18 Selenium

Selenium is a complex trace element. It's an essential nutrient for both animals and humans, but toxic at high concentrations. Deficiency is generally caused by low concentrations in soil, forage and food, while toxicity problems usually result from build-up in body tissues and biomagnification in the food chain (Hartikainen 2005).

2.18.1 Chemical structure and functions

Selenium is a chemical element found in many different forms. Naturally occurring selenium is inorganic, and includes among other selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}). In living systems selenium is in its organic form incorporated into selenoproteins. The most common are selenomethionine and selenocysteine (Pedersen et al. 2009). The selenoproteins often have important enzymatic functions, and selenocysteine often function as the active site where it has an redox function (Rayman 2000). The best-known example of this redox function is the enzyme glutathione peroxidases: it reduces hydrogen peroxides and hydroperoxides to harmless products, and thereby acting as a defend mechanism against damage caused by free radicals (Pedersen et al. 2009). Twenty-five selenoproteins have been reported to make up the selenoproteome. These

include peroxidases: cellular (cGSHPx), extracellular (eGSHPx), phospholipid hydroperoxide (phGSHPx), and gastrointestinal (giGSHPx) (Norden 2012).

2.18.2 Selenium soil content and availability

From 1950 animal selenium deficiency diseases were identified in a large scale of livestock in different part of the world. Most known is the white-muscle disease in lambs and calves, reproduction problems and restricted growth. For humans deficiency diseases (Keshan disease, Kashin-Beck disease) have been recognized in some regions, especially China, where the soil is extremely low in selenium (Rayman 2000). Selenium enters the food chain trough plants, which take it up from the soil. Deficiency of selenium is therefore often caused by low soil content of selenium (Rayman 2000) or poor availability of the selenium compounds to be taken up by plants (Aasen 1997).

The distribution of selenium in soil is very uneven, with observed variations ranging from almost zero to 1250 mg/kg soil. In most soils, however, the concentration of selenium is between 0.01 and 2 mg/kg (Hartikainen 2005). In the Nordic countries selenium values are in the range of 0.1-0.4 mg/kg. Higher values have been observed in forest soil from humus-samples taken from eastern Norway and Nord-Trøndelag, with values ranging from 0.42-0.63 mg selenium per kg. Samples obtained from coastline shows higher values than inland samples, probably due to higher amount of rain, which adds selenium, in the coastline areas (Aasen 1997).

Due to mostly low selenium levels in Norwegian soil, the content in feed like grain and grass cultivated in Norway is also low. Middle values are found to be 0.009 and 0.025 mg selenium per kg dry matter for grain and grass respectively. Regarding feed to livestock a selenium content of 0.25-0.50 mg per kg dry matter is desired (Aasen 1997). Enrichment of selenium in the form of sodium selenite (Na_2SeO_3) to concentrated feed was required as of 1979 in Norway, thus selenium deficiencies in domestic animals are no longer an issue (Pedersen et al. 2009).

The amount of selenium in the soil is not the only factor, which affects the level of selenium in plants, also the availability of the selenium is important. A broad range of oxidation states can be found in selenium, at least in theory. The Se is +6 in selenates, +4 in selenites, 0 in elemental Se, and -2 in inorganic and organic selenides (Hartikainen

2005). In soil the oxidation level is somewhat dependent on the pH level. Selenate is the most common form found in alkaline soil, while neutral and acid soil have a higher occurrence of selenite. Plants have the ability to absorb both types of Se, but selenite often binds to clay particles, iron and humus, thus reducing the availability for uptake by plants (Aasen 1997).

2.18.3 Recommendations and intake

Recommendations for selenium intake is 50 µg/d for women and 60 µg/d for men in Norway (Helsedirektoratet 2014). This intake is assessed to achieve a maximal GSHPx activity in serum. Intakes of 80-120 µg/d are needed for maximal GSHPx activity in red blood cells and platelets. It is not apparent, however, that maximal GSHPx activity in all tissues is necessary for optimal health (Norden 2012).

Because of the low content of selenium in Norwegian soil, plants and cereals are not a good source of selenium. In the Nordic countries fish, meat, eggs and milk are the major sources, and have higher selenium content since feed is fortified with selenium. People who live on a vegetarian diet, or eat little meat, can be susceptible to selenium deficiencies (Norden 2012).

2.18.4 Increasing selenium content of meat

One effective way to increase the selenium content in plants, and thereby in domestic animals and humans, is to fortify fertilizers with selenium as done in Finland (Hartikainen 2005), where the working group of the Ministry of Agriculture and Forestry proposed to supplement multinutrient fertilizers with selenium. In 1991 an initial level of 16 mg/kg was used, then reduced to 6 mg/kg and raised to 10 mg/kg again in 1998. This fertilization induced a drastic change in selenium concentration in agricultural products, and for meat and meat products, the selenium concentration increased 13-fold during 1985–1991. This also resulted in an increased selenium intake for the whole population in Finland (Hartikainen 2005) and today the selenium status for the Finnish population are on an optimal level (Alfthan et al. 2014).

2.19 Zinc

Zinc is found to be part of more than 300 enzymes that are involved in synthesis, metabolism, and turnover of macromolecules, nucleic acids and some vitamins. Enzymes that contain zinc includes alkaline phosphatase, alcohol dehydrogenase and superoxide dismutase (Norden 2012), the latter protecting the body from oxidative damage by

being an important enzymatic antioxidant (Bowen 2003). Zinc is included in many processes in the body, and intake seems to be related to maintenance of normal bone density, cognitive function, fertility and reproduction (Norden 2012).

2.19.1 Recommendations and intake

Norway's Directory of Health recommends a daily intake of 9 mg zinc for men, and 7 mg for women (in the age 18-30 years) (Helsedirektoratet 2014). Recommendations for other age groups can be accessed through Table 2 in Helsedirektoratet (2014). According to data calculated from recent national dietary surveys by the NNR, estimates of the average zinc consumption in Norway are 12.5 mg per 10MJ (Norden 2012). According to the most recent dietary survey by Helsedirektoratet (2012) the average intake of energy is 10.9 MJ/d for men and 8.0 MJ/d for women (Helsedirektoratet 2012). According to Pedersen et al. (2009) the recommendation for zinc intake is associated with uncertainty regarding the facts that the body can adapt to different intake levels, the measurement of zinc status is not optimal, and the composition of the diet influence the zinc uptake.

2.19.2 Zinc content and bioavailability from different sources

The content and bioavailability of zinc in food varies widely (Damodaran et al. 2008). According to Norden (2012) meat, milk and milk products are good sources of zinc, both because they contain a large amount of zinc, and because they have a good bioavailability. In the study by Scherz and Kirchhoff (2006) the zinc content of various raw food from different countries of the world was compared and variations displayed. An extract of the findings is presented in Table 8.

Table 8: Zinc contents of individual animal and plant foods, and variation seen between different countries (µg/100 g edible portion). Modified table from (Scherz & Kirchhoff 2006).

Food	Mean value	Variation
Cow milk	384	310-445
Beef, muscle	4010	1050-5650
Pork muscle	2520	1490-3600
Chicken muscle	1130	800-1540
Cod	395	325-450
Wheat	2870	2190-4160
Carrots	270	150-400
Banana	164	100-640

This table displays that beef meat is a good source of zinc but large variations are seen between different countries' reported zinc levels, ranging from 1500-5650 µg in beef muscle.

How well minerals are absorbed in the gastrointestinal tract also has a huge impact on mineral bioavailability (Lopez et al. 2002). According to Norden (2012) whole grain cereals are also a good source of zinc, but the absorption is reduced because of the presence of inhibitory compounds like phytic acid (main phosphorous storage compound in plants) in the grains. Phytic acid forms insoluble complexes with zinc, which prevents it from being absorbed, thus the bioavailability of zinc decreases (Lopez et al. 2002).

Lopez et al. (2002) suggest that minerals must be ionized before uptake trough the intestinal membrane, and that this ionization makes the mineral very unstable, thus susceptible to bind with phytic acid from cereals of plant seeds. These complexes are very stable and accordingly, the ion gets unavailable for intestinal uptake, because it is no longer in an ionic state (Lopez et al. 2002).

When the intake of zinc is close to the requirements, a prediction of the inhibitory effect of phytic acid on zinc has been suggested to be the molar ratios of PA-to-ZN (Lopez et al. 2002), however, Fordyce et al. (1987) suggested that the prediction should include calcium, since high levels of calcium can increase the inhibitory effects of phytic acid on zinc, forming a Ca-Zn-PA complex which is even less soluble that phytate complexes. Hence, PA * Ca/Zn ratio can be a better prediction of zinc bioavailability.

There are ways to improve zinc bioavailability; the total amount of dietary zinc can be increased or food can be fermented to enhance zinc absorption, because fermentation decreases the phytate content. Dietary proteins has also been suggested to facilitate zinc absorption, even in the presence of phytic acid (Sandstrom et al. 1989).

2.20 Red meat and colorectal cancer risk

In western societies colorectal cancer (CRC) is one of the major causes of cancer death. Genetic factors are important for the formation of CRC in individuals, but it also appears that environmental factors are important (Sesink et al. 1999; Yi et al. 2013). Recent meta studies have concluded that there seems to be a related risk to red and processed meat

consumption, whereas processed meat is more closely linked to the risk of CRC (Bastide et al. 2011; Larsson & Wolk 2006). This association is based on epidemiological data, where western types of diets (high in meat and fat, and low in fiber) are associated with a high risk for CRC. The mechanisms suggested for this association are many.

2.20.1 Suggested mechanisms between meat and CRC

The meta analysis and review of Bastide et al. (2011) gives an overview of current mechanisms. First, meat that is fried at high temperatures contains mutagenic heterocyclic amines. However, the consumption of chicken also is a major contributor to heterocyclic amines, but white meat is not associated with CRC (Bastide et al. 2011; Parr et al. 2013). A second hypothesis suggest that an increased risk of CRC is caused by the high saturated fat content of red- and processed meat (Bastide et al. 2011). The report of the World Cancer Research Fund (2007) concluded that there is limited but suggestive evidence that animal fat intake increases the risk of CRC, while recent meta-analysis, however, showed no effect of this relationship (Alexander et al. 2009; Clinton et al. 1992).

Sesink et al. (1999) hypothesized that the heme content of red meat could explain the association between high intakes of red meat and the increased risk of CRC, being involved in diet-induced colonic epithelial damage which results in increasing epithelial proliferation. The hypothesis was tested by conduction of a cancer study in rodents. Rats were either fed a purified control diet, or a purified diet supplemented with 1.3 μmol/g of hemin (ferriheme), protoporphyrin IX, ferric citrate, or bilirubin, for 14 days. The results showed significant increase in colonic epithelial proliferation in heme fed rats, compared to control rats. The fecal water of heme fed group was also found to be highly cytotoxic compared to controls(Allam et al. 2011). They concluded that dietary heme leads to the formation of an unknown, highly cytotoxic factor in the colon lumen (Sesink et al. 1999). In later years many studies have supported the hypothesis of heme iron from meat, including a large prospective study by (Cross et al. 2010) that investigated the potential mechanisms between meat consumption and CRC risk. However, the mechanisms implicated in the promotion of CRC by heme are poorly understood.

Two suggested mechanisms based on the catalytic effect of heme iron is presented: the formation of N-Nitroso compounds (NOCs), which are carcinogenic compounds formed

by a reaction between nitrite and free amino acids or amines in meat,, and reactive oxygen spices (ROS) (Oostindjer et al. 2014).

2.20.2 N-nitroso compounds (NOCs)

NOCs are a generic term, including hundreds of compounds, where most compounds have been found to have carcinogenic properties. NOCs occur in the environment, plants can synthesize some, but most are formed by nitrosation of amines. Nitrosation is the reaction where a secondary amine reacts with a nitrosating agent, commonly nitrite salts, creating nitrosamines (Loeppky & Michejda 1994).

The potassium and sodium salt of nitrite and nitrate are commonly used in curing mixtures for meat. Nitrite is found to be the functional constituent, and it is a precursor of nitric oxide (NO), which is an essential for most curing reactions in meat. Nitrite is added to some processed meat to enhance flavor, inhibit microorganisms (at higher levels of addition only) , develop wanted meat color and to retard development of oxidative rancidity (Damodaran et al. 2008). The gastric secretion of hydrochloric acid in the stomach of humans, provide an environment where nitrosamines can form (Loeppky & Michejda 1994), the nitrite in the meat forms NO that can react with secondary amines, and to some extent primary and tertiary amines like prolin, histidine and tryptophan in the stomach. This reaction results in the formation of NOCs (Damodaran et al. 2008).

However, the carcinogenicity of NOC formed in the gut after eating heme from red and processed meat is unknown, since they are not all carcinogenic (Bastide et al. 2011). In addition, most research done in animal models uses purified compound testing. This led to the question how nutrients and compounds from other food items in the meal modulate the carcinogenic compounds (Oostindjer et al. 2014). As an example, calcium salt is able to precipitate heme iron, thus limiting the amount of peroxidation, which limits the catalyzing effect heme iron has on NOC formation (Allam et al. 2011).

Nitrate salts also occur naturally in many foods, including vegetables such as spinach, and the accumulation of large amounts of nitrate in plant tissue grown on heavily fertilized soils can be of concern (Damodaran et al. 2008). Still no association between vegetables and CRC is found (World Cancer Research Fund 2007).

2.20.3 Reactive oxygen spices (ROS)

ROS are a type of radical derived from oxygen that are generated constantly in biological systems. They are a part of normal aerobic life, and are formed in mitochondria when oxygen is reduced in the electron transport chain (Bowen 2003). But compounds in food can also catalyze ROS formation. Some ROS include superoxide (O2•), hydroxyl (HO•), alkoxyl (RO•), peroxyl ($RO•_2$), aryloxyl (ArO•), nitric oxide (•NO), nitrogen dioxide (NO2•), thiyl (RS•), thiyl peroxyl (RSOO•), sulfonyl (RSO2OO•), and carbon- centered radicals (R•) (Niki 2014). Radicals have high chemical reactivity, and when overproduced, they can inflict damage on cells, nucleic acid and macromolecules such as lipids and proteins (Bowen 2003).

The heme in meat is suggested to catalyze lipid peroxidation in vivo (Tappel 2007). The heme is part of the complex pigment molecule called myoglobin, which binds oxygen and function as a storage mechanism for oxygen in the muscle and exists in meat. The myoglobin is built of a single polypeptide chain that is folded around the heme, which contains an oxygen-binding site (Mathews et al. 1999). This myoglobin also contains an iron, that is found to be toxic to living cells when it is free (Damodaran et al. 2008). In vivo, the iron is chelated by a tetrapyrrole ring system, called protoporphyrin IX, as seen in Figure 7. The complex of protoporphyrin IX with Fe^{2+} is called heme. Hemin is the term used when the iron of heme is in the ferric state (3+) and bound to a chloride (protoporphyrin IX with Fe^{3+} bound to chloride) (Mathews et al. 1999).

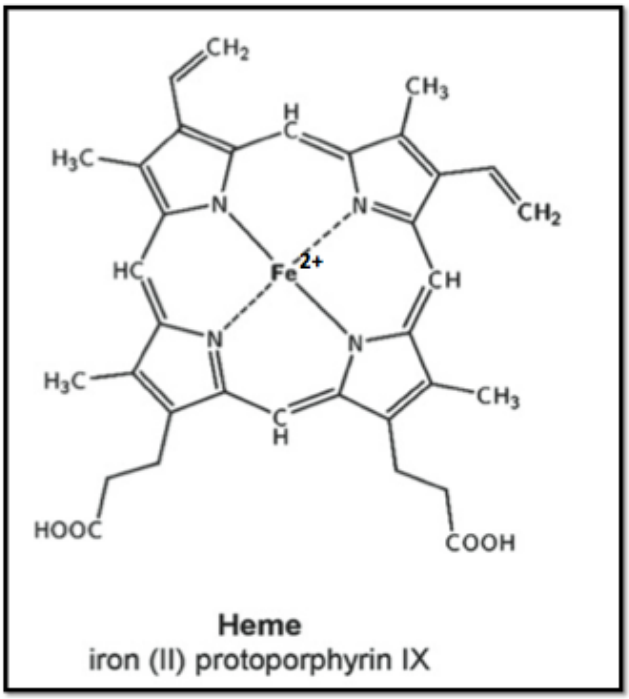


Figure 7: Chemical structure of heme. Modified figure of Bastide et al. (2011)

After being eaten the heme proteins are hydrolyzed to amino acids and peptides, and the iron is coordinated to sulfur, nitrogen or oxygen of amino acids and peptides. The heme group are then absorbed and transported by the blood to organs and tissue.

2.20.4 Suggested pathway

One major pathway of reactions between lipids and heme is suggested by Tappel (2007):

LOOH (lipid hydroperoxide) + Fe ligands (heme) → LOOFe ligands →

LO^{*} (lipid alkoxy radical) + $^{*}OFe$ ligands (heme oxyradical).

Further is it suggested that the alkoxy radical and the heme oxyradicals can react further and create chain reactions (Tappel 2007). ROS can be catalyzed by heme when PUFA is present, such as fatty acids from phospholipids found in cell membranes.

Under normal conditions hemin is absorbed in the digestive system through heme transport proteins. But if the concentration of hemin digested is high, not all will be absorbed. This results in hemin being present in the digestive system and the feces, where it can be able to catalyze ROS (Oostindjer et al. 2014). ROS can increase the risk of cancer because it has been seen implicated in DNA damage, and have the ability to disrupt normal cell proliferation of the gut epithelial cells (Perse 2013).

However, in vivo there exist mechanisms that can reduce or repair this damage. Additionally, a diet rich in vegetables and fruit may contain enough antioxidants to trap ROS. However, if the diet is unbalanced with a large portion of meat, the protective mechanisms may not be sufficient (Perse 2013). In addition, the heme in vivo is also protected by cells and subcellular location, which prevents oxidative damage (Tappel 2007).

2.20.5 Protective factors in meat with respect to cancer

Meat is a complex food, and do not only contain catalytically iron, but it also contains nutrients that are protective against cancer, such as folate, vitamin A, selenium and zinc. All these nutrients are suggested to be protective, as well as the bioavailability of the nutrients are better in meat than in vegetables (Biesalski 2005).

Both vitamin A, β-carotene, selenium and zinc is believed to have protective factors, mostly due to their antioxidant function. Selenium is part of the active site of the glutathione peroxidase enzyme – which function is to reduce hydrogen peroxides, acting as the body's defense mechanism against damage from free radicals (Biesalski 2005; Pedersen et al. 2009). Zinc is part of metalloenzymes that are important for replication and growth of cells, and it may also contribute to antioxidant defense, and β-carotene functions as an antioxidant in the body. Lastly, folate has been shown to decrease the risk of cancers in patients according to Biesalski (2005), however, the protective factor were not evident over 15 years, thus the nutrient needs to be present in the diet for a long period of time (Biesalski 2005).

However, when a meal with meat takes place, it is most probably not eaten alone. It may be combined with vegetables, or dairy products that also can contain protective factors against cancer. One example is dairy products which contains calcium that is believed to have chelating effect of heme iron (Allam et al. 2011). Further a healthier gut environment can be obtained by eating a lot of fiber rich fruit and vegetables (Oostindjer et al. 2014).

Food containing vitamin E may also help prevent the development of CRC, mostly due to its antioxidant function (Oostindjer et al. 2014). Vitamin E functions as a chain-breaking antioxidant and thus a radical scavenger (Niki 2014). The RRR-α-tocopherol reacts with

peroxyl radicals, by the chromane ring that has redox properties. This breaks the radical chain reaction, causes a relatively stable lipid hydroperoxide, and protects the lipids from oxidation (Sies & Stahl 1995).

Some vitamin E is stored in all cell membranes in the body (Pedersen et al. 2009). Here it interacts with the phospholipids, attaching the chromanol ring among the polar head groups of phospholipids, while the phytol side chain interacts with phospholipids UFA chain. This position is optimal in protecting the PUFAs in phospholipids from peroxidation by ROS (Liu et al. 1995). In addition, vitamin E is also found in plasma and red blood cells, where it protects lipids and low density lipoproteins against per oxidative damage (Sies & Stahl 1995). In the review article of Traber and Atkinson (2007) two studies showed that vitamin E supplements decreases lipid peroxidation in subjects under oxidative stress. On the other hand vitamin E showed no effect in studies were subjects where not under oxidative damage.

All nutrients functioning as antioxidants may help prevent CRC because they can prevent the peroxidation and nitrosation, which is suggested to be the mechanisms for meat induced CRC (Oostindjer et al. 2014).

2.21 Nutrient variations in meat

The composition of meat is quite variable. According to Lawrie and Ledward (2006) species, breed, sex, age, nutritional status and activity of the animal are major factors affecting the gross composition of meat. In addition, factors like anatomical location of retail cut, post slaughter processes, storage and cooking contribute to variety of meat composition (Damodaran et al. 2008).

2.21.1 Breed

Breed is the most influential trait on the biochemistry and constitution of the muscle, after species. Regarding cattle big differences can be seen between the milk producing breeds and the meat-producing breeds, whereas beef-type cattle has a higher percentage of intramuscular fat. Since fat is a highly heritable trait, large differences can be observed for this trait between breeds (Lawrie & Ledward 2006).

2.21.2 Age

When animal ages, the composition of the muscle does vary, irrespectively of species, breed or sex. The main change with aging is the growth of the animal, which often means increased fat deposition, and the SFA is the fatty acid increasing most, altering the P:S ratio when aging (Kerry & Ledward 2009).

All parameters seem to increase with age, except from water. However, different muscles change in different rates. Muscles change with age until the components reach adult life values, and different components reaches these values at different times. The most evident changes with age in the *l. dorsi* muscle is the increase of intramuscular fat until the age of 40 months, the consequently decrease moisture content, and the rapidly increase of myoglobin until 24 months of age. The concentration of myoglobin increases in a two-phase manner. For cattle there is first a rapid phase of 3 years, followed by a second phase where the increments are more gradual (Lawrie & Ledward 2006).

2.21.3 Sex

In general males have less intramuscular fat than females (Lawrie & Ledward 2006), and grow to a larger mature size (Warriss 2010). Castrates are shown to have more intramuscular fat than bulls. There also seems that the depot fat of steers have more saturated fat than heifers. In addition heifers seems to have a large proportion of oleic acid compared to steers (Lawrie & Ledward 2006).

2.21.4 Anatomical location

The most complex and largely unknown source of variation in meat is caused by differences in anatomical locations in the muscle. Muscles can broadly be classified as “red” or “white” according to function. But in a mammalian body there are over 300 muscles, all differs because of the different actively they have. Between muscles there have been showed large differences in moisture and fat content, degree of saturation, nitrogen content, collagen content, content of sodium, potassium and myoglobin among others (Lawrie & Ledward 2006).

Earlier the differentiation between muscles where not so important since wholesale and retail cut of beef where large, and represented an aggregate of muscles. In later years, specific portions of the meat is prepared and packed for individual consumers, and a product may arise from only one muscle. In these cases, the consumer should know the difference in composition between muscles (Lawrie & Ledward 2006).

2.21.5 Training and exercise

How active an animal is can change some features in the muscles. The most logical is that active animals have a higher level of myoglobin in the muscles. This because myoglobin is the body's short-term oxygen store, and when an animal is more active, the level of myoglobin will increase compared to an inactive animal. Training can also alter the amount and type of protein, whereas moderate inactivity seems to cause a reduction in sarcoplasmic and myofibril proteins (Lawrie & Ledward 2006).

2.21.6 Inter-animal variability

Lastly, one of the least understood factors for variation between animals is the intrinsic factor. Even between animals of the same sex, eating the same food, variations can be seen. The differences may be explained by recessive genes, but no reason have been found so far (Lawrie & Ledward 2006).

2.21.7 The effect of feeding

Feeding is an important way to alter fatty acid composition of beef. Even if rumen hydrogenate most of the dietary PUFA, some increase in linoleic and linolenic acid have been seen when feeding different plant oils and different forages to beef (Kerry & Ledward 2009). In northern Europe and Norway fresh grass is an important feed for cattle. Fresh grass contains a lot of linolenic acid and can enhance the n-3 fatty acids in beef. Several studies have shown a reduction of the n-6/n-3 ratio in muscle from bulls, steers or heifers that have consumed grass or silage diet, compared to a concentrate diet. Both the type of grass in the diet, the length of time on grass before slaughter, can affect the total fatty acid composition of the muscle (Kerry & Ledward 2009).

2.21.8 Examples of variation in proximate, fat, vitamin and minerals

According to Damodaran et al. (2008) the proximate composition of lean tissue is somewhat variable. In general, it is assumed that water accounts for 70% of the muscle weight, and that protein ranges from 18-23%, while ash and mineral content is approximately 1.0-1.2%. The content of fat also varies. Few food composition tables include variation of nutrients, but in

Table 9 the proximate variation of moisture, ash, protein and total fat can be seen as described in the Danish and French composition tables.

Table 9: Mean value and variations in the four primary components of meat. Data are gathered from the Danish and French food composition tables of raw minced and grounded beef, and applies for 100 g edible food (*AFSSA* 2008; *DFCD* 2009).

Nutrient name	Denmark		France	
Proximate	**Mean value**	**Variation**	**Mean value**	**Variation**
Moisture (g)	65.5	60.0 - 71.0	65.9	42.0-77.7
Ash (g)	0.9	0.8 - 1.0	N/A	N/A
Protein (g)	19.3	18.0 - 21.0	18.7	16.1-22.7
Total Fat (g)	16	6.7 - 27.4	13.6	1.55-20.5

The lipid content and composition of meat is the most variable of the four primary components (Damodaran et al. 2008). The composition is influenced by both genetic and environmental factors. Some of the factors affecting fatness and fatty acid composition between and within breeds include: feed, species and breed, fatness, sex, age and/or live weight at slaughter. In addition differences in fat content and fatty acid composition between muscles must also be accounted for (De Smet et al. 2004).

According to Wood et al. (2008) and De Smet et al. (2004) the effect of diet and breed should always be judged against the amount of fat. Fat deposition is a highly heritable trait, and the fatty acid composition varies with fat content, independent of breed and dietary factors. When fatness increases in cattle, the SFA and MUFA level increase, while PUFA decreases. This affects the P/S ratio. Differences in fatty acid composition between the two major lipid fractions, and their relative contribution to total lipids in cattle, can explain the effect of fatness on the P/S ratio (De Smet et al. 2004).

The major lipid class in adipose tissue (>90%) is triacylglycerol or neutral lipid. In muscle, however, there are a significant proportion of phospholipids. The phospholipids have a much higher PUFA content than triacylglycerol, and long chained n-3 and n-6 are thus mainly found in the muscle (Warren et al. 2008; Wood et al. 2008).

Since phospholipids are an essential component of the cell membranes, the level is relative constant regardless of fat content and the PUFA content are also strictly controlled in order to maintain membrane properties. The amount of triglycerides on the other hand is strongly related to total fat content. So, if the fat content increases; the level of triglyceride will increase while the level of phospholipids is constant, hence the proportion of n-6 and n-3 will decrease. For example, lean animals have higher levels of

18:2n-6 and lower level of 18:1cis-9, but as body fat increases the neutral lipids will predominate the fatty acid composition, thus causing a decline in P/S ratio (Wood et al. 2008).

The effect of breed on lipid composition can also be influenced by the segregation of major genes (e.g double-muscled gene in cattle) (De Smet et al. 2004). Variation in fat level, live weight, age and production system can confound the contribution of genetics to observed variation. It is therefore difficult to assess if genetics or other factors are responsible for the observed variation (De Smet et al. 2004; Wood et al. 2008). Lipid composition can vary from muscle to muscle within a species, and because of this meat cuts can affect the fat composition (Damodaran et al. 2008).

According to Lawrie and Ledward (2006) males have generally less intramuscular fat than females. In addition, most parameters seems to increase (except water), with increased age of the animal. The level of intramuscular fat is observed to increase until and beyond 40 months of age (Lawrie & Ledward 2006). Variations seen in fatty acid composition in different minced meat samples in France are given in Table 10.

Table 10: Fatty acid variations in minced meat samples standardized for 15% fat in the French Food Composition table (*AFSSA* 2008), values applies for 100 g edible food.

Nutrient name	France	
Fats	**Mean value**	**Variation**
Sum of SFA (g)	5.88	5.25-6.9
C12:0 (g)	0.009	0.0085-0.01
C14:0 (g)	0.39	0-0.44
C16:0 (g)	3.25	2.87-3.61
C18:0 (g)	1.78	1.62-1.98
Sum of MUFA (g)	6.18	5.29-7.8
C18:1 (g)	4.65	1.07-5.55
Sum of PUFA (g)	0.538	0.33-0.7
C18:2 (g)	0.206	0.17-0.26
C20:4 (g)	0.026	N/A-0.13
C18:3 (g)	0.048	0.03-0.17
EPA (C20:5) (g)	0.003	0-N/A
Cholesterol (mg)	110	48-135

France has also included the variation for some vitamins and minerals, and the range of variations are presented in Table 11 and 12 below.

Table 11: Vitamin variations in minced meat samples standardized for 15% fat in the French Food Composition table (*AFSSA* 2008), and mean values and variations for beef, mince, raw with 16% fat from the Danish Food Composition Table (*DFCD* 2009). Values applies for 100 g edible food

Nutrient name	France		Denmark	
Vitamins	**Mean value**	**Variation**	**Mean value**	**Variation**
Retinol (µg)	11.7	0-20	12.4	7.10 - 17.0
Vitamin D (µg)	0.35	0.1-0.6	0.6	N/A
Vitamin E (mg)	0.415	0.2-0.65	0.40	0.120 - 0.870
Vitamin C (mg)	0.5	0-1	N/A	N/A
Thiamin (mg)	0.109	0.03-0.23	0.046	0.030-0.120
Riboflavin (mg)	0.203	0.08-0.39	0.155	0.128-0.180
Niacin (mg)	4.1	3.9-7.5	3.7	3.10-4.30
Pantothenic acid (mg)	0.54	0.47-0.6	0.31	0.120-0.470
Vitamin B_6 (mg)	0.202	0.18-4	0.235	0.160-0.330
Folic acid (µg)	5.35	2-6.9	9.72	4.00-16.0
Vitamin B_{12} (µg)	1.9	1.0-8	1.90	1.00-3.00

N/A = not available

Table 12: Mineral variations observed in minced meat samples standardized for 15% fat in the French Food Composition Table (*AFSSA* 2008), and mean values and variations for beef, mince, raw with 16% fat from the Danish Food Composition Table (*DFCD* 2009).

Nutrient name	France		Denmark	
Minerals	**Mean value**	**Variation**	**Mean value**	**Variation**
Calcium (mg)	10.2	3.0-15	N/A	N/A
Iron (mg)	2.58	1.4-3.6	2.1	1.60-2.50
Magnesium (mg)	15.3	13.6-26	18	16.0-19.0
Phosphorus (mg)	155	130-240	N/A	N/A
Potassium (mg)	226	161-440	N/A	N/A
Sodium (mg)	110	48-135	N/A	N/A
Zinc (mg)	4.82	2.4-6.1	4.2	2.70-5.50
Copper (mg)	0.09	0.05-0.15	N/A	N/A
Manganese (mg)	0.04	0.006-N/A	N/A	N/A
Selenium (µg)	6.06	3.0-51	N/A	N/A
Iodide (µg)	6.53	0.6-6.8	N/A	N/A

N/A = not available data

3 Materials

3.1 Laboratory equipment

3.1.1 Heme analysis

Chemicals	**Supplier**
Distilled water	-
Acetone for analysis	Emsure (KGaA 64271)
Hydrocholic acid 37%	Aldrich
Myoglobin from equine skeletal muscle (95-100%)	Sigma-Life

Instruments	**Supplier**
Plate reader – Synergy H4 hybrid reader	Biotek
96 well micro plate	Biotek
Scale	Sartorius
Blender	IKA A11 basic blender
Vortex – genie2	Scientific industries
Centrifuge	CT15RE VWR Himac
Pipette 20-200µl	Thermoscientific
Pipette 20-200 µl	Thermoscientific
Pipette 1-5ml	Thermoscientific

3.1.2 T-bars analysis

Chemicals	**Supplier**
Distilled water	-
Triachloracetic acid (TCA)	Merck KGaA
2-thiobarbituric acid (TBA)	Merck KGga
Hydrocholic acid 37%	Aldrich

Instruments	**Supplier**
Plate reader – Synergy H4 hybrid reader	Biotek
96 well micro plate	Biotek
Scale	Sartorius
Blender	IKA A11 basic blender
Magnetic stirrer	Heigar, RTC basic IKA labortechnic
Centrifuge	CT15RE VWR Himac
Water bath	Julabo TW20

3.1.3 DPPH

Chemicals	**Supplier**
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	Sigma-aldrich
Ethanol absolute (EtOH)	VWR international

Trolox

Instruments	Supplier
Plate reader – Synergy H4 hybrid reader	Biotek
96 well micro plate	Biotek
Scale	Sartorius
Blender	IKA A11 basic blender
Centrifuge	CT15RE VWR Himac
Magnetic stirrer	IKA (big-squid)
Pipette 1-10ML	Thermoscientific finnpipette
Pipette 20-200 µl	Thermoscientific finnpipette

3.1.4 Total PV

Chemicals	Supplier
Ringer's solution	Merck
Streptomycin	Sigma–Aldrich
Chloroform	Merck
Methanol	Merck
Butylated hydroxyl-toluene (BHT)	Alfa Aesar
Sulfuric acid (H_2SO_4)	Merck
Sorbitol	Sigma–Aldrich
Xylenol Orange (XO)	Alfa Aesar
Iron (II) sulphate ($FeSO_4$)	Merck
Sodium dithionite (DTT)	VWR Inc
Triphenylphosphine (TPP)	Alfa Aesar
Guanidine hydrochloride (GuHCL)	Sigma-Aldrich

Instruments	Supplier
Plate reader – Synergy H4 hybrid reader	Biotek
96 well micro plate	Biotek
Blender	IKA A11 basic blender
Centrifuge	CT15RE VWR Himac
Vortex – genie2	Scientific industries
Water bath	Julabo TW20

4 Methods

This thesis is part of the project "Identification of the healthiest beef meat". The four primary objectives of the project are to:

1. Establish a Norwegian knowledge platform for production of the healthiest beef meat
2. Ranking beef meat raw material with respect to healthiness
3. Investigate the health effects of beef meat using animal/in vitro models
4. Preform an intervention and animal feeding study.

A part of the objective "ranking beef meat raw material with respect to healthiness" is covered in this thesis. The nutritional composition of eighteen cattle of the breed Norwegian Red was analyzed in the form of standardized 14% minced meat. Minced meat was chosen as samples, since it represents a large part of the animal (40% in Norway). Analyses included all values found in the Norwegian food composition table (*Norwegian Food Composition Database* 2013) in addition to analyses of fatty acid composition, some special nutrients and oxidation indicators of the meat.

Eighteen animals, presumed to be more representative for Norwegian meat consumption than earlier studies on nutritional value, from eight different grazing regions in Norway were selected for the analysis based on an estimation of high producing regions and regions differing from each other in roughage feed. From all regions one cow and one bull were selected, except region 5 in Sogn og Fjordane where one cow and one young cow were achieved. One cow and one bull were selected because it reflects the meat intake in Norway as seen in Table 17. In addition, a difference in the nutritional value of meat based on variance in feed or feed composition was to be identified based on feed information gathered by questioners. Lastly, comparing 13 different countries minced meat composition based on the given values in their respective countries food composition tables were carried out.

I have participated in the recruiting process of Norwegian Red Cattle (NRC) producers, the collection of samples, and registration of animal information. I also analyzed the DPPH, TBARS, and hemin content, following training, and collected data from Food Composition Tables from other countries through literature search.

4.1 Applied database for estimation of possible sample regions

Animalia's database "Statistical overview of the classification of cattle in Norway, year 2012" (Røe 2013) was used to identify the largest cattle producing regions in Norway, the most commonly produced breeds in Norway and the most common age and sex categorys slaughtered in Norway. The database describes 287 238 animals that are assumed to be ~75% of the animals slaughtered for human consumption in Norway in 2012. The registrations in the database included county, municipality, age category, breed, weight and kg fat for all slaughtered animals.

4.2 The selected regions for sampling

The regions selected for meat sampling where selected based on two criteria's:

1. The region has a large cattle production – this criteria tries to ensure possible representative selection of all cattle meat in Norway. The cattle production/ region was identified from a database maintained by Animalia (Røe 2013).
2. Regions were selected based on assumed variation in roughage feed. This was done in cooperation with Yngve Rekdal at Norwegian Forest and Landscape Institute. 75% of the meat is assumed to originate from cattle fed on intensive fertilized grassland, while 25% originates from rough grazing.

Table 13 below defines the regions identified for sampling combined with arguments for identified region. The main focus related to identifying regions is based on quantity of production and the regions presented represents ~70% of the cattle raised in Norway.

Table 13: Selected regions to participate in the study and arguments for selected region. Yngve Rekdal provided arguments.

Counties selected	Regions selected	Argument for selected region
Rogaland	Region 1	High Intensive production. Fertilized grassland.
	Region 2	High intensive production. Fertilized grassland. Fjord landscape.
Møre og Romsdal	Region 3	Fertilized grassland. Coastal landscape.
	Region 4	Rough grazing . Valley landscape.
Sogn og Fjordane	Region 5	Rough grazing.
Oppland	Region 6	Forest landscape. Fertilized grassland. Some pasture in forest.
Nord-Trøndelag	Region 7	High intensive production. Rough grazing. Lowland landscape.
Nordland	Region 8	Rough grazing

When selecting regions within large scale producing counties the most important criteria were size of the production region. The largest producing region where chosen first. Thereafter, the second region should be selected based on its size, but also based on the presumption that it varies in feed from the first region selected. So, the second region within counties is not necessarily the second largest region in that county, but it is the largest region in the counties that in addition differs from the first region selected regarding roughage feed.

4.3 Recruiting producers of Norwegian Red Cattle

All the farms participating in this study was randomly selected in their region and recruited by telephone. The participants had to fulfill the following six inclusion criteria:

1. Produces Norwegian Red Cattle.
2. Delivers young bulls and cow directly from farm to slaughterhouse
3. Used locally produced roughages
4. Willing to inform project leaders when relevant animals are sent to slaughter
5. Give detailed information about the selected animals feed
6. Give access to forage analyses

4.4 Method for obtaining feed information

All farms participating in the study were sent a questioner by email and post. Questions included forage, silo feed, and additional supplements or feed. The full questioner can be seen in Appendix 1. Additional information given to the farmer can also be seen in Appendix 2.

4.5 Collection of meat samples

The animals were slaughtered at local slaughterhouses in accordance to normal procedures. Thereafter, the carcasses were removed from their normal production line, chilled and transported to Animalia's pilot plant for cutting and deboning in Oslo. To make the samples relatively representative 40% of the edible part of the carcass was cut to make "beef 14% samples" according to standard cutting patterns. Lastly the meat was transported to NMBU where it was divided into samples (see Appendix 3 for procedure) and frozen at -80°C degrees. Ten days was used from slaughtering to freezing.

4.6 Collecting data from Food Composition Databases

An overview of different countries food composition data of minced meat was collected. The minced meat compared had a fat level ranging from 13.6-17%, excluding Czech Republic, which have a fat content of 8%. All food composition tables in the following Table 14 was accessed through the European Food Information Resource (EuroFIR) database (*EuroFIR* 2014). The reason that such a broad fat range was used is due to the fact that there is no standardized fat percentage requested in any Food Composition Database.

Table 14: A complete list of all countries food composition tables accessed through the EuroFIR database (*EuroFIR* 2014) Countries included in the comparison, name and reference of database and search word used are given.

Country	Food composition database	Search word	Reference
France	The French food composition database (AFSSA)	Beef, ground, 15% fat, raw	(*AFSSA* 2008)
Canada	Canadian Nutrient Files	Beef, ground, medium, raw	(Government of Canada 2012)
Denmark	Danish Food Composition Databank (DFCD)	Beef, mince, raw	(*DFCD* 2009)
Slovakia	Slovak Food Composition Data Bank	Beef, minced	(*Compiled online food* 2008-2013)
Sweden	NFA Food Composition Database	Beef, Minced, Meat, Fat 15%, Raw	(*The National Food* 2014)
Iceland	ISGEM (The Icelandic Food Composition Database)	Beef, minced, hrátt	(*ISGEM* 2009)
Czech Republic	Czech Food Composition Database (CFCD)	Beef, Production meat, 8% fat, raw	(*CFCD* 2013)
Germany	Food Composition and Nutrition Tables	Minced meat	(Medpharm 2014)[1)]
Netherlands	NEVO	Minced beef raw	(*NEVO* 2013)[2)]
United Kingdom	Composition of Foods integrated dataset	Beef, mince, raw	(*Composition of Foods integrated dataset* 2002)

1)To access the German food composition table, it is necessary to register for a 10 days trial.
2) To access the data from NEVO the dataset must be requested from their web page.

The food composition table from USA was accessed through the United States Department of Agriculture (USDA). The Norwegian and Finnish food composition tables was accessed through NORFOODs (2014), the Nordic food database. Method of searching is seen in Table 15.

Table 15: A complete list of countries food composition tables accessed through the UDSA and NORFOODs database. Countries included in the comparison, name and reference of database and search word used are given.

Country	Food composition database	Search word	Reference
USA	United States Department of Agriculture	Beef, ground, 85% lean meat / 15% fat, raw	(United States Department of Agriculture 2014)
Finland	Finnish Food Composition Databank	Minced meat, beef 17 % fat	(*Fineli* 2013)
Norway	Norwegian Food Composition table	Beef, minced meat, max 14 % fat, raw	(*Norwegian Food Composition Database* 2013)

4.7 Analysis of proximate, lipids, minerals, fat soluble vitamins and water soluble vitamins

The analyze methods used to produce values for proximate, lipids, minerals, fat-soluble and water soluble vitamins is presented in Appendix 4.

4.8 Other analyses

4.8.1 Heme iron analysis

Myoglobin stock solutions were made dissolving 0.02, 0.04, 0.06 and 0.08 mg myoglobin pure (from equine skeletal muscle, 95-100%) in 10 ml distilled water. Standard myoglobin solution was made dissolving 0.155ml myoglobin stock solution in 0.233 ml distilled water, 1.55 ml acetone and 0.063 ml 37% HCL. Both myoglobin stock solution and standard myoglobin solution were measured spectrophotometric at 525nm using Synergy H4 hybrid reader with software version 2.03.1. Each myoglobin molecule carries one heme.

Meat samples was taken out from -80°C freezers and blended in an IKA A11 basic blender. An amount of 0.155 g meat was dissolved in 0.233 ml distilled water, 1.55 ml acetone, 0.063 ml 37% HCL for all samples. The samples were vortexed for 20 seconds

at room temperature, and then centrifuged at 1600 g for 10 minutes at 4°C. Then 200 µl of the supernatant was extracted and absorbance was measured at 407nm at 20°C using Synergy H4 hybrid reader with software version 2.03.1. All samples were performed in duplicates, and run against appropriate blank (in 0.233 ml distilled water, 1.55 ml acetone, 0.063 ml 37% HCL). The calculations of hemin concentrations can be seen in Appendix 5.

4.8.2 T-BARS

Frozen meat samples (-80°C) were broken up and homogenized by IKA A11 basic blender and 2 grams of meat were measured in 50 ml falcon tubes. The meat sample was put in a Julabo TW20 water bath for 50 minutes at 70°C. After heating, 10 ml TBA stock solution was added to each sample. For 500 ml stock solution 1.875 grams of TBA, 75g of TCA and 21.25ml 1 N HCL was mixed before adding distilled water so that the total volume was 500 ml. The stock solution was then put on a magnetic stirrer to solubilize for 20 minutes. The sample was put on water bath again at 99.9°C for 10 minutes. Thereafter the samples were rapidly cooled down in ice water for 20 minutes. 1.5 ml of each sample was transferred to Eppendorf tubes without transferring fat particles. The Eppendorf tubes were centrifuged at 16 000rpm at 4°C in 25 minutes. Then 200µl was transferred to micro plate 96/u-PP Eppendorf-plate and the absorbance at 532nm was measured. All samples were performed in duplicates and run against appropriate blank containing all the reagents minus the meat sample. The calculations mg/kg TBARS is given in Appendix 6.

4.8.3 DPPH

A DPPH stock solution of 0.25mg/ml was made by dissolving 0.025g of DPPH in 100ml EtOH at constant stirring over night at 4°C and kept in aluminum foil, away from light. A DPPH working solution (0.050mg/ml) was made from the stock solution. To make 10ml, 2 ml of stock is added to 8ml of EtOH. The working solutions absorbance at 515nm should be around 0.8 in absorbance, and were checked before samples were made.

The meat samples were taken out from -80°C freezer and homogenized by an IKA A11 basic blender. 0.5gram sample were weighed in 15ml falcon tubes, and added 4 ml of DPPH working solution. Samples were shaken vigorously by hand and kept in the dark, with aluminum foil, at room temperature for 50 minutes. Then samples was shaken

again and transferred to 2 ml micro tubes and centrifuged for 5 minutes at 35000rpm at 20°C in CT15RE Himac. Lastly 200µl of the supernatant were pipetted into a 96 well microplate and absorbance were measured at 515nm at 20°C. All samples were performed in triplicate and run against appropriate blank. The calculations of DPPH scavenging potential are given in Appendix 7.

4.8.4 Total PV

See Appendix 8: protocol for PV measurements

4.8.5 Statistics

Most of the analytical data was obtained late in the period allowed in the spring/June parallel education period. A simple statistical approach was used to evaluate if there were any true variation between animals. This approach was:

If 4x standard deviation of the analysis was larger than the difference between max and min value, the variation was regarded as significant. This will normally be a sufficiently strict criteria with probability below 0.005 for a one sided test and less than 0.05 in a 2 sided test. Standard deviations were calculated in EXCEL. It was used standard deviations from experimental data and not the predicted standard deviation from routine use of the methods (see Appendix 9 for Fødevarestyrelsen's data and more details).

Statistical comparison to data from other countries cannot be made due to the fact that the analysis are done in different laboratories, except for the Danish values and Norway 2014 (except vitamin K).

5 Results

5.1 Production of cattle in Norway

Total sum of slaughtered animals and number of animals slaughtered per breed in Norway in 2012 are presented in Table 16. Twenty-seven different breeds were registered for slaughter and Norwegian Red Cattle (NRC) is the main breed used for cattle production. NRC represents 75.4% of the total slaughtered animals, crossbreed is the second largest produced breed with 13.9% and Hereford represents 2.5% of Norwegians cattle production.

Table 16: Numbers of slaughtered animals per breed in Norway in 2012 and total number of slaughtered animals (Røe 2013).

Breeds	Number of animals	Breeds	Number of animals
Norwegian Red Cattle	216567	Telemarksfe	278
Crossbreeds	39847	Brown Swiss	168
Hereford	7292	Blonde d'Aquitaine	130
Charolaise	6112	Sør-og Vestlandsfe	108
Aberdeen Angus	3721	Raukolle	103
Limousine	2889	Dølafe	81
Holstein	2814	Jarlsbergsfe	57
Unknown	1774	Galloway	46
Jersey	1277	Dexter	43
S, Tr and Nordl*	1268	Dairy Simmental	27
Meat Simmental	1173	Piemontese	13
Highland	737	RDM	4
Vestlandsfe	414	Salers	2
Tiroler grauvieh	293	**Total animals**	**287238**

* Sidet Trønder- og Nordlandsfe

The division of different age and sex categories of the slaughtered animals in Norway in 2012 is displayed in Table 17 below. Young bulls are the most slaughtered animals in Norway, followed by cow and young cow. Young bull, bull and castrates make up 47.8% of the total slaughtered animals, and Cow, young cow and heifer make up 46.0% of the total. The amount of calf slaughtered is 6.3%.

Table 17: Cattle production in Norway in 2012 divided by category: young bull, cow, young cow, heifer, calf, bull and castrate (Røe 2013)

Age and sex category of cattle	Number
Young bull	126687
Cow	59078
Young cow	51987
Heifer	20981
Calf	18030
Bull	8700
Castrates	1775

5.2 Production places of cattle in Norway

The cattle production of different counties in Norway is displayed in Table 18. Rogaland, Oppland and Nord-Trøndelag are Norway's largest cattle producing counties, while Finnmark and Oslo have the smallest production.

Table 18: Production places in Norway divided by county and numbers of animals slaughtered per county (Røe 2013).

County	Number of animals
Rogaland	48 080
Oppland	40 012
Nord-Trøndelag	30 888
Sør-Trøndelag	26 476
Møre og Romsdal	24 857
Nordland	21 580
Hedmark	18 914
Sogn og Fjordane	18 453
Hordaland	13 352
Buskerud	8 021
Vest-Agder	6 664
Akershus	6 512
Østfold	5 767
Troms	4 609
Telemark	4 343
Vestfold	3 920
Aust-Agder	2 458
Finnmark	2 332
Oslo	0

5.3 Grazing, concentrate and roughage in Norway

The farmer's answers to the questioners about feed (Appendix 1) are given in this section, where questions about grazing, concentrate and roughage was given. Of the 18 questioners sent out to the producers, one for each animal, 13 questioners were returned.

5.3.1 Grazing

Results from the questioners about grazing are presented in Table 19. According to the questioners, six out of thirteen animals had been grazing outdoors during the last 20 weeks before slaughter. This included all the cows, except one in municipality 6. None of the young bulls had been grazing outside. The longest duration of pasture was 20 weeks, and the shortest was seven weeks. One animal was grazing until the day of slaughter: the young cow in region 1. Most of the farmers in this study used loose housing systems, instead of stanchion barns. Results are lacking for the cow and young bull in Rogaland, municipality 1a, and for the cow in municipality 2. Lastly, results from region 8 are also lacking.

Table 19: Results from questioner (Appendix 1) about grazing. Questions included barn type and how many weeks the animal had been on pasture the last 20 weeks before slaughter. Information about the slaughter date where given by the slaughterhouse.

Region	Municipality	Cow/ bull	Barn type	Pasture (weeks)*	Last day on pasture	Slaughter date
Rogaland	1a	Cow	N/A	N/A	N/A	13.05.13
Rogaland	1a	Young bull	N/A	N/A	N/A	13.05.13
Rogaland	1	Young bull	Loose housing system	0	-	27.09.13
Rogaland	1	Young cow	Stanchion barns	18	27.09.13	27.09.13
Rogaland	2	Young bull	Loose housing system	0	-	01.11.13
Rogaland	2	Cow	N/A	N/A	N/A	21.02.14
Møre og Romsdal	3	Young bull	Loose housing system	0	-	01.11.13
Møre og Romsdal	3	Cow	Stanchion barns	8	11.08.13	01.11.13
Møre og Romsdal	4	Young cow	Loose housing system	20	20.09.13	10.12.13
Møre og Romsdal	4	Young bull	Loose housing system	0	-	06.03.14
Sogn og Fjordane	5	Young cow	Stanchion barns	12	30.08.13	20.02.14
Sogn og Fjordane	5	Cow	Loose housing system	7	01.08.13	04.10.13
Oppland	6	Young bull	Loose housing system	0	-	20.03.14
Oppland	6	Young cow	Loose housing system	0	-	21.03.14
Nord-Trøndelag	7	Cow	Loose housing system	18	20.09.13	04.10.13
Nord-Trøndelag	7	Young bull	Loose housing system	0	-	04.10.13
Nordland	8	Young bull	N/A	N/A	N/A	N/A
Nordland	8	Young cow	N/A	N/A	N/A	N/A

*During the last 20 weeks before slaughter
N/A : Not available
- none

5.3.2 Concentrate

Results from the questioner about concentrate are presented in Table 20. The questioner had questions about amount of concentrate consumed per day, type and producer of feed. Results are lacking for one cow in municipality 2 and from region 8. The amount of concentrate given varies from 0.5 kg/day to 10+2 kg/day. The feed types

are coded, and their nutritional content will not be displayed in this project because of confidentiality agreement. The nutritional content of the feeds are used to see if they can explain some of the observed variance between the different samples of minced meat.

Table 20: Results from questioner (appendix 1) about concentrate. Questions included type and producer of feed and amount of given feed per day in the last 20 weeks before slaughter. The feed types are coded.

Region	Municipality	Cow/ bull	Amount (kg/d)*	Type
Rogaland	1a	Cow	5	Feed 9 + Feed 8 (6 weeks before slaughter)
Rogaland	1a	Young bull	2.5	Feed 2
Rogaland	1	Young bull	2	Feed 2
Rogaland	1	Young cow	5	Feed 5
Rogaland	2	Young bull	6	4 kg of feed 5 +5 kg of feed 3 +2 kg of feed 12
Rogaland	2	Cow	N/A	N/A
Møre og Romsdal	3	Young bull	2.5	Feed 3
Møre og Romsdal	3	Cow	3	Feed 4
Møre og Romsdal	4	Young cow	0.5	Feed 10
Møre og Romsdal	4	Young bull	4	Feed 10
Sogn og Fjordane	5	Young cow	8	Feed 11
Sogn og Fjordane	5	Cow	10+2	Feed 1 and protein feed
Oppland	6	Young bull	4	Feed 1
Oppland	6	Young cow	1	Feed 1
Nord-Trøndelag	7	Cow	4	3 kg of feed 7 + 1 kg of feed 4
Nord-Trøndelag	7	Young bull	4	Feed 3
Nordland	8	Young bull	N/A	N/A
Nordland	8	Young cow	N/A	N/A

*During the last 20 weeks before slaughter
N/A: not available

5.3.3 Roughage

Results from the questioner about roughage are presented in Table 21. The questioner included questions about amount of roughage consumed per day. Results are lacking for

the cow in municipality 2 and both animals in region 8. The amount of roughage is given in different measurement values. The cow and bull in region 4, the young bull in region 2 and the cow and young bull in region 1a had free access of roughage, while the young bull and young cow from region 1 had five and eight armfuls respectively. In region 5, 6 and 7 the amount is given in kg and the variation was 10-50 kg of roughage a day.

Table 21: Results from questioner (Appendix 1) about the amount of roughage eaten per day, the last 20 weeks before slaughter.

Region	Municipality	Cow/bull	Amount per day*
Rogaland	1a	Cow	Free access
Rogaland	1a	Young bull	Free access
Rogaland	1	Young bull	4 feed units
Rogaland	1	Young cow	4 feed units
Rogaland	2	Young bull	Free access
Rogaland	2	Cow	N/A
Møre og Romsdal	3	Young bull	5 armfuls
Møre og Romsdal	3	Cow	8 armfuls
Møre og Romsdal	4	Young cow	Free access
Møre og Romsdal	4	Young bull	Free access
Sogn og Fjordane	5	Young cow	40 kg
Sogn og Fjordane	5	Cow	25kg
Oppland	6	Young bull	20 kg
Oppland	6	Young cow	14 kg
Nord-Trøndelag	7	Cow	50 kg
Nord-Trøndelag	7	Young bull	N/A
Nordland	8	Young bull	N/A
Nordland	8	Young cow	N/A

*During the last 20 weeks before slaughter
N/A: not available

5.3.4 Other feed/feed supplements

The questioner about feed also asked about other feed/supplements given to the animals included in this study and the results are presented in Table 22. Questions about amount of potatoes, carrots, rutabaga, minerals, medication and others where given. Results are lacking for the cow in region 1a and for minerals and medication for the young bull in region 1a. Results are also lacking for the cow in municipality 2 and both animals in region 8.

The young bull from region 1a got 5 kg hay/d, while the young bull from region 1 got iron and whey supplements but the amount is unknown. The bull and cow from region 4 got multi supplements, while the young cow from region 5 got a mineral supplement.

The cow from region 5 got penicillin during the last 20 weeks before slaughter and the young bull and young cow from region 6 both got approximately 10 kg of Rutabaga per day.

Table 22: Results from questioner (Appendix 1) about other types of feed (potatoes, carrots, rutabaga, minerals, medications or other) given to the cattle, the last 20 weeks before slaughter.

Region	Munici-pality	Cow/bull	Other type of feed*	Minerals*	Medication*
Rogaland	1a	Cow	N/A	N/A	N/A
Rogaland	1a	Young bull	5 kg hay per/d	N/A	N/A
Rogaland	1	Young bull	Whey, iron	None	None
Rogaland	1	Young cow	None	None	None
Rogaland	2	Young bull	None	None	None
Rogaland	2	Cow	N/A	N/A	N/A
Møre og Romsdal	3	Young bull	None	None	None
Møre og Romsdal	3	Cow	None	None	None
Møre og Romsdal	4	Young cow	None	Multi supplement	None
Møre og Romsdal	4	Young bull	None	Multi supplement	None
Sogn og Fjordane	5	Young cow	None	Mineral for cattle	None
Sogn og Fjordane	5	Cow	None	None	Penicillin
Oppland	6	Young bull	Rutabaga (10kg)	Yes	None
Oppland	6	Young cow	Rutabaga (10kg)	Yes	None
Nord-Trøndelag	7	Cow	None	None	None
Nord-Trøndelag	7	Young bull	None	None	None
Nordland	8	Young bull	N/A	N/A	N/A
Nordland	8	Young cow	N/A	N/A	N/A

* During the last 20 weeks before slaughter
N/A: Not available

5.4 Nutritional value of minced meat in different countries

The following results show a comparison between thirteen different countries' nutritional composition table, displaying their official values for minced meat. Norway's official values from the food composition table are termed Norway 2005, and additionally, values for Norwegian minced meat analyzed in this project and presented in the same tables are termed Norway 2014. The tables are made based on collected

data from each countries different food composition table, and in some cases data are calculated. This will be specified in each table.

All countries in this comparison has an official value of fat content ranging from 13.1% to 17% fat, except from Czech Republic that has a fat content of 8%. Table 23-28 give values for proximate, lipids, minerals, fat-soluble vitamins, water-soluble vitamins and amino acids. The abbreviation N/A used in the tables means “not available” in the database used for searching, and Tr means traces.

5.4.1 Proximate

The nutritional proximate value for all thirteen countries and values from this project is presented in Table 23. Moisture content varies in the ranges of 62.0-71.7 g/100g, whereas Slovakia and United Kingdom had the lowest registered values, and Czech Republic the highest. The ash content is relatively similar for all countries, ranging from 0.8-1.0 g/100g. Variations in protein content ranges between 18.7-20.5 g/100g, with France displaying the lowest value of protein, and Germany the highest. Total fat content ranged from 8.0-17.0 g/100g, with Czech Republic containing the lowest value, followed by France who had a fat content of 13.6 g/100g. The minced meat from Finland contained the highest amount of fat. The carbohydrate content was given as a logical zero in almost all nutrition tables. The same was done for alcohol. France and Netherlands analyzed for carbohydrate content and found traces and 0.2 g/100g respectively. Variation seen in energy (KJ) content ranged from 636-952 KJ, with Czech Republic having the lowest and Finland the highest value.

Table 23: Proximate values for minced meat from 13 different countries and new results from Norwegian minced meat analyzed in this project, called "Norway 2014".

Nutrient name	Value per 100 g of edible portion of minced meat													
Proximate	France	Canada	Denmark	Slovakia	Sweden	Iceland	Netherlands	Czech Republic	USA	Germany	United Kingdom	Finland	Norway 2005	Norway 2014
Moisture (g)	65.9	64.3	65.5	62.0	66.4	65.8	65.1	71.7	65.7	64.2	62.0	N/A	66.0	67.1[1)]
Ash (g)	N/A	0.8	0.9	0.0	0.9	0.9	1.0	1.0	0.9	N/A	N/A	N/A	N/A	N/A
Protein (g)	18.7	18.9	19.3	19.7	19.4	19.0	18.9	20.0	18.6	20.5	19.7	19.0	18.8	18.5[2)]
Total Fat (g)	13.6	16.1	16.0	16.2	15.0	13.8	16.5	8.0	15.0	14.0	16.2	17.0	14.0	13.1[1)]
Carbohydrate (g)	Tr	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	N/A	0.0	0.0	0.0	N/A
Alcohol (g)	0.0	0.0	0.0	0.0	0.0	N/A	0.0	0.0	0.0	N/A	0.0	0.0	0.0	N/A
Energy (kcal)	199	226	N/A	223	205	N/A	225	152	215	208	225	N/A	201	179[3)]
Energy (kJ)	830	945	920	934	856	N/A	937	636	898	867	934	952	838	748[3)]

1) n = 16. Results from one young bull from region 1 and one young bull from region 8 were lacking.
2) n= 15. One outlier was taken out; the value recorded was biological impossible
3) Calculated based on protein and fat content
N/A – not available data.

Germany is excluded from the tables below because no other information than proximate was available for access in the EuroFir database.

5.4.2 Lipids

Lipid values for twelve countries and values from this project is presented in Table 24. All countries except from Slovakia gave values for sum of saturated fatty acid (SFA) content. Sum of SFA varied between the countries; from 4.060 g/100g in Czech Republic to 7.300 g/100g in Denmark. The official value for Norwegian minced meat was 5.800 g/100g, while Norwegian values from this project was 5.904 g/100g. Czech Republic had the highest value of SFA (50.8 %) as percentage of total fat while Finland had the lowest content of SFA with 38.2 %. Official values from Norway, Finland, Iceland, and Slovakia had no available data regarding individual SFA, while Denmark and Sweden had no available data on SFA C15:0 and C17:0. France had not available data on SFA C15:0, C17:0 and C20:0. Values for C4:0-C10:0 and DHA, and sum of TFA were not acquired in this project (Norwegian values 2014).

The most abundant SFA in all countries, where SFA data were available, was C16:0 (palmitic acid) followed by C18:0 (stearic acid). Variation between countries for C16:0

were 2.140-4.290 g/100g from Czech Republic and Denmark respectively. Variation in C18:0 ranged from 1.52g/100g in The Czech Republic to 2.48 in Denmark. Variations observed in SFA C14:0 (myristic acid) were 0.25-0.515g/100g and SFA C15:0 (pentadecylic acid) ranged from 0.040 g/100g to 0.624 g/100g. For both SFA (C14:0 and C15:0) Czech Republic had the lowest and Netherlands the highest content. For the SFA C17:0 (Margaric acid), Canada had the highest value of 0.220 g/100g and Czech Republic the lowest value of 0.090 g/100g.

The SFA C12:0 (lauric acid) and C20:0 (arachidic acid) were found in small amounts in all countries official data. Zero values were reported in Denmark and Sweden for both SFA. Variations in C12:0 were from 0.000-0.016 g/100g with the highest values from Netherlands. Values for C20:0 varied from 0.000 g/100g to 0.020 g/100g with Canada and United Kingdom reporting the highest values. The data for SFA C4:0-C9:0 was either not reported or not detected in any of the food composition tables. Canada, Netherlands, Czech Republic and United Kingdom had given values for SFA C10:0 (capric acid) in their food composition tables ranging from 0.010-0.312 g/100g.

Values for sum of MUFA were also available in all food composition tables except from Slovakia. Variation in MUFA content between countries ranged from 2.930 g/100g to 7.300 g/100g whereas Czech Republic had the lowest content and Denmark and Netherlands had the highest value. Presented in percentage of total fat, Check republic had the lowest value of 36.6% while Sweden had the highest percentage of MUFA: 46.0%. The official Norwegian values of MUFA were the second lowest after Czech Republic with minced meat samples containing 5.400 g/100g MUFA. The minced meat analyzed in this project had a total MUFA content of 5.720 g/100g. Further, Slovakia, Iceland, Finland and the official values from Norway had no available values for different monounsaturated fatty acids and France only had values for C18:1, while the Swedish food composition table only displayed values for C16:1 and C18:1. A values for C17:1 was only reported in this project, with a value of 0.078 g/100g.

The MUFA with highest abundance were C18:1 (oleic acid), with values ranging from 2.600 -6.440 g/100g, with Denmark displaying the highest value, and Czech Republic the lowest. MUFA C14:1 varied in the range of from 0 g/100g in United Kingdom to 0.165 in

Denmark. For C16:1 a variation of 0.28-0.752g/100g were given, with Czech Republic having the lowest content, and Canada the highest. The variation in MUFA C20:1 were 0 – 0.083g/100g, whereas United Kingdom had a value of zero and Denmark having the highest value.

The sum of PUFA was also given in all food composition tables except in the tables from Slovakia. Reported values varied within the range of 0.220-0.538g/100g between the countries, with Czech Republic having the lowest amount and France the highest. When sum of PUFA were calculated as percentage of total fat Iceland had the lowest value of 1.8% while Sweden had the highest value of 4.7%. The P:S ratio for all countries was calculated in this project and values ranged from 0.045 in Denmark and Iceland to 0.106 in Sweden.

The amount of n-6 PUFA varied from 0.170-0.429 g/100g between countries, with Iceland displaying the lowest value, and Norwegian values from this project the highest. The n-3 PUFA reported in minced meat from the different countries varied from 0.040 g/100g in Czech Republic to 0.300 g/100g in Sweden. The n-6:n-3 ratio was calculated in this project and displayed a variation from 1.0 in Sweden to 4.5 in Czech republic.

The n-6 PUFA 18:2 was seen in the highest amount in all countries ranging from 0.170 g/100g in Czech Republic to 0.400 g/100g in Sweden. Variations for C20:4 were in the range of 0.000-0.038 g/100g whereas Sweden and United Kingdom reported zero content of C20:4, and Canada the highest. The n-6 PUFA C20:2 was only analyzed in this project (Norway 2014) and no other countries had reported values for this PUFA in their respective food composition tables. Values for Norway 2012 were 0.014 g/100g.

The n-3 PUFA C18:3 where reported in highest amount in all countries with variations from 0.04 g/100g in Czech Republic to 0.100 g/100g in Sweden. EPA, DPA and DHA were all reported in small quantities or at zero values in most countries. EPA ranged from 0.000 – 0.015 g/100g, were Canada, Denmark, Sweden, Netherlands and United Kingdom had zero values, and Norwegian values from 2014 the highest content. Only USA and Norway 2014 detected DPA levels in the meat with values of 0.012 and 0.019 g/100g respectively. Only USA detected values of DHA with a content of 0.001 g/100g.

The amount of C20:3 was only analyzed in this project for minced meat, with value of 0.017 g/100g. Variations in TFA content were reported from 0.030 g/100g in Czech Republic to 0.935 g/100g in USA. When TFA was calculated to percentage of total fat Czech Republic had the lowest TFA value of 0.4% while USA had the highest with 6.2%.

Cholesterol level varied from 51.7 mg/100g to 68.0 mg/100g. Finland had the minced meat with lowest cholesterol levels, while USA had the highest values. In Canada and Finland the amount of plant sterol were included in the food composition tables. Zero mg/100g were given in Canada, while 0.8 mg/100g where reported in Finland. In United Kingdom the amount of phytosterols were given at a value of 0.4 mg/100g. Stigmasterol values were given in the Canadian and the United Kingdoms food composition tables, both with a content of 0 mg/100g.

Table 24: Lipid values for minced meat from 12 different countries and new results for Norwegian minced meat analyzed in this project, called "Norway 2014".

Nutrient name	Value per 100 g of edible portion of minced meat						
Lipids	**France**	**Canada**	**Denmark**	**Slovakia**	**Sweden**	**Iceland**	**Netherlands**
Total Fat (g)	13.6	16.1	16.0	16.2	15.0	13.8	16.5
Sum of SFA (g)	**5.880**	**6.670**	**7.300**	**N/A**	**6.600**	**5.570**	**7.000**
Sum of SFA (%)	**43.2**	**41.4**	**45.6**	**N/A**	**44.0**	**40.4**	**42.4**
C4:0-C10:0[1)] (g)	0.000	0.030	0.000	N/A	0.000	N/A	0.312
C12:0 (g)	0.009	0.010	0.000	N/A	0.000	N/A	0.016
C14:0 (g)	0.390	0.465	0.495	N/A	0.400	N/A	0.515
C15:0 (g)	N/A	0.090	N/A	N/A	N/A	N/A	0.624
C16:0 (g)	3.250	3.648	4.290	N/A	3.500	N/A	3.947
C17:0 (g)	N/A	0.220	N/A	N/A	N/A	N/A	0.156
C18:0 (g)	1.780	2.188	2.480	N/A	2.000	N/A	2.184
C19:0 (g)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C20:0 (g)	N/A	0.020	0.000	N/A	0.000	N/A	0.016
Sum of MUFA (g)	**6.180**	**7.120**	**7.300**	**N/A**	**6.900**	**6.150**	**7.300**
Sum of MUFA (%)	**45.4**	**44.2**	**45.6**	**N/A**	**46.0**	**44.6**	**44.2**
C14:1 (g)	N/A	0.170	0.165	N/A	N/A	N/A	0.156
C16:1 (g)	N/A	0.752	0.660	N/A	0.600	N/A	0.748
C17:1 (g)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C18:1 (g)[2)]	4.650	5.988	6.440	N/A	5.900	N/A	6.380

C20:1 (g)	N/A	0.030	0.083	N/A	N/A	N/A	0.031
Sum of PUFA (g)	**0.538**	**0.430**	**0.331**	**N/A**	**0.700**	**0.250**	**0.400**
Sum of PUFA (%)	**4.0**	**2.7**	**2.1**	**N/A**	**4.7**	**1.8**	**2.4**
Sum of n-6 PUFA (g)	N/A	0.285	0.248	N/A	0.300	0.170	0.300
C18:2 (g)[3)]	0.206	0.295	0.248	N/A	0.400	0.160	0.300
C20:2 (g)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C20:4 (g)	0.026	0.038	N/A	N/A	0.000	N/A	0.016
Sum of n-3 PUFA (g)	N/A	0.065	0.083	N/A	0.300	0.090	0.100
C18:3 (g)	0.048	0.065	0.083	N/A	0.100	0.070	0.070
C20:3 (g)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
EPA (C20:5) (g)	0.003	0.000	0.000	N/A	0.000	0.010	0.000
DPA (C22:5) (g)	N/A	0.000	0.000	N/A	0.000	N/A	0.000
DHA (C22:6) (g)	0.000	0.000	0.000	N/A	0.000	N/A	0.000
n-6:n-3 ratio	**N/A**	**4.4**	**3.0**	**N/A**	**1.0**	**1.9**	**3.0**
P:S ratio	**0.091**	**0.064**	**0.045**	**N/A**	**0.106**	**0.045**	**0.057**
Sum of TFA (g)	**N/A**	**0.662**	**0.556**	**N/A**	**0.500**	**0.370**	**0.500**
Sum of TFA (%)	**N/A**	**4.1**	**3.5**	**N/A**	**3.3**	**2.7**	**3.0**
Cholesterol (mg)	67.0	60.0	67.0	60.0	61.7	63.0	52.9
Plant sterol (mg)	N/A	0.0	N/A	N/A	N/A	N/A	N/A
Phytosterol (mg)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Stigmasterol (mg)	N/A	0.0	N/A	N/A	N/A	N/A	N/A

1) Values only describing fatty acid C10
2) Number describe different ways of measuring C18:1: France (n-9 cis, oleic), Canada (not specified), Denmark (n-9: 6.440 g and cis n-7: 0 g), Sweden (not specified), Netherlands (Cis + total), Czech Republic (18:1 n-9: 2,60 g, 18:1 n-9 trans: 0,03) ,USA (undifferentiated), United Kingdom (5.690 value = Cis C18:1, whereof 5.44 were cis/trans C18:1n-9), Norway 2014 (value describes trans + n-9 + n-7)
3) Number describe different ways of measuring C18:2: France (9c, 12c n-6 linoleic), Canada (18:2 = 0.294 g and 18:2 n-6 = 0.247), Denmark (n-6), Sweden (not specified), Iceland (c9,12), Netherlands (C18:2 n-6 – cis), Czech republic (n-6), Finland (cis n-6), USA (undifferentiated), United Kingdom (2.30 g/100g is the value for C18:2, whereof 2.27 g/100g are cis n-6 C18:2) Norway 2014 (value describes: C18:2 + CLA + C18:2 trans).
4) Calculated in this project by using the formula: ((Sum of SFA (or MUFA , PUFA or TFA))/total fat)*100
N/A: not available

Table 24 continued: Lipid values for minced meat from 12 different countries and new results for Norwegian minced meat analyzed in this project, called "Norway 2014".

Nutrient name	Value per 100 g of edible portion of minced meat					
Lipids	**Czech Republic**	**USA**	**United Kingdom**	**Finland**	**Norway 2005**	**Norway 2014**
Total Fat (g)	8.0	15.0	16.2	17.0	14.0	13.1
Sum of SFA (g)	**4.060**	**5.870**	**6.940**	**6.500**	**5.800**	**5.904**
Sum of SFA (%)[4)]	**50.8**	**39.1**	**42.8**	**38.2**	**41.4**	**45.1**
C4:0-C10:0[1)] (g)	0.010	0.000	0.010	N/A	N/A	N/A
C12:0 (g)	0.010	0.011	0.010	N/A	N/A	0.013
C14:0 (g)	0.250	0.443	0.460	N/A	N/A	0.341

C15:0 (g)	0.040	0.073	0.100	N/A	N/A	0.062
C16:0 (g)	2.140	3.290	3.720	N/A	N/A	3.170
C17:0 (g)	0.090	0.169	0.170	N/A	N/A	0.117
C18:0 (g)	1.520	1.875	2.460	N/A	N/A	2.059
C19:0 (g)	N/A	N/A	N/A	N/A	N/A	0.019
C20:0 (g)	0.010	0.014	0.020	N/A	N/A	0.019
Sum of MUFA (g)	**2.930**	**6.555**	**6.940**	**7.000**	**5.400**	**5.720**
Sum of MUFA (%)[4)]	**36.6**	**43.7**	**42.8**	**41.2**	**38.6**	**43.7**
C14:1 (g)	0.040	0.116	N/A	N/A	N/A	0.104
C16:1 (g)	0.280	0.534	0.610	N/A	N/A	0.468
C17:1 (g)	N/A	N/A	N/A	N/A	N/A	0.078
C18:1 (g)[2)]	2.600	5.751	5.690	N/A	N/A	5.043
C20:1 (g)	0.010	0.049	0.000	N/A	N/A	0.026
Sum of PUFA (g)	**0.220**	**0.432**	**0.480**	**0.400**	**0.300**	**0.535**
Sum of PUFA (%)[4)]	**2.8**	**2.9**	**3.0**	**2.4**	**2.1**	**4.1**
Sum of n-6 PUFA (g)	0.180	N/A	0.360	0.300	N/A	0.429
C18:2 (g)[3)]	0.170	0.342	0.180	0.213	N/A	0.384
C20:2 (g)	N/A	N/A	N/A	N/A	N/A	0.014
C20:4 (g)	0.010	0.036	0.000	N/A	N/A	0.032
Sum of n-3 PUFA (g)	0.040	N/A	0.120	0.100	N/A	0.106
C18:3 (g)	0.040	0.054	0.090	0.043	N/A	0.055
C20:3 (g)	N/A	N/A	N/A	N/A	N/A	0.017
EPA (C20:5) (g)	N/A	0.002	0.000	0.002	N/A	0.015
DPA (C22:5) (g)	N/A	0.012	0.000	N/A	N/A	0.019
DHA (C22:6) (g)	N/A	0.001	0.000	0.000	N/A	N/A
n-6:n-3 ratio	**4.5**	**N/A**	**3.0**	**3.0**	**N/A**	**4.0**
P:S ratio	**0.054**	**0.074**	**0.069**	**0.062**	**0.052**	**0.091**
Sum of TFA (g)	**0.030**	**0.935**	**0.810**	**0.400**	**0.300**	**N/A**
Sum of TFA (%)[4)]	**0.4**	**6.2**	**5.0**	**2.4**	**2.1**	**N/A**
Cholesterol (mg)	N/A	68.0	60.0	51.7	60.0	59.2
Plant sterol (mg)	N/A	N/A	N/A	0.8	N/A	N/A
Phytosterol (mg)	N/A	N/A	0.4	N/A	N/A	N/A
Stigmasterol (mg)	N/A	N/A	0.0	N/A	N/A	N/A

1) Values only describing fatty acid C10
2) Number describe different ways of measuring C18:1: France (n-9 cis, oleic), Canada (not specified), Denmark (n-9: 6.440 g and cis n-7: 0 g), Sweden (not specified), Netherlands (Cis + total), Czech Republic (18:1 n-9: 2,60 g, 18:1 n-9 trans: 0,03) ,USA (undifferentiated), United Kingdom (5.690 value = Cis C18:1, whereof 5.44 were cis/trans C18:1n-9), Norway 2014 (value describes trans + n-9 + n-7)
3) Number describe different ways of measuring C18:2: France (9c, 12c n-6 linoleic), Canada (18:2 = 0.294 g and 18:2 n-6 = 0.247), Denmark (n-6), Sweden (not specified), Iceland (c9,12), Netherlands (C18:2 n-6 – cis), Czech republic (n-6), Finland (cis n-6), USA (undifferentiated), United Kingdom (2.30 g/100g is the value for C18:2, whereof 2.27 g/100g are cis n-6 C18:2) Norway 2014 (value describes: C18:2 + CLA + C18:2 trans).
4) Calculated in this project by using the formula: ((Sum of SFA (or MUFA , PUFA or TFA))/total fat)*100
N/A: not available

5.4.3 Minerals

Mineral values for twelve countries and values from this project are presented in Table 25. The content of calcium ranged from 4.0 mg/100g in Czech Republic to 15 mg/100g

in USA. Variation in iron content between countries ranged from 1.40 mg/100g in Slovakia and United Kingdom to 2.58 mg/100g in France. Only the Dutch food composition and the project results from Norway had values of heme iron, with values of 19.9 mg/100g and 13.2 mg/100g respectively. The magnesium content varied from 15.3 to 23.0 mg/100g, with France displaying the lowest value, and Czech Republic the highest. Phosphorous ranged from 146 mg/100g in Finland to 191 mg/100g in Czech Republic. Potassium levels varied from 226 mg/100g in France to 370 mg/100g in Iceland.

Sodium levels had a variation from 56 mg/100g in Finland to 360 mg/100g in the Norwegian official values, however, the Norwegian official values are sampled from minced meat with added salt (NaCl) thus not representing the natural sodium value of minced meat. The second largest sodium level was reported in the French food composition table with 110 mg/100g. In Slovakia and Finland, the amount of sodium chloride was also given with values of 0.20 g/100g and 0.14 g/100g respectively. The amount of zinc varied from 3.6 mg/100g in Finland to 4.96 mg/100g in the Norwegian minced meat from this project.. Amount of copper varied from 0.15 mg/100g in Iceland to traces in United Kingdom. Manganese content varied from 0.04 mg/100g in France to traces in United Kingdom. The selenium content varied from 5.3 µg/100g in Sweden to 15.8 µg/100g in USA. The amount of iodine varied from 1µg/100g in Norway's official values to 9 µg/100g in Slovakia and United Kingdom.

Denmark was the only country displaying levels for nickel with a content of 0.7 µg/100g, while Iceland included levels of lead, arsenic and fluoride. Values were 2.8 µg/100g for lead, 0.3 µg/100g for mercury and 1.5 µg/100g for arsenic. USA included fluoride levels, whereas a value of 22.4 µg/100g was given and lastly United Kingdom had values for chloride, with a content of 76 mg/100g.

Table 25: Mineral values for minced meat from 12 different countries and new results for Norwegian minced meat analyzed in this project, called "Norway 2014".

Nutrient name	Value per 100 g of edible portion of minced meat												
Minerals	**France**	**Canada**	**Denmark**	**Slovakia**	**Sweden**	**Iceland**	**Netherlands**	**Czech Republic**	**USA**	**United Kingdom**	**Finland**	**Norway 2005**	**Norway 2014**
Calcium (mg)	10.2	10.0	N/A	9.0	7.0	8.6	8.0	4.0	15.0	9.0	8.1	8.0	7.6
Iron (mg)	2.58	1.88	2.10	1.40	1.94	1.71	2.00	2.20	2.09	1.40	2.10	1.80	2.30
Heme iron (mg)	N/A	N/A	N/A	N/A	N/A	N/A	19.9[2)]	N/A	N/A	N/A	N/A	N/A	13.2
Nonheme iron (mg)	N/A	N/A	N/A	N/A	N/A	N/A	0.2	N/A	N/A	N/A	N/A	N/A	N/A
Magnesium (mg)	15.3	19.0	18.0	17.0	20.0	20.0	20.0	23.0	18.0	17.0	17.6	19.0	19.2
Phosphorus (mg)	155	156	N/A	160	160	200	167	191	171	160	146	160	161
Potassium (mg)	226	267	N/A	260	280	370	313	274	295	260	262	290	308
Sodium (mg)	110	59	N/A	80	83	60	110	65	66	80	56	360	69
Sodium chloride (g)	N/A	N/A	N/A	0.20	N/A	N/A	N/A	N/A	N/A	N/A	0.14[1)]	N/A	N/A
Zinc (mg)	4.82	4.00	4.20	3.90	4.91	4.52	4.33	4.70	4.48	3.90	3.60	3.80	4.96
Copper (mg)	0.09	0.09	N/A	N/A	N/A	0.15	0.06	N/A	0.07	Tr	N/A	0.05	N/A
Manganese (mg)	0.04	0.02	N/A	N/A	N/A	0.02	N/A	N/A	0.01	Tr	N/A	N/A	<0.05
Selenium (µg)	6.1	15.7	N/A	N/A	5.3	3.8	8.0	N/A	15.8	7.0	15.2	7.0	8.0
Iodide (µg)	6.5	N/A	N/A	9.0	3.0	1.5	2.5	N/A	N/A	9.0	3.0	1.0	2.0
Nickel (µg)	N/A	N/A	0.7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Lead (µg)	N/A	N/A	N/A	N/A	N/A	2.8	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mercury (µg)	N/A	N/A	N/A	N/A	N/A	0.3	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Arsenic (µg)	N/A	N/A	N/A	N/A	N/A	1.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fluoride (µg)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	22.4	N/A	N/A	N/A	N/A
Chloride (mg)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	76	N/A	N/A	N/A

1. Termed "salt" in the in the Finnish food composition table
2. Value is given as 1.6 mg/100g in the Dutch food composition table. This value was, however extremely low, and was most likely describing how much iron 100 mg of heme contained. So by dividing 1.6mg on 0.0806 (which are the percentage of weight from hemin) the hemin content were calculated.
Tr = Traces
N/A: Not available

5.4.4 Fat soluble vitamins

Fat soluble vitamin values for twelve countries and values from this project is presented in Table 26. The amount of retinol equivalents varied from 0 to 20 RE between countries, with Netherlands having the highest values and Canada and USA had zero content. The amount of retinol was higher than beta-carotene in France and Czech Republic, while the relationship was reversed in Sweden, Iceland, Netherlands and Norway. Retinol content varied from 0.0 µg/100g to 16.0 µg/100g, where Denmark had the highest value and Canada and the USA had a value of 0.0 µg/100g. Variations in beta-

carotene content ranged from 0.0 µg/100g in France, Canada, Czech Republic, and USA to 38.6 µg/100g in the Norwegian minced meat analyzed in this project.

Variations in vitamin D content ranged from 0.1 µg/100g in Netherlands and USA to 0.7 µg/100g in United Kingdom. Vitamin E content ranged from 0.25mg/100g in Czech Republic to 0.50mg/100g in Netherlands, Finland and the official values from Norway. Lastly the level of vitamin K where only given in five countries, results from Norway 2014 displaying the highest level of 9.3 µg/100g, Sweden with a level of 8.0 µg/100g, Finland with 1.8 µg/100g and lastly, Canada and USA, both with values of 1.8 µg/100g.

Table 26: Fat soluble vitamins values for minced meat from 12 different countries and new results for Norwegian minced meat analyzed in this project, called "Norway 2014".

Nutrient name	Value per 100 g of edible portion of minced meat												
Fat soluble vitamins	**France**	**Canada**	**Denmark**	**Slovakia**	**Sweden**	**Iceland**	**Netherlands**	**Czech Republic**	**USA**	**United Kingdom**	**Finland**	**Norway 2005**	**Norway 2014**
Retinolequivalents (RE)	N/A	0	12.4	N/A	13.0	6.8	20.0	N/A	0.0	Tr	18.6	8.0	N/A
Retinol (µg)	11.7	0.0	12.4	N/A	11.0	4.0	16.0	6.0	0.0	Tr	N/A	6.0	11.0
Beta-carotene (µg)	0	0	N/A	N/A	28	34	27	0	0	Tr	22[1)]	21	38.6
Vitamin D (µg)	0.4	0.1	0.6	0.5	0.2	0.5	0.1	N/A	0.1	0.7	0.2	0.2	N/A
Vitamin E (mg) (*α-toc)*	0.42	N/A	0.40	0.17	0.70	0.23	0.50	0.25	0.37	0.17	0.50	0.50	0.43
β-tocopherol (µg)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	N/A	N/A	N/A	<10
γ-tocopherol (µg)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	N/A	N/A	N/A	17.7
δ-tocopherol (µg)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	N/A	N/A	N/A	<10
Vitamin K (µg)[2)]	N/A	1.3	N/A	N/A	8.0	N/A	N/A	N/A	1.3	N/A	1.8	N/A	9.6

1) Termed "total carotenoids" in Finnish food composition table.
2) Values termed vitamin K in food composition tables measure different types of K vitamins. Canada: not specified. Sweden: Vitamin K is calculated as the sum of K2 and K3. USA: value is vitamin K1. Finland: value is "total" vitamin K. Norway 2014: sum of vitamin K_1 and K_2.
N/A: not available

5.4.5 Water soluble vitamins

Water soluble vitamin values for twelve countries and values from this project is presented in Table 27. The vitamin C content is mostly 0.00 mg/100g for most countries, except from France and Netherlands where values of respectively 0.5 mg/100g and 8.00 mg/100g are given. Thiamin levels varied from 0.03 mg/100g in the official value from Norway and the Netherlands, to 0.15 mg/100g in Iceland. Riboflavin levels varied from 0.24 mg/100g in Iceland to 0.13 mg/100g in Slovakia, Netherlands, United Kingdom, Finland and the values for Norway 2014. Niacin equivalents (NE) ranged from 10.00 to

3.70, whereas Iceland had the highest content and Denmark the lowest. Niacin levels where highest in Iceland with a content of 6.20 mg/100g and lowest in Netherlands with 3.50 mg/100g.

Pantothenic acid varied from 0.31 mg/100g in Denmark to 0.61 mg/100g in Canada. The content of vitamin B_6 varied from 0.20-0.42mg/100g, whereas France had the lowest value, and Finland displayed the highest value. The level of biotin was only given in Denmark and United Kingdom, values were 1.3 µg/100g and 1.0 µg/100g, respectively. Amount of folic acid ranged from 3.00 µg/100g in Norway and Sweden to 14.00 µg/100g in United Kingdom. Vitamin B_{12} levels varied from 1.0-3.0 µg/100g. Both values belong to Norway, and the lowest value is from the official value in the food composition table, while the highest value is the result from this project. USA was the only country including total choline and betaine in the food composition table. Values for total choline were 61.2 mg/100g, and 7.2 mg/100g for betaine.

Table 27: Water soluble vitamins values for minced meat from 12 different countries and new results for Norwegian minced meat analyzed in this project, called "Norway 2014".

Nutrient name	Value per 100 g of edible portion of minced meat									
Water soluble vitamins	**France**	**Canada**	**Denmark**	**Slovakia**	**Sweden**	**Iceland**	**Netherlands**	**Czech Republic**	**USA**	**United Kingdom**
Vitamin C (mg)	0.50	0.00	N/A	N/A	0.00	0.00	8.00	0.00	0.00	0.00
Thiamin (mg)	0.11	0.12	0.05	0.06	0.06	0.15	0.03	0.04	0.04	0.06
Riboflavin (mg)	0.20	0.20	0.16	0.13	0.15	0.24	0.13	0.16	0.15	0.13
Niacin (mg)	4.10	4.93	3.70	5.80	4.40	6.20	3.50	4.60	4.65	5.80
Niacin equivalents (NE)	N/A	8.81	3.70	5.80	7.90	10.00	N/A	N/A	N/A	9.40
Pantothenic acid (mg)	0.54	0.61	0.31	0.49	N/A	N/A	N/A	N/A	0.55	0.49
Vitamin B_6 (mg)	0.20	0.22	0.24	0.37	0.26	0.32	0.27	0.31	0.35	0.37
Pyridoxal (mg)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pyridoxine (mg)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pyridoxamine (mg)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Biotin (µg)	N/A	N/A	1.3	N/A	N/A	N/A	N/A	N/A	N/A	1.0
Folic acid (µg)	5.35	6.00	9.72	14.00	3.00	7.30	5.60	N/A	6.00	14.00
Vitamin B_{12} (µg)	1.90	1.75	1.90	2.00	1.57	1.40	1.90	N/A	2.17	2.00
Choline, total (mg)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	61.2	N/A
Betaine (mg)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	7.20	N/A

1) Folic analysis were not finished by the time of submitting this project, however, the numbers for folic analysis will be present in later stages of the project.
N/A: Not available

Table 27 continued: Water soluble vitamins values for minced meat from 12 different countries and new results for Norwegian minced meat analyzed in this project, called "Norway 2014".

Nutrient name	Value per 100 g of edible portion of minced meat		
Water soluble vitamins	**Finland**	**Norway 2005**	**Norway 2014**
Vitamin C (mg)	0.00	0.00	N/A
Thiamin (mg)	0.08	0.03	0.05
Riboflavin (mg)	0.13	0.15	0.13
Niacin (mg)	5.10	4.20	4.12
Niacinequivalents (NE)	9.2	N/A	N/A
Pantothenic acid (mg)	N/A	N/A	N/A
Vitamin B_6 (mg)	0.42	0.26	0.30
Pyridoxal (mg)	N/A	N/A	0.18
Pyridoxine (mg)	N/A	N/A	0.01
Pyridoxamine (mg)	N/A	N/A	0.13
Biotin (µg)	N/A	N/A	N/A
Folic acid (µg)	5.00	3.00	N/A[1)]
Vitamin B_{12} (µg)	1.40	1.00	3.00
Choline, total (mg)	N/A	N/A	N/A
Betaine (mg)	N/A	N/A	N/A

1) Folic analysis were not finished by the time of submitting this project, however, the numbers for folic analysis will be present in later stages of the project.
N/A: not available

5.4.6 Amino Acids

The data on amount of amino acids were only available in the Canada, Czech Republic and USA food composition tables and values are given in Table 28. In all three countries minced meat contains all the essential amino acids.

Table 28: Amino acid values of minced meat from Canada, Czech Republic and USA.

Nutrient name	Value per 100 g of edible portion of minced meat		
Amino Acids	**Canada**	**Czech Republic**	**USA**
Tryptophan* (g)	0.233	N/A	0.096
Threonine* (g)	0.792	1.016	0.721
Isoleucine* (g)	0.810	1.055	0.821
Leucine* (g)	1.515	1.819	1.450
Lysine* (g)	1.578	1.179	1.541
Methionine* (g)	0.441	0.608	0.479
Cystine (g)	0.182	0.352	0.192
Phenylalanine* (g)	0.717	0.913	0.724
Tyrosine (g)	0.590	0.876	0.573
Valine* (g)	0.916	1.107	0.913
Arginine* (g)	1.276	1.412	1.207
Histidine* (g)	0.602	0.812	0.605
Alanine (g)	1.234	1.290	1.161
Aspartic acid (g)	1.727	2.054	1.675
Glutamic acid (g)	2.972	3.421	2.790
Glycine (g)	1.403	1.000	1.251
Proline (g)	0.958	0.814	0.941
Serine (g)	0.732	0.867	0.743
Hydroxyproline (g)	N/A	N/A	0.356

*Essential amino acids in mammals

5.5 External analysis and variation

5.5.1 Proximate variation

The amount of collagen, fat, proteins and water in the minced meat from Norway 2014 were analyzed at Animalia's pilot plant for cutting and deboning in Oslo. The average value and max-min values were calculated from 16 animals and the values for Norwegian minced meat are presented in Table 29. The content of fat varied from 9.5 g/100g to 13.1 g/100g, the content of protein varied from 18.5 g/100g to 20.3 g/100g, collagen varied from 2.3 g/100g to 2.9 g/100g and lastly, the water content varied from 64.2 g/100g to 70.0 g/100g.

Table 29: Average, min and max values for proximate values in Norwegian minced meat. Average values is calculated from n=16 samples, except from protein where one outlier was excluded and n=15.

Nutrient name	Value per 100 g of edible portion of minced meat		
Proximate	**Average**	**Max**	**Min**
Collagen (g)	2.5	2.9	2.3
Fat (g)	13.1	15.7	9.5[1)]
Protein (g)	19.3	20.3	18.5
Water (g)	67.1	70.0	64.2

1) The low fat content is not due to analyze errors, but higher amount of fat were not obtained in the minced meat because of one animal with low fat content.

5.5.2 Fatty acids variation

The fatty acid analyzes were performed at Fødevarestyrelsen in Denmark and results are presented in Table 30. Fatty acid content was calculated from mg/100g of fatty acids to g/100g of edible food as seen in Appendix 10. The average, max and min value were calculated for all analysis, and the results for fatty acids can be seen in Table 30 below. To see which results really had a true variance, the criteria explained in Appendix 11 were used. According to that estimation the fatty acids: C14:0, C16:0, C18:0, C16:1, C18:1trans, C18:1n-7, C18:1 n-9, C18:2trans, C18:2, CLA, C20:4, C18:3 and cholesterol had a true variance: i.e the values from the two most extreme animals were different.

Table 30: Average, min, max and st.dev value from the fatty acid analysis of Norwegian minced meat. All analysis where done in parallels. The values are analyzed at Fødevarestyrelsen in Denmark. All fatty acids marked with a star (*) have true variance according to the criteria explained in Appendix 11.

Nutrient name	Value per 100 g of edible portion of minced meat			
Fatty acids	**Average**	**Max**	**Min**	**St.dev**
Sum of SFA (g)	**5.800**	**7.150**	**4.796**	
C12:0 (g)	0.013	0.014	0.011	0.016
C14:0 (g)*	0.341	0.466	0.251	0.018
C15:0 (g)	0.062	0.099	0.042	0.018
C16:0 (g)*	3.170	3.508	2.821	0.007
C17:0 (g)	0.117	0.155	0.084	0.122
C18:0 (g)*	2.059	2.834	1.561	0.061
C19:0 (g)	0.019	0.042	0.013	0.009
C20:0 (g)	0.019	0.032	0.013	0.011
Sum of MUFA (g)	**5.720**	**6.985**	**4.618**	
C14:1 n-7 (g)	0.104	0.181	0.039	0.169
C16:1 (g)*	0.468	0.710	0.312	0.027
C17:1 (g)	0.078	0.105	0.052	0.157
C18:1trans (g)*	0.374	0.638	0.190	0.021
C18:1 n-9 (g)*	4.500	5.069	3.908	0.009
C18:1 n-7 (g)*	0.169	0.243	0.100	0.025
C20:1 (g)	0.026	0.039	0.017	0.022

Sum of PUFA (g)	**0.535**	**0.742**	**0.355**	
Sum of n-6 PUFA (g)	*0.429*	*0.608*	*0.280*	
C18:2trans (g)*	0.119	0.166	0.085	0.005
C18:2 (g)*	0.212	0.280	0.137	0.007
CLA (g)*	0.053	0.099	0.025	0.007
C20:2 (g)	0.014	0.015	0.013	0.016
C20:4 (g)*	0.032	0.049	0.019	0.001
Sum of n-3 PUFA (g)	*0.106*	*0.134*	*0.076*	
C18:3 (g)*	0.055	0.067	0.036	0.006
C20:3 Homo (g)	0.017	0.026	0.013	0.010
EPA (C20:5 n-3) (g)	0.015	0.016	0.014	0.001
DPA (C22:5) (g)	0.019	0.026	0.012	0.011
Cholesterol (mg)*	59.20	81.10	52.00	4.21

5.5.3 Mineral variation

The mineral analyzes were performed at Fødevarestyrelsen in Denmark and the average, max and min value and standard deviation were calculated for all analyzes. The results can be seen in Table 31 below. All samples had a variation between them according to the criteria explained in Appendix 11, except for manganese were no such evaluation was possible. The nutrient reference value (NRV) is also given for each mineral, and the average value of the different nutrients in Norwegian minced meat were used to calculate % of NRV. Zinc has the highest % of NRV followed by iron.

Table 31: Average, min, max and st.dev value for the mineral analysis of Norwegian minced meat. All analysis where done in parallels and analyzed at Fødevarestyrelsen in Denmark. The nutrient reference value (NRV) for minerals and calculated % of NRV is also presented. All minerals marked with a star (*) have true variance according to the criteria explained in Appendix 11.

Nutrient name	**Value per 100 g of edible portion of minced meat**					
Minerals	**Average**	**Max**	**Min**	**St.dev**	**NRV**	**% of NRV**
Iron (mg) *	2.30	3.06	1.39	0.076	14	16.4
Manganese (mg)	<0.05	<0.05	<0.05	0.000	2	--
Zinc (mg) *	4.96	6.50	3.90	0.082	10	49.6
Sodium (mg) *	69	84	59	1.046	N/A	--
Calcium (mg)*	7.6	13.5	4.7	0.684	800	1.0
Magnesium (mg) *	19.2	22.1	16.7	0.382	375	5.1
Phosphorus (mg) *	161	184	142	3.510	700	23
Potassium (mg) *	308	343	272	3.985	N/A	15.4
Selenium (µg) *	8	12.5	4.7	0.377	55	14.5
Iodine (µg) *	2.0	4.0	0.8	0.000	150	1.3

-- calculation of % NRV was not possible

5.5.4 Fat soluble vitamin variation

The fat soluble vitamin analysis were performed at Fødevarestyrelsen in Denmark and the average, max and min value and standard deviation were calculated for all analysis. The results for fat-soluble vitamins can be seen in Table 32 below. All fat-soluble vitamins have a real variation according to the principle explained in Appendix 11, except β-tocopherol and δ-tocopherol where this could not be calculated. The nutrient reference value (NRV) is also given for each fat soluble vitamin, and the average value of the different nutrients in Norwegian minced meat were used to calculate % of NRV. Vitamin K has the highest % of NRV value.

Table 32: Average, min and max value for the fat-soluble vitamin analysis of Norwegian minced meat. All analysis where done in parallels and analyzed at Fødevarestyrelsen in Denmark. The nutrient reference value (NRV) for fat soluble vitamins and calculated % of NRV is also presented. All fat soluble vitamins marked with a star (*) have true variance according to the criteria explained in Appendix 11.

Nutrient name	Value per 100 g of edible portion of minced meat					
Fat soluble vitamins	**Average**	**Max**	**Min**	**St.dev**	**NRV**	**% of NRV**
Retinol (µg) *	11.0	16.8	5.6	0.000	800	1.4
β–carotene (µg) *	38.6	98.0	13.3	0.000	N/A	--
a-tocopherol (mg) *	0.433	0.980	0.163	0.009	12	3.6
b-tocopherol (µg)	-	-	-	0.000	N/A	--
γ-tocopherol (µg) *	17.7	30.7	10.6	0.361	N/A	--
δ-tocopherol (µg)	-	-	-	0.000	N/A	--
Vitamin K_1 (µg)*[1)]	3.9	7.2	1.4	0.178	75	12.8
Vitamin K_2 (µg)*[1)]	5.7	11.4	1.7	0.170		

- : Content is less than 10 µg per 100 g.
-- : calculations of % of NRV was not possible
1) The method for vitamin K_1 and K_2 analysis is not accredited.

5.5.5 Water soluble analysis – variation

The water-soluble vitamin analysis were performed at Fødevarestyrelsen in Denmark and the average, max and min value and standard deviation were calculated for all analysis. The results can be seen in Table 33 below. All water-soluble vitamins had a true variation based on the criterion given in Appendix 11. The nutrient reference value (NRV) is also given for each water soluble vitamin, and the average value of the different nutrients in Norwegian minced meat were used to calculate % of NRV. Vitamin B_{12} and Niacin had the highest % of NRV values.

Table 33: Average, min and max value for the water-soluble vitamin analysis of Norwegian minced meat. All analysis where done in parallels. The values were analyzed at Fødevarestyrellsen in Denmark. The nutrient reference value (NRV) for water soluble vitamins and calculated % of NRV is also presented. All water soluble vitamins marked with a star (*) have true variance according to the criteria explained in Appendix 11.

Nutrient name	Value per 100 g of edible portion of minced meat					
Water soluble vitamins	**Average**	**Max**	**Min**	**St.dev**	**NRV**	**% of NRV**
Thiamin (mg) *	0.05	0.07	0.04	0.001	1.1	4.6
Riboflvin (mg) *	0.13	0.155	0.108	0.002	1.4	9.3
Vitamin B_6 (mg) *	0.3	0.34	0.26	0.004	1.4	21.4
*Pyridoxal (mg) **	0.18	0.24	0.13	0.005	N/A	--
*Pyridoxine (mg) **	0.01	0.03	0.01	0.001	N/A	--
*Pyridoxamine (mg) **	0.13	0.17	0.09	0.002	N/A	--
Niacin (mg) *	4.12	4.90	3.20	0.204	16	25.8
Vitamin B_{12} (µg) *	3.00	4.60	1.90	0.151	2.5	120

-- calculation of % NRV was not possible

5.6 Other analysis

5.6.1 Heme

The concentration of myoglobin in the meat samples was calculated based on the equation of the standard curve from the standard myoglobin solution as seen in Appendix 5. Results are shown in Table 34. The average apparent myoglobin content for all samples were 3.21 mg/ml. Variations between samples ranged from 1.90-5.41 mg/ml. The highest amount of myoglobin observed came from a cow in Sogn og Fjordane. According to the criterion used in this thesis (Appendix 11) all the samples show a true variation between the extreme samples. Expressed as hemin content the average value was 13.2 mg/100g.

Table 34: Myoglobin content (mg/ml) and hemin content (g/100g) in all minced meat samples based on calculations from the standard curve of myoglobin solution. All values are the average of duplicates.

Region	Municipality	Cow/ bull	Myoglobin concentration (mg/ml)	Hemin (mg/100g)
Rogaland	1a	Cow	2.96	12.2
Rogaland	1a	Young bull	1.90	7.8
Rogaland	1	Young bull	3.90	15.9
Rogaland	1	Young cow	4.58	18.9
Rogaland	2	Young bull	2.25	9.3
Rogaland	2	Cow	2.73	11.2
Møre og Romsdal	3	Young bull	4.46	18.4
Møre og Romsdal	3	Cow	3.01	12.4
Møre og Romsdal	4	Young cow	3.52	14.5
Møre og Romsdal	4	Young bull	2.18	9.0
Sogn og Fjordane	5	Young cow	4.30	17.7
Sogn og Fjordane	5	Cow	5.41	22.3
Oppland	6	Young bull	2.54	10.5
Oppland	6	Young cow	2.61	10.8
Nord-Trøndelag	7	Cow	2.12	8.7
Nord-Trøndelag	7	Young bull	4.40	18.1
Nordland	8	Young bull	2.61	10.8
Nordland	8	Young cow	2.28	9.4

5.6.2 TBARS

The TBA stock solution with added meat sample was measured using a spectrophotometric at 532nm. From the absorption the level of malonaldehyde in 2 grams of sample were calculated according to an extinction coefficient of: 1.56 x 105 M-1cm-1 as seen in Appendix 6. The results are given in Table 35. The mean value of malondialdehyde equivalents was 0.194 mg/kg, with a range of 0.037-0.576 mg/kg.

Dividing results of TBARS between sexes, the average value for young bull were 0.295 mg/kg while cow/young cow had an average value of 0.114 mg/kg.

Two samples, one young bull from municipality 1 and one young bull from municipality 3, had a maldondialdehyde equivalents level above 0.5 mg/kg and was above the threshold levels for rancid taste (Raharjo & Sofos 1993). All other samples where below the detection limit. According to calculations with standard deviation (Appendix 11) the more extreme samples were different.

Table 35: Calculated malonaldehyde in 2 grams of meat sample. Calculations were done from absorbance at 532 nm, according to the extinction coefficient of: 1.56 x 105 M-1cm-1, and further calculated to mg/kg.

Region	Municipality	Cow/bull	Calculated TBARS (mg/kg)
Rogaland	1a	Cow	0.237
Rogaland	1a	Young bull	0.518*
Rogaland	1	Young bull	0.258
Rogaland	1	Young cow	0.216
Rogaland	2	Young bull	0.125
Rogaland	2	Cow	0.088
Møre og Romsdal	3	Young bull	0.576*
Møre og Romsdal	3	Cow	0.132
Møre og Romsdal	4	Young cow	0.087
Møre og Romsdal	4	Young bull	0.384
Sogn og Fjordane	5	Young cow	0.096
Sogn og Fjordane	5	Cow	0.054
Oppland	6	Young bull	0.231
Oppland	6	Young cow	0.147
Nord-Trøndelag	7	Cow	0.045
Nord-Trøndelag	7	Young bull	0.191
Nordland	8	Young bull	0.073
Nordland	8	Young cow	0.037

* Has a value above 0.5 mg maldondialdehyde equivalents.

5.6.3 DPPH

The DPPH working solution with added meat sample was measured spectrophotometrically at 515 nm. Thereafter the DPPH-scavenging % was calculated using the equation seen in Appendix 7. The results are given in Table 36 below. The DPPH-scavenging percentage had a mean of 71.9% and samples varied between 67.3-75.9%. According to calculation based on the principle seen in Appendix 11, the more extreme samples were different.

Table 36: Percent DPPH scavenging potential for all minced meat samples.

Region	Municipality	Cow/bull	DPPH-scavenging (%)
Rogaland	1a	Cow	72.4
Rogaland	1a	Young bull	70.2
Rogaland	1	Young bull	70.5
Rogaland	1	Young cow	74.6
Rogaland	2	Young bull	68.5
Rogaland	2	Cow	74.2
Møre og Romsdal	3	Young bull	72.8
Møre og Romsdal	3	Cow	74.6
Møre og Romsdal	4	Young cow	67.3
Møre og Romsdal	4	Young bull	68.4
Sogn og Fjordane	5	Young cow	68.3
Sogn og Fjordane	5	Cow	72.4
Oppland	6	Young bull	75.9
Oppland	6	Young cow	68.8
Nord-Trøndelag	7	Cow	74.9
Nord-Trøndelag	7	Young bull	72.2
Nordland	8	Young bull	74.7
Nordland	8	Young cow	74.2

5.6.4 Total peroxide value (PV)

The lower (unipolar peroxides), upper (polar peroxides) and inter phase (protein bound peroxides) were all measured spectrophotometrically at 590 nm. The results presented in Table 37 below are the total PV value (mmol/kg) for all phases. In Appendix 10 the mean values and St. Deviations for all phases can bee seen. According to calculation based on the principle seen in Appendix 11 there are significant differences between min and max values. The differences are between the minced meats polar and protein bound peroxides, as seen in Appendix 12.

The mean value of PV were 0.740 mmol/kg, and values ranged from 0.481 to 1.172 mmol/kg, of which the young bull from region 1 had the highest total PV and the cow from region 3 had the lowest total PV value.

Table 37: Total peroxide value (PV) for all minced meat samples.

Region	Municipality	Cow/bull	Total PV (mmol/kg)
Rogaland	1a	Cow	0.956
Rogaland	1a	Young bull	0.718
Rogaland	1	Young bull	1.172
Rogaland	1	Young cow	0.892
Rogaland	2	Young bull	0.603
Rogaland	2	Cow	0.575
Møre og Romsdal	3	Young bull	0.624
Møre og Romsdal	3	Cow	0.481
Møre og Romsdal	4	Young cow	0.907
Møre og Romsdal	4	Young bull	0.729
Sogn og Fjordane	5	Young cow	0.706
Sogn og Fjordane	5	Cow	0.817
Oppland	6	Young bull	0.805
Oppland	6	Young cow	0.685
Nord-Trøndelag	7	Cow	0.967
Nord-Trøndelag	7	Young bull	0.536
Nordland	8	Young bull	0.619
Nordland	8	Young cow	0.529

6 Discussion

This project is the largest systematic collecting of beef meat, regarding nutrient content, ever been done in Norway. The pool of animals selected for this study is presumed to be more representative for the larger share of beef meat produced in the whole country compared to other projects, and the collection of animals is done based on the production scale of counties and difference in roughage regions in Norway, in addition to reflect the typical beef meat consumers can buy in the chops both regarding sex and age of the animal. Based on these collected animals/carcasses new and updated information about the nutrition quality of minced meat has been obtained. Since it is believed that age, sex and feed are large variables and factors that can affect the nutritional value of beef a variation is expected to be seen between the samples.

This is however, only the first set of result from the larger study “healthier beef meat”. In later stages more animals will be included, so the average results will be more accurate, and more information about extreme values will hopefully be obtained. More specific questions regarding roughage can be gathered and when the number of animals

increases, statistical tests will be performed to reveal what factors may affect the nutritional value of minced meat.

The collected feeding data are too limited to perform valid comparisons between factors that can affect nutritional value and the differences between nutritional content of Norwegian minced meat. However, it appears that feeding may vary in Norway based on the 18 animal collected so far.

Nevertheless, this thesis presents updated values for all components found in the official Norwegian Food Composition tables, except values for: copper, vitamin D, retinol equivalents, vitamin C and folic acid. Vitamin D and folic acid analysis is a part of the project and these analytical data will come later. In addition, for the first time analyses for fatty acid profile, manganese, b-tocopherols, γ-tocopherol, δ-tocopherol, vitamin K_1, vitamin K_2, niacin, pyroxidal, pyridoxaine, pyridoxamine and collagen value are presented. The variations seen between animal samples are given, which are information not been published in the official Norwegian Food Composition table. This is valuable information as many products are declared with an average and for some variables random sampling from shops is sometimes used and large deviations from declared values are possible when the biological variation is not considered.

6.1 Variation in nutrient content in meat from different Norwegian Red Cattles

6.1.1 Fatty acids

For the fatty acids analyzed in this project there is a 95% probability that the variation seen for: C14:0 (0.251-0.466 g/100g.), C16:0 (2.821-3.508 g/100g), C18:0 (1.561-2.834 g/100g), C16:1 (0.321-0.710 g/100g), C18:1trans (0.190-0.638 g/100g), C18:1n-7 (0.100-0.243), C18:1 n-9 (3.908-5.069 g/100g), C18:2trans (0.085-0.166 g/100g) , C18:2 (0.127-0.280 g/100g), CLA (0.025-0.099 g/100g.), C18:3 (0.036-0.067 g/100g), C20:4 (0.032-0.049 g/100g) and cholesterol (59.2-81.2 mg) represents true variance. Official values for variations registered for fatty acids are limited and only found in the French food composition table where the variance for C14:0 (0.000-0.440 g/100g), C16:0 (2.87-3.61 g/100g) and C18:0 (1.620-1.980 g/100g) are listed (French et al. 2000).

These results indicate that there is a difference in the fatty acid content between different animals in Norway. The variance of the fatty acid C18:0 seems to be bigger in Norway than in France, while the variation of C14:0 are larger in France than in Norway. The variation seen for C16:0 is quite similar for both countries. The latter may be an indication that variations observed in Norway actually are quite possible, even though it may seem large.

Differences between fatty acid content and composition in animals are both influenced by genetic and environmental factors (Damodaran et al. 2008). According to Lawrie and Ledward (2006) and Kerry and Ledward (2009) the total amount of body fat increases with increasing age of the animal. When the fatness increase the P:S ratio will also be affected because the SFA and MUFA values tend to increase, and PUFA decreases (De Smet et al. 2004). This happens because the phospholipid fraction of the fat, which contains most of the PUFA, are relatively constant regardless of fat level, thus: the level of PUFA decreases (Wood et al. 2008). Also many animals become fatter (more total fat) with age. In addition, males seems to have less intramuscular fat than females (Lawrie & Ledward 2006). Feeding will also affect the composition and an increased intake of concentrate and decreased intake of pasture has shown to increase SFA levels (French et al. 2000).

No causality between the observed variance and factors like feed, sex or age is possible to obtain in this project, since the dataset is still small, and more elaborate statistical analysis was waived. However, some observations might be of interest to further investigate in a larger dataset. That applies to the MUFAs C16:1, C18:1trans and C18:1n-7. For MUFA C16:1 seven animals had a value above average of 0.468 g/100g. Of these seven animals, six were cows/young cows, and one was a young bull. The same were the case for MUFA C18:1n-7; of the seven animals above average value (0.169g/100g), six were either the cows or the one young cow while one was a young bull. For MUFA C18:1trans the relationship were reversed. Of the seven animals above average value (0.374), six where young bulls, and one was the young cow. Later, when data from more animals are available it would be interesting to look at the content of these fatty acids compared to sex and grazing versus concentrate feeding.

6.1.2 Minerals

For minerals analyzed in this project there was a 95% probability that the variation in extreme values seen for iron (1.39-3.06 mg/100g), zinc (3.90-6.50 mg/100g), sodium (59-84 mg/100g), calcium (4.7-13.5mg/100g), magnesium (16.7-22.1 mg/100g), phosphorus (142-184 mg/100g), potassium (272-343 mg/100g), selenium (4.7-12.5 µg/100g) and iodine (0.8-4.0 µg/100g) are representing real variation between the minced meat samples. Thus, mineral values vary between different animals in Norway. Variations of mineral values are also given in the French Food Composition table, with variances for iron (1.4-3.6 mg/100g), zinc (2.4-6.1 mg/100g), sodium (48-135 mg/100g), calcium (3.0-15 mg/100), magnesium (13.6-26 mg/100g), phosphorous (130-240 mg/100g), potassium (161-440 mg/100g), selenium (3.0-51 µg/100g) and iodine (0.6-6.8 µg/100g) (*AFSSA* 2008). The Danish food composition table also contains variance for iron (1.66-2.50 mg/100g), zinc (2.70-5.40 mg/100g) and magnesium (16.0-19.0 mg/100g) (*DFCD* 2009).

The variances in the French Food Composition table for sodium, magnesium, phosphorous, potassium, selenium and iodine seem to be larger than the variances found for the same minerals in this study. However, because only one breed is included in this study the effect of breed on variation has been excluded. How many different breeds that are included in the French food composition tables is not known. According to the French food composition table, the variability in nutritional composition of food is due to many factors: "animal species and plant cultivars, geographic origin, breeding conditions, raw materials, industrial and home recipes and formulas, food processes, storage and preparation of food etc" (French et al. 2000). Variations for iron, zinc and magnesium registered in the Danish Food Composition table are all smaller than the variation found in the Norwegian samples.

Why the variation seen in the Danish minced meat are smaller than Norwegian samples are not known either. It may be due to less variations between pasture regions since Denmark is a smaller country that both France and Norway. Another theory may be that there is a difference in the sample collection interval done for minced meat. In this project a period of 10 months was used to collect samples, thus a large variation in feed used for cattle were included. However, neither the collection interval, nor the variation

between pasture regions in Denmark is known. According to the Danish National Food Institute variations seen in vitamin and mineral fraction are in direct relationship with variance seen in proximate fractions (*DFCD* 2009). According to the report making the bases for iron, zinc and magnesium values in the Danish Food Composition table for minced meat, the 20 samples collected to analyze these minerals had a fat content variation of 16-20% (Fødevaredirektoratet 2000) while in this project a variation from 9.5 to 15.7% of fat were used in the analyzes. Maybe the larger variation in fat content in this project is reason for the larger variation in iron, zinc and magnesium values.

The three animals with highest content of iron in this project were all cows. According to Lawrie and Ledward (2006) iron content increases with the age of the animals so maybe a significant trend of higher iron content of cows versus young cows and bulls will be evident in later stages of the study. Myoglobin levels can also vary between different anatomical locations in cattle (Lawrie & Ledward 2006). This can, however, not explain the variation seen in the Norwegian dataset, since all animals have been cut by the same standard procedure. Lastly, the amount of added iron in concentrate feed can also affect the amount of iron found in minced meat (Lawrie & Ledward 2006). Later in this project when a larger dataset is obtained and the response rate from farmers, regarding feed are higher, there may be a possibility to se a relationship between iron in meat and iron in concentrate feed.

The average values presented for sodium in this thesis (69 mg/100g) varies a lot from the official data of minced meat that has a sodium content of 360 mg/100g. The high amount of sodium in the Norwegian official values is due to the addition of salt in the minced meat as sold to consumer. Thus, no value for natural sodium content in Norwegian minced meat was available before this project. The five animals with highest sodium content were all young bulls, so a difference between sexes can be looked at in later stages of this project.

The variation in minced meat selenium content seen in both Norway and France may be due to different concentrations and availability of selenium in soil, and to different supplementation practice to the feed concentrates (Hartikainen 2005; Aasen 1997). Dietary selenium also seems to affect the concentration of muscle values when provided

as organically bound selenium. However, there seems to be a long supplementing period before effects are seen in the meat (Kerry & Ledward 2009). Since the selenium content in beef varies based on soil content and availability, there would be interesting to look for trends in selenium content in relation to pasture region when the dataset increases.

Despite the large variation seen for zinc value, no trends were evident in this dataset regarding sex, age are feeding. This relationship should be looked at when the dataset increases later in the project.

6.1.3 Vitamins

For fat soluble vitamins analyzed in this project there is a 95% probability that the variation seen for retinol (5.6-16.8 µg/100g), β–carotene (13.3-98.0 µg/100g), α-tocopherol (0.163-0.980 mg), γ-tocopherol (17.7-30.7 µg/100g), vitamin K_1 (1.4-7.2 µg/100g) and vitamin K_2 (0.170-1.700 µg/100g) was a true variation. In the case for ß-tocopherol and δ-tocopherol true variation was not calculated since these variables had no reported value. Variances for fat soluble vitamins listed in the French food composition table are: retinol (0.0-20.0 µg/100g) and α -tocopherol (0.200-0.650 mg/100g) while the Danish food composition table had the following variance for vitamin E: 0.120-0.870 mg/100g. The variation in retinol content was larger in the French Food Composition Table than in the Norwegian, while the Norwegian value for α-tocopherol varied more than the French and Danish values.

Improving the vitamin E level in beef cattle (mainly α-tocopherol) has been done for a long time by supplementing the feed to improve antioxidation stability (Kerry & Ledward 2009). How much vitamin E is incorporated into the tissue seems to be affected by amount and duration of supplementation (Kerry & Ledward 2009). In addition it seems like pasture fed beef contains more α-tocopherols, carotenoids and flavonoids. In the study by Mercier et al. (2004) beef cattle that were finished on pasture had a higher vitamin E content, and a lower lipid oxidation when compared to concentrate fed cattle. It also seems that feeding cattle with 2500 IU/day have the same effect on tissue concentrations as grass feeding (Kerry & Ledward 2009). In this project information aboute pasture the last 20 weeks before slaughtered where gathered to see if there were a trend between pasturetime and levels of, amoung other vitamin E, in the

meat. If any of these factors affects the nutritional composition of minced meat in this project is still not possible to say.

For water soluble vitamins true variance were seen for: thiamin (0.04-0.07 mg/100g), riboflavin (0.108-0.155 mg/100g), vitamin B_6 (0.26-0.34 mg/100g), pyridoxal (0.18-0.24 mg/100g), pydridoxine (0.01-0.03 mg/100g), pyridoxamine (0.13-0.17 mg/100g), niacin (4.12-4.90m mg/100g) and vitamin B_{12} (3.00-4.60 mg/100g). In the French food composition table variance levels are given for: thiamin (0.03-0.23 mg/100g), riboflavin (0.08-0.30 mg/100g), vitamin B_6 (0.18-4.0 mg/100g), niacin (3.9 – 7.5 g/100g) and vitamin B_{12} (1.0-8.0 mg/100g). Variances are also found in the Danish food composition table with values for: thiamin (0.030-0.120 mg/100g), riboflavin (0.128-0.180 mg/100g.), vitamin B_6 (0.160-0.330 mg/100g), niacin (3.10-4.30 mg/100g) and vitamin B_{12} (1.00-3.00 mg/100g).

With exception of the different forms of vitamin B_6 both Denmark and France have given variations for all the vitamins analyzed in this project. For all water soluble vitamins the largest variations were found in the French Food Composition Table. The Danish and Norwegian variations were most alike, except from the vitamin B_{12} value of Norwegian minced meat, where the lowest registered value in this project were the highest value registered in the Danish Food Composition Table.

According to Damodaran et al. (2008) the content of water-soluble vitamins are strongly affected by: species, age, sex and nutritional status of the animal. Large variations are seen especially for vitamin B_{12} and vitamin B_6 in this study. The reason for this variance cannot be found in this project by now.

6.2 For which nutrients are Norwegian minced meat a good source?

In the next part of this chapter the definition for the expression “good source” are set according to the criteria’s for allowed nutrient content claim usage. Nutrient content claims can be put on a food if it meets certain criterias as described in the norwegian regulation on “nutrition and health claims made on foods” (Helse- og omsorgsdepartementet 2012) and in Livsmedelsföretagen et al. (2012). The average value of the different nutrients in Norwegian minced meat obtained in this project are used to calculate which of the nutrients that are fulfilling the nutrition claim criterias.

The regulation accepts the calculations to be based on either the content of a certain nutrient per 100 grams or per serving of the food. The calculations in this thesis are based on 100 g minced meat.

For solid foods minimum 10 E% of the food needs to come from proteins to be classified as a "source of protein". When the amount of protein constitute 20 E% the classification "rich source of protein" can be used (Helse- og omsorgsdepartementet 2012). The Norwegian minced meat contains 18.2 g/100g which correspons to 41.3 E%. Norwegian minced meat can therefore claim to be a "rich source of proteins". Meat is the most important single source of protein in Norway (Helsedirektoratet 2012). To have a diet containing a high amount of protein gives the body important building blocks in the form of amino acids (Damodaran et al. 2008). In addition, proteins give high satiety (Pereira & Vicente 2013), which is important in a time where an increasing percentage of the population are struggling with overweight (Hånes et al. 2012).

For a food to be classified as "a source" of vitamins and minerals it need to contain 15% of the nutrient recommendation value (NRV) per 100 g. To be classified as a "rich source of" a percentage of at least 30% is needed per 100 g (Helse- og omsorgsdepartementet 2012). Based on theese criterias, minced meat from Norway can claim to be a source of iron (16.4%) , phousphorus (23%), potassium (15.4%), niacin (25.75%) and vitamin B_6 (21.4%) according to average nutritional contents found for these minerals in this project. In addition, Norwegian minced meat can be claimed to be a rich source of zinc (49.6%) and vitamin B_{12} (120%).

These classification and nutrient claims are made based on the average content of each nutrient. However, when meat is ingested it rarely contains the average value of nutrients listed in the food composition tables. The nutrient content will differ from each pack of minced meat that is consumed. But, when eaten over time the nutrient content will get closer to the average value of nutrient, if representative sampling for analysis is made. For some nutrients, such as iron where different recommendations are given for women and men (Helsedirektoratet 2014), and some part of the population suffers from iron deficiencies (WHO 1993-2005) and inadequate intake (Helsedirektoratet 2012) a

sorting of minced meat based on iron levels could be a way to offer meat with higher levels of iron to special groups.

This hypothetical sorting of minced meat would enable a certain amount of minced meat to contribute to a bigger part of the NRV. To see how much potential there is in the Norwegian market, some calculations of NRV have been done on the maximum amount reported of some nutrients in the minced meat of this project.

For example, in this study there is a large difference between the amounts of vitamin B_{12} in animals. On average, 100g of minced meat from Norway contains a lot of vitamin B_{12}, it actually covers 120% of the nutrient recommendation value, and 150% of the daily-recommended intake of 2 µg/d (Helsedirektoratet 2014). However, if minced meat were sorted based on maximum and minimal values of vitamin B_{12} levels a 100 g of minced meat would either contain 4.60 µg of B_{12} (184% of the NRV), or 1.90 µg (76% of NRV).

There is also a big difference between the official value of minced meat where vitamin B_{12} has a value of 1 µg, and in this project were the value are 3 µg, and thus tree times higher. This big difference in values can either be explained by difference in analysis methods, or by the fact that bacteria's can produce vitamin B_{12} in minced meat when stored. According to Martens et al. (2002) the genera of vitamin B_{12} producing bacteria's are: *Aerobacter, Agrobacterium, Alcaligenes, Azoto- bacter, Bacillus, Clostridium, Corynebacterium, Flavobacterium, Micromonospora, Mycobacterium, Norcardia, Propionibacterium, Protaminobacter, Proteus, Pseudomonas, Rhizobium, Salmonella, Serratia, Streptomyces, Streptococcus* and *Xanthomonas* (Perlman 1959). Since the minced meat in this project is vacuum packed before storage at -70 degree Celsius, it is most likely that it contains a microflora of lactic acid bacteria in the genera of *Lactobacillus, Carnobacterium, Leuconostoc and Clostridia (Adams & Moss 2008).* LAB have been used in the industry for other foods to improve the vitamin B_{12} level of foodstuffs (Burgess et al. 2009). However, this is just speculations for now.

The amount of zinc also varies quite a lot between max and min values in Norway and the difference corresponds to either covering 65% of the NRV or 39% of the NRV. The variation seen between vitamin K also constitutes a difference of either covering 4.11%

of the NRV, or 24.8%. Average vitamin K levels in minced meat are not high enough to assess the health claim “source of”, but if the average content can be increased towards the maximum value this claim could be applied. The same is the case for selenium: the average content of Norwegian minced meat are 14.5% of the NRV, thus its just below the limit for allowed labeling. However, if the average level of selenium can be increased slightely towards the maximum value observed (from 8.0 towards 12.5 g) a 100 g of minced meat woud cover 22.7% of the NRV and thus be classified as a source of selenium. This could be done by increasng selenium content in concentrate or by giving supplements directly to the animal. For other nutrients, like niacin, increasing the content to the maxium level found in this project or systematic sorting of meat based on the highest niacin content, could enable using the health claim “rich source of”, because the maximum value found in minced meat constitute 30.6% of the NRV.

The discussion above opens for possible market advantages for beef meat, provided either sorting or strict feeding control. In some case a micronutrient can provide substantial amounts of the NRV or total recommended intake of that nutrient at max values, even if reduced consumption would be encouraged. In a marked where food and nutrition knowledge amongst people are gradually reduced, and media are filled with advertisments claiming you have to take supplements to maintain good health, adjusted cattle feeding and meat sorting can provide opportunities for meat and meat product to still be part of a healthy and nutritious diet.

Even though a food is not classified as a good source of a nutrient according to the claim regulation, it can provide a substantial part of the diet. According to Norkost 3, meat and meat products contributes with 21 % of the vitamin A intake (Helsedirektoratet 2012), even though it is not allowed to carry a nutrition claim about vitamin A. The same is the case for thiamin and riboflavin where meat respectively contribute to 21 and 15% of the vitamin intake (Helsedirektoratet 2012). Foods can probide a substinal part of the diet, eventhoug it does not contain large amount of that nutrient, in two ways. Firstly, if a food is eaten in large amounts it will still be a good source of nutrients, eventhough an food is low on a specific nutrient. Secondly, if the intake of a certain nutrient is lower than the recommendations, one foodgroup which are not in principle a good source contribute to a substainial proportion. Thus, when looking on how large part a nutrient

contributes to the intake in a population, this tells nothing aboute how the intake is relative to the recommendations.

6.3 How are Norwegian values compared to other countries?

The total fat content in minced meat varies from 8 % in Czech republic to 17% in Finland. In this study, the minced meat was supposed to be standardized to 14% fat. This standardization would make it easier to compare fat-soluble vitamins in the minced meat, which varies with different total fat content. In addition all countries selected for comparison should have a total fat content that were not far from 14%. This is true for all countries except Czech Republic. However, the average fat content in this study were 13.1 g/100 g compared to the 14.0 g/100g in official values. The standardization was affected by the fact that some carcasses in this thesis had such a low fat content that minced meat with 14 g/100 g fat was not possible to obtain. This might not be a unique situation for this project: e.g. in France the search word "beef, ground, 15% fat, raw", represents a minced meat containing 13.6% fat.

When looking at fatty acid composition in percent of total fatty acids, the Norwegian values of SFA, MUFA and PUFA were 45.5%, 43.7% and 4.1%, respectively. This division between saturated, monounsaturated and polyunsaturated are similar to the standard composition reported by Kerry and Ledward (2009) were SFA constitute 45-49% of total fat, MUFA constitute 43-50% of total fat and PUFA 2-10% of total fat. However, when comparing the Norwegian percentage with values from other countries it is evident that there is a potential for improvement, especially with regards to SFA content. Both Finland and USA has a low percentage of SFA in their minced meat, 38.2 and 39.1% respectively. These are SFA numbers as low as seen in pork (Kerry & Ledward 2009). Thus, the potential to reduce SFA in Norwegian beef appear present from these data. Another improvement option for Norwegian meats is regarding percentage of MUFA. The amount of MUFA varies from 36.6-46.7% between all countries. However, in all countries except from Czech Republic, Norwegian official values and Norwegian values from this project had percentage of MUFA that were higher or similar to SFA percentage.

Looking at the percentages of SFA, MUFA and PUFA for all countries there is a variation in how many percent they constitute in total. For calculated values in this project the

percent of SFA, MUFA and PUFA were 92.9%, quite similar to the amount in France, Denmark and Sweden. The values for Finland,however, and for the official values for Norway the percent is only 81.8 and 82.1%. This difference can maybe be explained by the conversion factor used when calculating the amount of fatty acids from mg/100g of fatty acids to g/100g food. However, the conversion factor used by other countries or Norwegian official values are not known. Because of this difference, the differences seen between SFA, MUFA and PUFA levels may not be as big as it appears to be. However, when looking at P:S and n-6:n-3 ratios, this effect will not make any difference on the results, since ratios nulls out this effect.

In no country a P:S ratio above 0.46 was observed. The P:S ratio of fat is suggested to be above 0.46 to have a positive impact on health, with regards to cancer and CHD (Warren et al. 2008). The ranges were from 0.045 in Denmark and Iceland, to 0.106 in Sweden. So, the country with the best P:S value were still far away from the desired limit. These results correspond well with literature, stating that ruminant muscle has a low P:S ratio (Lawrie & Ledward 2006). Norway had a value of 0.091, which were the second best P:S ratio together with France that had similar value. Even though the amount of SFA in Norwegian minced meat still are high compared to countries as Finland and USA, the amount of PUFA in Norwegian minced meat is the highest of all countries, with a value of 4.1%. If this high amount of PUFA is due to large amount of roughage intake and grass consumption when animals pasture would be interesting to know. However, to make a comparison, the amount of roughage and time of pasture in Norway and other countries as well would be needed. It also would also be interesting to see if Norwegian cows/young cows have an even higher PUFA percentage than bulls, since they are the only one on pasture according to this study.

Regarding the n-6:n-3 ratio, all countries except France, Slovakia, USA and the official values for Norway had given enough information to make it possible to calculate the n-6:n-3 ratios. The n-6:n-3 ratio varied from 1.0 in Sweden to 4.5 in Czech republic. The n-6:n-3 ratio have been shown to have positive impact to lifestyle diseases such as CHD and cancer if the ratio is below 4.0 (Warren et al. 2008). Thus, the minced meat from all countries, except from Canada and Czech Republic, has a good n-6:n-3 ratio. The ratio of Norwegian minced meat from this study was exactly on the limit, with a ratio of 4.0.

Seeing how low the n-6:n-3 ratio can become in a county like Sweden, the ratio can most likely be improved in Norway as well.

Focusing on separate fatty acids, the fatty acid content of C15:0 in the Dutch food composition table stands out. The amounts given are more than 6 times higher than other values, and are suspiciously high. There is reason to believe that this may be an error in the Dutch food composition table. If not, the C15 content in meat should be attempted increased, at least according to the recent publication of Forouhi et al. (2014) indicating that a higher content of odd-chain fatty acids, like C15:0, were inversely associated to incident type 2 diabetes.

When comparing Food Composition tables from different countries, a proper comparison of the fatty acid C18:1 and C18:2 is hard to obtain. There is different ways of reporting C18:1 and C18:2 content per 100g between countries. In the French Food Composition Table the one value given for C18:1 represented both the n-9 cis and the oleic version of the fatty acid. For the Dutch Food Composition Table the value represents the cis version of the fatty acid and the "total value" of the fatty acid. In USA the value represented an "undifferentiated value", while Canada and Sweden did not specify what the value included. In the Food Composition Tables from Denmark, Czech Republic, United Kingdom and for the new results for C18:1 values from Norway obtained in this project, two separate numbers were available: both for C18:1 n-9 and C18:1 n-7.

Since different countries Food Composition Tables report different isomers, and some countries did not specify what the value for C18:1 represented all the C18:1 values were added up and organized into one cell. The same problem was present for C18.2. This makes comparing values from different Food Composition Tables complicated and there should be a standardized way to report fatty acid content in the EU.

When comparing the Norwegian mineral values obtained in this project with the values of all other countries, Norway has the highest content of zinc. However, for all other minerals, other countries have a higher value. Thus, therefor it should be possible to achieve higher values of minerals in Norwegian minced meat as well. Looking at the

mineral value variations in this projects and comparing them with the variation between countries some observations can be made. The following minerals: calcium, magnesium, phosphorous, potassium, selenium and iodine have a higher average value in other countries than the maximum value observed for the same mineral in this project.

One example is the amount of selenium which has a daily recommended intake of 60 µg for men and 50 µg for women in Norway (Helsedirektoratet 2014). The minced meat in USA contains 15.8 µg selenium/100g, compared to 8.0 µg/100g in Norwegian minced meat, and a maximum value of 11.5 µg/100g. The difference between the average values of the USA minced meat and the Norwegian minced meat in terms of NRV are that the minced meat from USA contains 28.73% of the nutrient recommendation value, compared to 14.55% in Norwegian minced meat. This also means that 100g of minced meat can contribute to as much as 31.60% of the daily-recommended intake of selenium for women, and 26.3% for men, if the selenium content of Norwegian minced meat were at the same level as USA. Since the soil in USA has a much higher content of selenium than the Norwegian soil (Aasen 1997), this will require selenium supplementation either via the concentrate or as supplements.

In the case of sodium an average of 69 mg/100g are found in Norway and the lowest registered value in this project were 59 mg/100g. As already mentioned, the big difference seen between official Norwegian values and this project is due to the addition of salt to industrial produced minced meat. With reduction of sodium content being one of the key nutrition advices focused on in the report "recommendations on diet, nutrition and physical activity" from Helsedirektoratet (2014), and with meat and meat products as the largest contributor to the daily intake of sodium (Helsedirektoratet 2012), it would be easy to improve the sodium level of minced meat by taking out the added sodium.

In the case of iron, the minced meat from France had a higher value than Norwegian minced meat, but iron content were not higher than Norway's maximum registered value. The reason for this high maximum iron content is not known, but it can be related to how much iron is added to feed, or maybe the iron content is high because there is a

large proportion of older animals in the Norwegian analyze. However, the age of the animals used in the French analyze is not known.

As for fat soluble vitamins, Norwegian values are highest for β-carotene and vitamin K. However, the comparison of vitamin K is not a simple one, and it do not give the correct picture. This is because the different countries food composition tables define the vitamin K value differently. For Sweden vitamin K means the sum of vitamin K_2 and vitamin K_3, while in USA the value are only for vitamin K_1. Finland uses the term "total" vitamin K, but exactly what the "total" includes is not stated. The value given from Canada does not have a specified origin. Lastly, for the Norwegian numbers an addition of vitamin K_1 and K_2 have been used. In the case of retinol and vitamin E, the Norwegian maximum value is larger than the values found in other countries.

For water-soluble vitamins: the amount of thiamin, riboflavin, vitamin B_6, niacin and vitamin B_{12} are higher in minced meat from other countries than the average value of Norwegian minced meat. In addition, these values are also higher than the maximum value found for the same nutrients in Norwegian minced meat.

The amount of vitamin B_6 found in minced meat from Finland are 0.42 mg/100g compared to the Norwegian value of 0.30 mg/100g. The Norwegian minced meat contains 21.43% of the NRV, while the Finnish contains 30%, thus Finnish minced meat can be labeled a "rich source" of vitamin B_6. The same is the case for niacin, were Icelandic minced meat contains 6.2 mg/100g which corresponds to 38% of the NRV, and thus minced meat from Iceland is a "rich source" of vitamin B_6. Norwegian minced meat is only allowed to claim to be "a source of".

6.4 Oxidation indicators

The measurements of oxidation indicators are not values found in the food composition table, with exception of heme iron than can be found in the food composition table from the Netherlands. The values of heme, TBARS, DPPH and total PV are all important for the cancer hypothesis, and the minced meat analyzed in this project will be further analyzed to investigate health effects by using animals/in vitro models.

6.4.1 Heme iron

Results from the myoglobin analysis showed an average myoglobin content for all samples of 3.21 mg/ml, with a variation from 1.90-5.41 mg/ml. Other studies have reported around the same concentrations of myoglobin, however with other cattle breeds. Charolaise and Limousine had 2.77 mg/ml and 2.72 mg/ml respectively, while breeds such as Gelbvieh, Red angus and Simmental steaks had a higher myoglobin content of 3.62, 3.43 and 3.71 mg/ml respectively (King et al. 2010).

To the author's knowledge, only the Dutch food composition table (*NEVO* 2013) includes hemin values for their minced meat, with a value of 19.85 mg/100g. This is higher than the hemin value of Norwegian minced meat with a content of 13.2 mg/100g. According to literature the amount of heme is affected by, among others, the age of the animal, where heme increases with increasing age (Lawrie & Ledward 2006). The animal with the largest heme concentration in this study is in fact a cow, but all other animals that had heme values above average were in the category of young bulls or young cows.

Literature has also pointed out that iron, and especially heme has a catalytic effect on peroxidation (Damodaran et al. 2008; Monahan et al. 1993). According to the results, a young bull from region 1 had the lowest content of hemin at 7.8 mg/100g. According to literature, and the peroxidation theory, it can be assumed that the peroxidation seen in this sample then would be much less than other animals because of its low concentration of heme (Oostindjer et al. 2014) However, the same young bull also had the second largest TBARS value of 0.518. The animal with highest heme concentration (22.3 mg/100g) was a cow from region 5. This same sample has a TBARS value of 0.054 mg/kg, and is far below the limit of 0.5mg maldondialdehyde equivalents. So as far as the two outer points goes in this study, the results do not correspond well with literature. However, the dataset is too small to draw any conclusions and no statistical testing has been performed.

Also, the TBARS only looks at the creation of malondialdehyde, and a lot of other aldehyde products can also be created by lipid oxidation (Sun et al. 2001). The sample with highest content of heme also had a DPPH-scavenging capacity of 72.4%, so as other

authors point out, a complete meal can also include antioxidant components and other prooxidants, and there is more nutrients affecting the peroxidation than heme-iron alone (Oostindjer et al. 2014).

6.4.2 TBARS

Results from TBARS analysis showed that all samples had a mean value of 0.194 mg/kg maldondialdehyde equivalents, with a variation from 0.037 to 0.576 mg/kg. Two of the samples had a malondialdehyde level above 0.5 mg/kg, an amount that corresponds with threshold levels for tasting off-flavors when meat is tested by a trained sensory panel (Raharjo & Sofos 1993). From these results the average TBARS value are much higher for young bulls (0.295 mg/kg) compared to cows/young cows (0.114 mg/kg). Gender effects are not described in the literature as a factor that influences malondialdehyde formation. The study by Pepe (2011) also showed no gender effects when studying malondialdehyde formation in rat-liver. The five highest malondialdehyde levels all comes from bulls. In addition, since no bull had been on pasture, the trend can also result from differences in pasture time, not only differences in sex. If in fact the relationship seen is due to pasture time, this corresponds with the finding of Mercier et al. (2004) where the pasture fed animals had lower TBARS level that animals finished on a mixed diet. However, in the research of Mercier et al. (2004) the animals with lowest TBARS value were finished on pasture, and nothing else, which is not the case in this analyze.

In this thesis the malondiadehyde level for detection was set to 0.5 mg/kg, though it is difficult to come up with one particular number that corresponds with the threshold levels. However, numbers from 0.3-1.0 mg/kg in beef are reported to be limits for detectable off-flavors (Raharjo & Sofos 1993).

The TBA test is widely used to quantify malondialdehyde, which is a degradation product of lipid hydroperoxides, and is used as a marker for lipid peroxidation in muscle tissue, because of its simplicity and speed (Raharjo & Sofos 1993). However, the method has some weaknesses: malondialdhyde is not the only decomposition product that can react with TBA. Other substances can also react with TBA and contribute to its absorbance, thus the meted may not specifically quantify malondialdehyde (Raharjo & Sofos 1993; Sun et al. 2001). The test is also based on spectrophotometric

measurements and is not (that) sensitive (Raharjo & Sofos 1993), and often are more sensitive and specific methods like HPLC (high performance liquid chromatographic) method or GC (gas chromatographic) suggested to analyze malondialdehyde (Raharjo & Sofos 1993). Other criticism of the method has been discussed in the review of Raharjo and Sofos (1993).

6.4.3 DPPH

The DPPH scavenging potential varied between meat samples from 67.3 to 75.9% and had a mean of 71.9%. These results tell about the endogenous antioxidant capacity (radical scavenger) of the meat (Serpen et al. 2012). It were expected that the minced meat with lowest DPPH % had a high content of micronutrients such as vitamin C, β-carotene and vitamin E which is supposed to increase the antioxidant capacity of the meat (Sies & Stahl 1995). However, the sample with the lowest DPPH% had a value belowe average for all nutrients that can affect the antioxidant capacity (selenium, β-carotene, vitamin E, zinc and calcium). However, the minced meat with the highest DPPH% of 75.9 actually had the lowest level of α-tocopherol. In later stages of this project it would be interesting to look if there is a trend between the amount of nutrients that is believed to affect the antioxidant capacity and the DPPH% value.

One cluster that was observed in the results was the fact that all the cows had a DPPH % above average value. No other groupings seemed to be made in the results, but calculation shows that there is a true difference between the DPPH % values in this study. Later in the project it should be looked into if the older animals or cows, have a better antioxidant capacity than young cows and young bulls.

There are a lot of factors that can affect the DPPH% value of the minced meat. Meat is a heterogeneous and complex food, and some authors (Perez-Jimenez & Saura-Calixto 2005) suggests that the antioxidant activity cannot be evaluated by a single method only. Hence, other radical scavenge capacity assays should also be included in further research, such as maybe ABTS and Fe^{+3} as done in the article by Serpen et al. (2012) where the total antioxidant capacity of raw and cooked meats where investigated.

6.4.4 Total PV

The total PV varied between meat samples from 0.481 to 1.172 mmol/kg and had a mean value of 0.740 mmol/kg. The PV value measures hydroperoxides in the meat, a

primary product of lipid oxidation (Grau et al. 2000). Not many studies have been published on the PV value of beef. However, one study found that lean beef meat contained around 1.1 mmol/kg total peroxides (Yi et al. 2013). The method in this study is the same, but the analysis of Yi et al. (2013) were done in the *M. semimembranosus* muscle.

The study of Yi et al. (2013) also found that the amount of peroxides were significantly related to hemin levels and fatty acid composition, however, this was mostly current for the lipid peroxide. In this phase no real variation were found in this project. In addition the study found that the protein bound peroxides were less explained by hemin concentrations. In this project, no clear trends could be seen between factors like hemin level, age, sex, or content of antioxidant nutrients related to variations in peroxide values.

Lastly, when the dataset increases the correlation between amounts of heme, amount of DPPH, total PV and TBARS should be looked at. According to the peroxidation theory beef meat containing large amount of heme and high percentage of DPPH should in theory contain a large total PV value, and TBARS value.

6.5 What to include in the food composition tables in the future?

The components measured in the Norwegian food composition table are not the exact same components as measured in other countries tables. As for Canada, Denmark, Sweden, Netherlands, Czech Republic, USA and United Kingdom present values for most fatty acids. Since research shows that there is a difference between each fatty acids effect on cholesterol levels (Astrup et al. 2011; Pedersen et al. 2009) this could be important information to include. In addition, the Norwegian health authorities recommends that 3% of the fatty acids should be linolenic and linoleic acid, whereas linolenic acid alone should contribute at least 0.5% (Helsedirektoratet 2014). When information about these fatty acids is missing in the Norwegian food composition table regarding minced meat, such planning of the diet is challenging.

The same is the case for amino acids: in this compilation only Canada, Czech Republic and USA had values. The amount of protein in a food only tells you the quantity, not the

quality of the proteins, which is decided by the containment of essential amino acids (Damodaran et al. 2008).

Further, only some countries (Canada, United Kingdom and Finland) list some types of plant sterols. The plant sterols is reported to lower the LDL-cholesterol (Katan et al. 2003) and a daily intake of 2 grams stanols or sterols would lead to a 10% reduction of LDL cholesterol according to a meta-analysis of 41 different trials (Katan et al. 2003). Because of the sterol's positive effect on cholesterol it is favorable to ingest for people with increased risk of heart disease. The addition of plant sterols for lowering cholesterol has already been done in some Norwegian foods, like the margarine "Vita Hjertegod: Pro-active". A study with 60 people with somewhat high cholesterol was conducted where participants were given 25 g of this margarine each day for 3 weeks (corresponding to 2 gram of plant sterols a day). This reduced the total cholesterol by 10% and the LDL-cholesterol by 15% compared to the cholesterol levels before the study (Mills DA). According to the Finnish food composition table, the minced meat contains 0.8 mg of plant sterols per 100g. This means that if 250 g of minced meat is consumed every day, the plant sterol content in minced meat could theoretically have cholesterol lowering effect. This is of course unacceptable amounts but it is not clear what is the max value of plant sterols in minced meat is.

Iron is an essential nutrient and iron content is given for minced meat in all countries. However, the iron can be found in two distinct forms: heme iron and non heme iron. To the author's knowledge, only the Netherlands have given information about the heme iron content. This is important information because of the difference in absorption (Hurrell & Egli 2010). Additionally, if the hypotheses concerning cancer and heme iron appear to be correct (Oostindjer et al. 2014), the Norwegian food composition table should include information about the heme-compounds in the food composition tables.

All countries except from Czech republic have a value for cholesterol, as seen in table 24. Cholesterol is an important structural component in the animal cell membrane, where it influences the fluidity integrity of the membrane (Damodaran et al. 2008). The reason for its inclusion in the food composition tables are probably due to the main view on cholesterol: that dietary cholesterol effects the cholesterol levels in the blood, thus

eating foods high in cholesterol can increase the risk of heart disease (Spence et al. 2010). In later years new publications have reached other conclusions (Rong et al. 2013) and the academic community are currently not agreeing on cholesterol effects regarding heart disease. Therefore it's reason to question if the amount of cholesterol in a food is important to include into food composition tables. Maybe this is a measurement not longer needed.

Cholesterol is one example of nutrient included in the food composition tables that can affect the health of the population in a negative manner. There are few other measurements that give information about negative components. Some countries like Iceland include toxins, in the form of heavy metals in their food composition tables, with values for lead, mercury and arsenic. According to Norwegian food safety authority toxins are unwanted components in the food and can cause cancer, damage genetic material, reduce learning ability, changes in hormone balance and fetal damage, trough long-term exposure even at low dosage (*Norwegian Food Composition Database* 2013). Based on this statement from the Norwegian food safety authority the Norwegian Food Composition Table should identify and include values for toxins in the Norwegian Food Composition Table.

7 Conclusion

There is a variation between animals from Norway with regards to vitamin, minerals, fatty acids and oxidation indicators. This reflects a potential that can be used for sorting and improving the nutrient content of minced meat. To sort minced meat based on special nutrients can be used for marketing purposes. The Norwegian minced meat is a source of iron, phosphorus, potassium, niacin and vitamin B_6, and a rich source of proteins, zinc and vitamin B_{12}. Compared to other countries improvements can be made regarding SFA content, n-6:n-3 ratio, calcium, magnesium, phosphorous, potassium, selenium, iodine, thiamin, riboflavin, vitamin B_6, niacin and vitamin B_{12} values. Regarding oxidation indicators the Norwegian minced meat contains 13.2 mg/100g hemin, has a TBARS level of 0.194 mg/kg, a DPPH value of 71.9% and a total peroxide value of 0.740 mmol/kg. When the dataset increases during the project "healthier beef meat" statistical data can be performed on parameters that can explain observed variations.

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Appendix 5: Calculations of fatty acids in 100 g food and 100 g total fatty acids. In Agriculture and Consumer Protection (ed.). FAO Corporate document repository. Available at: http://www.fao.org/docrep/008/y4705e/y4705e22.htm.

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Appendix 1 – questioner for obtaining feed information

FORSKNINGSPROSJEKTET

SUNNERE STORFEKJØTT

SPØRRESKJEMA OM FÔR

HVORFOR SPØR VI OM FÔRET?

Grunnen til at vi ber om informasjon om fôret som dyret har spist, er at vi ønsker å se om ulike fôrtyper påvirker innholdet av ulike næringsstoffer i kjøttet.

ANONYMITET

Alle deltakere vil være anonyme, unntatt for helt nødvendige prosjektmedarbeidere.

Alle deltakere vil få tilgang til analyser utført på de dyrene de har bidratt med i prosjektet, dersom de ønsker det.

INNSENDING

Vennligst returnert det utfylte skjemaet til

Ellen Skuterud

IKBM
Universitetet for miljø og biovitenskap

Kirkeveien 4

1432 ÅS

KONTAKTINFORMASJON PRODUSENT

NAVN: _____ PRODUSENTNUMMER: _____

ADRESSE: _____

TELEFON: _____ DATO: _____ **ID-NR PÅ DYRET:** _____

HVA SLAGS FJØS ER DYRET I?

☐ BÅSFJØS ☐ LØSDRIFTSFJØS

HAR DYRET VÆRT PÅ BEITE I LØPET AV DE SISTE 20 UKENE?

☐ NEI ☐ JA: HVOR MANGE UKER? _____ SISTE DATO UTENDØRS: _____

KRAFTFOR

OMTRENT TOTAL MENGDE PER DAG: _____

TYPE KRAFTFOR: _____

PRODUSENT: _____

DERSOM VI IKKE KLARER Å SKAFFE INNHOLDSDEKLARASJON DIREKTE FRA PRODUSENT, VIL VI KONTAKTE DEG PÅ NYTT FOR Å FÅ VITE SAMMENSETNINGEN AV KRAFTFORET.

GROVFÔR

HVOR ER FÔRET DYRKET? ☐ PÅ GÅRDENS JORDER ☐ KJØPT LOKALT ☐ KJØPT FRA ANDRE DELER AV LANDET

ER DET MULIG Å ANSLÅ TOTAL MENGDE GROVFOR DYRET HAR SPIST PER DAG VED INNEFÔRING? _____

ER DET GJORT ANALYSER AV SILOFÔRET I LØPET AV SISTE 12 MÅNEDER?:

☐ JA KAN VI FÅ TILSENDT KOPI AV ANALYSERESULTATENE?

☐ NEI DETTE VILLE VÆRE NYTTIG INFORMASJON FOR PROSJEKTET. ER DET MULIG AT DET KAN GJØRES?

ANNET FÔR / TILSKUDD

HAR DYRET FÅTT ANNET FÔR DE SISTE 20 UKENE? HVIS JA, ANSLÅ MENGDE PER DAG

☐ POTETER: MENGDE _____ ☐ GULRØTTER: MENGDE _____ ☐ KÅLROT: MENGDE _____

ANNET _____: MENGDE _____ ANNET _____: MENGDE _____

HAR DYRET FÅTT EKSTRA MINERALER? ☐ NEI ☐ JA, HVA OG HVOR OFTE? _____

HAR DYRET FÅTT MEDISINER I LØPET AV DE SISTE 20 UKENE?

☐ NEI ☐ JA, HVA OG NÅR? _____

ANDRE KOMMENTARER: _____

Appendix 2 – Information given to producers of NRC via email and postal mail

FORSKNINGSPROSJEKTET
SUNNERE STORFEKJØTT

Invitasjonsbrev

Dette er en invitasjon til å bli med på prosjektet *Identifisering av det sunneste storfekjøttet.*
Prosjektet har blant annet som formål å kartlegge ernæringssammensetning i norsk storfekjøtt. Norge har ikke oppdaterte data på dette. Denne informasjonen er viktig å ha ved deklarasjon av kjøttet, men også for å identifisere markedsfordeler knyttet til norsk storfekjøtt. Dersom du ønsker å delta i dette prosjektet tar du kontakt med Ellen Skuterud minst 2 uker før du ønsker å sende en ku og/ eller en ung okse til slakt.

I dette brevet finner du tre andre notater

1. Informasjonsark om prosjektet. Dette bør leses grundig da det forklarer hvilke kriterier som ligger til grunn for at nettopp din gård er valgt ut. Om dyrene som er aktuelle for dette prosjektet ikke tilfredsstiller disse kravene, må vi informeres, slik at vi kan finne en ny deltaker. Hver gård vil trolig kun delta med å levere dyr to ganger.

2. Informasjon knyttet til fôret. Mange forhold rundt fôret påvirker ernæringsverdien til kjøtt. Av den grunn ber vi deg om å fylle ut det vedlagte skjemaet knyttet til fôring. Dette skjemaet skal fylles ut og sendes til Ellen Skuterud når dyret forlater gården.

3. Transportskjema. Dette skjemaet påbegynnes av deg på gården og leveres deretter til sjåføren av slaktebilen. Skjemaet vil deretter følge dyret til kjøttprøvene er fremme på vårt laboratorium.

Vanlige spørsmål

Hvorfor er min gård valgt ut? Alle gårder er valgt ut tilfeldig innenfor gitte områder. I prinsippet kunne derfor nabogården din vært valgt ut i stedet, dersom den også tilfredsstiller kravene i prosjektet. Til sammen vil informasjonen fra alle gårdene gi et landsrepresentativt bilde av storfekjøttet som produseres i Norge. Ingen gårder får vite hvilke andre gårder som deltar.

Hvorfor må jeg gi beskjed til dere senest to uker før dyrene skal til slakt? Vi trenger å vite dette så lang tid i forveien fordi vi må ha tid til å planlegge logistikken fra gården din fram til vårt laboratorium. Etter at dyret kommer til slakteriet, blir det slaktet som normalt. Deretter blir det hentet ut av den vanlige produksjonslinjen og avkjølt. Så transporteres hele slaktet til Animalia i Oslo. Der skjæres det ned etter et bestemt mønster, før det tas ut prøver av kjøttet. Deretter blir prøvene fraktet til laboratoriet vårt på Ås. Alt dette må skje i løpet av 5-6 dager. Derfor må vi planlegge veldig nøye hva som må skje hvilken dag, så vi unngår at lørdager, søndager eller transportutfordringer stikker kjepper i hjulene for oss. Derfor setter vi stor pris på om du vil ha fleksibilitet på hvilken ukedag dyret sendes til slakt.

Hva får jeg ut av merarbeidet? Vi håper at de to gangene gården deltar ikke vil medføre så mye ekstraarbeid for deg. Når innhentingen av dyr og analysene er ferdig, vil vi beregne hva som er gjennomsnittlig norsk storfekjøttsammensetning, og hvordan analysene fra din gård var i forhold til gjennomsnittet. Denne informasjonen vil du få. Om vi ser at noe kan forbedres fôrmessig vil vi antyde det. Dersom analysene fra din gård blir slik at det er aktuelt å vurdere positiv markedsføring, må dette avtales med prosjektleder. Dette skyldes at det ofte er vanskelig å markedsføre slik informasjon riktig.

Prosjektet har behov for relevant informasjon om beiteområder og ensilering. Vi tror at det vedlagte spørreskjemaet vil gi den nødvendige informasjonen. Dersom det skulle bli behov for ekstra informasjon vil vi be om å få kontakte deg igjen.

Forskningsprosjekter tar lang tid og informasjon om resultatene fra dette prosjektet kommer tidligst neste høst. Fra innledende faser vet vi at dialogen med dere er viktig for at prosjektet blir vellykket. Hvis du lurer på noe underveis, bare ta kontakt med oss!

Vi håper at du vil delta i prosjektet og ser frem til å høre fra deg!

Med vennlig hilsen

Bjørg Egelandsdal
Professor og prosjektleder
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Mobil: 93 84 71 25

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Praktisk ansvarlig
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Ellen Hovland
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Universitetet for Miljø og Biovitenskap
Postboks 5003,1432 Ås

FORSKNINGSPROSJEKTET

SUNNERE STORFEKJØTT

Du har blitt kontaktet av oss i forbindelse med studien "Identifisering av det sunneste storfekjøttet", som forkortes *Sunnere storfekjøtt*. Dette er en stor norsk studie for å skaffe norsk kunnskap om sammenhengen mellom storfekjøtt og helse. Her vil vi fortelle om den delen av prosjektet du er involvert i. På baksiden av arket kan du lese om de andre delene.

UTVALGTE FÔR/BEITER

- Innefôring
- Kystbeite
- Fjellbeite
- Skogsbeite
- Særegne geografiske regioner

9 REGIONER

Det er valgt ut ni regioner i Norge
- Hå
- Vindafjord
- Dovre
- Vestre Toten
- Steinkjer
- Fræna
- Surnadal
- Gaular
- Sømna

ANALYSER

- Vitaminer
- Mineraler
- Fettsyrer
- Antioksidanter
- Totalt peroksiddannende potensial (TPFP)
- Totalt reduserende potensial (DPPH)
- Evt type kjøttfibre

BEHOV FOR NORSK KUNNSKAP

Det har så vidt vi vet ikke vært gjort tidligere forsøk på å identifisere sammensetningen av viktige ernæringskomponenter i typisk norsk storfekjøtt.
Dette er av interesse fordi
- Norge fortsatt bruker mye grovfôr i et langstrakt land, og ulikt grovfôr kan gi ulik sammensetning av kjøttet
- alder og kjønn på dyret kan påvirke næringsinnholdet i kjøttet
- kunnskap om sammensetningen er sentral i deklarasjon på matvarer og i matematiske modeller for hva som påvirker helsen vår
- det importeres storfekjøtt til Norge, og det er nyttig å kjenne til eventuelle forskjeller mellom norsk og importert storfekjøtt.

UTVALGET OG FREMDRIFT

I prosjektet vil det hentes ut kjøttprøver fra ung okse og ku, fordi disse to grupper representerer mer enn halvparten av det storfekjøttet vi spiser. Vi vil også avgrense undersøkelsen til Norsk Rødt Fe.

Det er valgt ut ni regioner. Kriteriene er at regionen er av en viss størrelse med hensyn på storfekjøttproduksjon og at den er geologisk interessant. Dette siste forutsetter at det benyttes lokalt grovfôr. Til sammen vil disse regionene gi et representativt bilde av det norske storfekjøttet.

Innhentingen av prøver vil finne sted de neste 12-18 månedene. Deretter vil dette sammenfattes til åpne rapporter.

KRITERIER FOR Å DELTA

1) Produserer storfekjøtt fra NRF
2) Leverer ungokse og ku direkte fra gården til slakteriet
3) Bruker lokalprodusert grovfôr
4) Villig til å informere prosjektledere/ -medarbeidere om når relevante dyr sendes til slakt
5) Gi detaljinformasjon om hvordan de utvalgte dyrene er fôret
6) Gi tilgang til eventuelt utførte analyser av surfôr

Prosjektet vil også sette pris på et bilde av beitende dyr fra gårdene.

Tusen takk for at du vil bidra!
Din innsats er en viktig brikke i dette forskningsprosjektet!

ANONYMITET

Alle deltakere vil være anonyme, unntatt for helt nødvendige prosjektmedarbeidere. Alle deltakere vil få tilgang til analyser utført på de dyr de har bidratt med i prosjektet, dersom de ønsker det.

INFORMASJON TIL STORFEKJØTTPRODUSENTER OM FORSKNINGSPROSJEKTET SUNNERE STORFEKJØTT
UNIVERSITETET FOR MILJØ OG BIOVITENSKAP, ÅS

TRE FORSKNINGSMILJØER OG EN SAMLET NORSK KJØTTBRANSJE STÅR BAK PROSJEKTET "SUNNERE STORFEKJØTT"

PROSJEKTLEDER

Overordnet prosjektleder for hele prosjektet er professor Bjørg Egelandsdal ved Institutt for kjemi, bioteknologi og matvitenskap ved Universitetet for Miljø og Biovitenskap (UMB) på Ås. Bjørg Egelandsdal leder en gruppe forskere som har spesielt fokus på muskelprotein i kjøtt og fisk.

FORSKNINGSMILJØER

En rekke forskningsmiljøer er knyttet til prosjektet

- Nofima, Ås
- Norges Veterinærhøyskole (NVH)
- Institutt for husdyr- og akvakultur vitenskap ved UMB
- Institutt for kjemi, bioteknologi og matvitenskap ved UMB

FINANSIELLE PARTNERE

Prosjektet er hovedfinansiert av Norges Forskningsråd, av midler fra Styret for fondet for forskningsavgift på landbruksprodukter (*FFL*) og Styret for forskningsmidler over jordbruksavtalen (*JA*).

I tillegg bidrar en samlet norsk kjøttbransje med økonomiske midler:

Opplysningskontoret for egg og kjøtt

Resultater fra noen forskningsstudier kan tyde på en sammenheng mellom høyt inntak av rødt kjøtt og tarmkreft. Dette er stort sett tall fra Amerika og andre land. For å forsøke å skaffe norske tall har hele den samlede kjøttbransjen gått sammen med tre norske forskningsmiljøer for å se på dette temaet. Det er et fireårig prosjekt, som varer fra 2013 til 2016.

UMB leder den delen av prosjektet som du/dere deltar i som handler om å skaffe mer **kunnskap om storfekjøttets sammensetning.**

UMB har også hovedansvaret for å arrangere en **workshop** i Oslo i november, hvor ca 40 nasjonale og internasjonale forskere på kjøtt og helse samles for å diskutere hvor langt forskningen på kjøtt og helse har kommet og hvordan vi skal forske smartest mulig fremover.

Nofima har en **kunstig tarm** hvor de kan etterligne menneskets fordøyelse. Den skal fordøye ulike typer kjøtt, for å se etter dannelse av kreftfremkallende stoffer.

Veterinærhøyskolen skal bruke en spesiell musestamme som lett får svulster i tarmen. Disse **musene** skal fôres med ulike typer storfekjøtt. Deretter skal de sjekkes for kreftfremkallende stoffer og svulster i tarmen, for å se om ulikt kjøtt har forskjellig helseeffekt.

Det skal også gjøres **fôringsforsøk** med ulike typer fôr til storfe på UMB for å se om kjøttets sammensetning kan påvirkes i en sunnere retning.

Til sist skal **forsøkspersoner** ved UMB spise vanlig storfekjøtt og det antatt forbedrede kjøttet, hvor det tas blod– og avføringsprøver for å se etter forskjeller i tegn på kreftfremkallende stoffer.

Hva kan resultatene brukes til?

Det viktigste er å kartlegge om det ser ut til å være en sammenheng mellom ubearbeidet rødt kjøtt og tarmkreft i Norge som i utlandet. Dersom det ikke er det, må det letes etter andre forklaringer.

Dersom det viser seg å være en sammenheng, kan dataene analyseres for å finne det beste fôret, den beste slaktealderen, osv. Dermed kan dette prosjektet bidra til å utvikle et sunnere storfekjøtt.

KONTAKT OSS GJERNE

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HAR DU LYST TIL Å FØLGE MED MER? KLIKK DEG INN PÅ WWW.UMB.NO/SUNNERE-STORFEKJOTT

Appendix 3 – Procedure for sample making

Kontroll Makros	Netto	Vann*	Protein*	Fett*	Fettsyresmst	Kolesterol	Retinol	Beta-karoten	α-tokoferol	Vit K_1	Vit K_2 MK-4	Tiamin(B1)	Riboflavin(B2)	Pyridoksin -Vit	Niacin	Vit B12	Folat**	Mineraler***	D_3	25-OH- D_3	D_2	25-OH- D_2	SUM
UTTAK		Antatt målt ved uttak: "Food Scan"			Samme "glass" NUNC rør					Samme "glass" NUNC rør		Samme "glass" NUNC rør			Samme "glass" NUNC rør				Samme "glass" NUNC rør				
Gram		Må gjøres etter homogenisering Ved henting 100 g			5 g x 2 (cis/ trans)		5g	5g	5g	2 g		15 g			10 g		5 g	20 g	20 g evt 2x10				150 g+ 100g
		Animalia			På IKBM: Disse 11 homogeniserte prøvene skal i NUNC rør og kan puttes alle sammen i en felles større pose som vakuumeres (skru korkene forsiktig til slik at man tar ut luft; test prosedyren), **må ha tydelige merkelapper** Fyll bare NUNC rørene helt opp, bedre å ta for mye enn for lite!																	
#					1,2		3	4	5	6		7			8		9	10	11				

* Kan man godta at disse analyseres hos Animalia med deres NIR utstyr?
Alt her er **homogenisert materiale.** NIFES sier at må ha 50% ekstra som sikkerhet og vi må pakke små mengder i NUNC rør **som fylles** .
210-Folatbestemmelse-mikrobiologisk**: prøvene tilsettes askorbinsyre (L (+) - Ascorbic acid) 50mg/g prøve før innfrysning. Denne strør du over prøven, og blander deretter inn i prøven.

***Mineraler=Ca, Fe, Na , K (?ut), Mg (?ut), Zn, Se, Cu, P, Pb, Ni (inn?), Ga (indikatorer for miljøforurensinger)

Appendix 4 – Method for analysis performed at Fødevarestyrelsen in Denmark

Type of analyze and principle of all analyzes for analyzes performed at Fødevarestyrelsen in Denmark (Table 1).

Table 1: Detektionsprincipper for metoder anvendt til analyse af kødprøver.

Analyse	**Akkredi-teret**	**Princip**
Fedtsyrer	Ja	GC metode med FID detektion
Cholesterol	Ja	(Atmospheric pressure chemical ionization) APCI-LC MS-MS metode
Retinol	Ja	HPLC metode med UV-detektion
β-caroten	Ja	HPLC metode med UV-detektion
Tocopheroler	Ja	HPLC metode med Fluorescensdetektion
Vitamin K (NIFES)	Nej	HPLC metode med Fluorescensdetektion
Vitamin B_1- og B_2	Ja	HPLC metode med Fluorescensdetektion
Pantothensyre	Ja	Traditionel mikrobiologisk assay og spektrofotometrisk måling af bakterievækst ved 650 nm
Vitamin B_6	Ja	HPLC metode med Fluorescensdetektion
Niacin	Ja	Mikrobiologisk assay på mikrotiterplader og spektrofotometrisk måling af bakterievækst ved 630 nm
Vitamin B_{12}	Ja	Traditionel mikrobiologisk assay med spektrofotometrisk måling af bakterievækst ved 650 nm
Mineraler (jern, zink, magnesium, phosphor, natrium, mangan, kalium, calcium)	Ja	Oplukning i mikrobølgeovn og efterfølgende måling på ICP-OES
Selen	Ja	Oplukning i mikrobølgeovn og efterfølgende måling på ICP-MS
Jod	Ja	Oplukning i mikrobølgeovn og efterfølgende måling på ICP-MS

Appendix 5 – Calculated myoglobin concentration

The absorbance of the standard myoglobin solution (Table 2) was measured to make a standard curve (figure 1) for calculating myoglobin concentration in the meat samples.

Table 2: Different concentrations (mg/ml) and following absorbance of the standard myoglobin solution used to make the standard curve for calculation of myoglobin concentration in meat sample.

Concentration (mg/ml)	Absorbance (407nm) of standard myoglobin solution
0	0.228
2	0.465
4	0.860
6	1.006
8	1.301

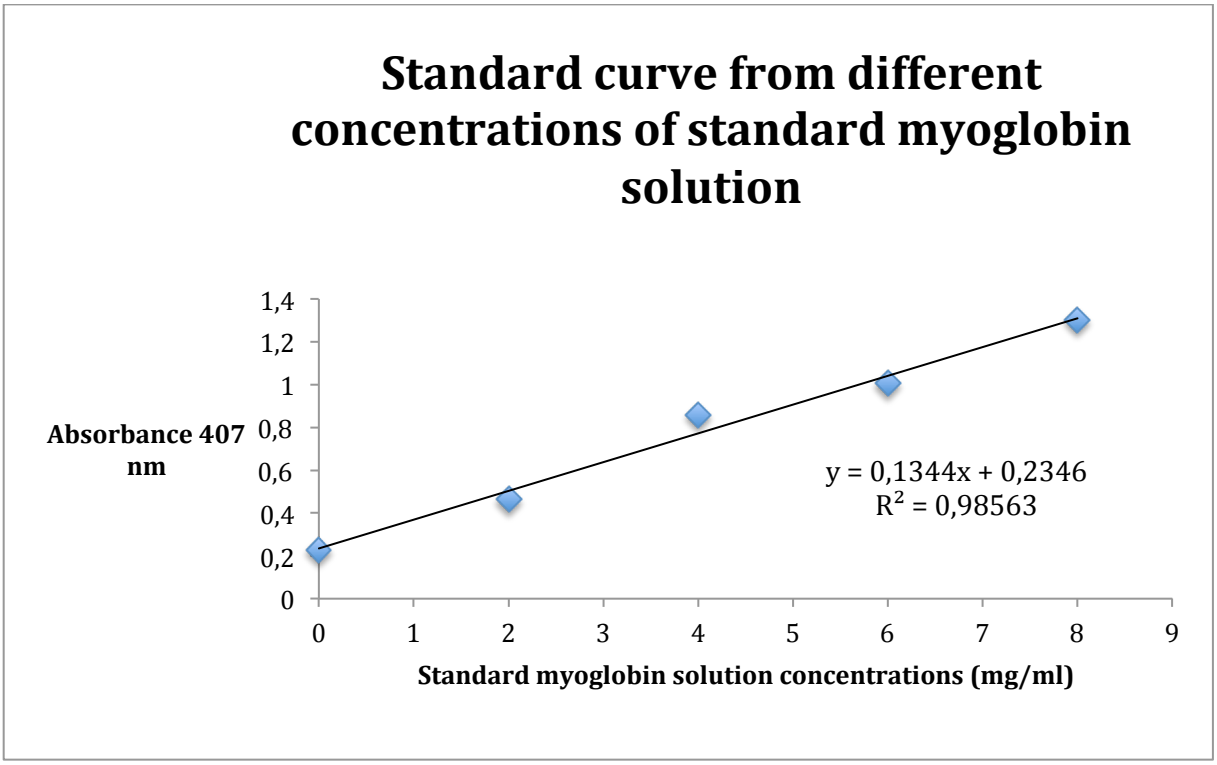


Figure 1: Myoglobin standard curve derived from the concentration (mg/ml) and absorbance (525nm) of a standard myoglobin solution. The equation used to calculate myoglobin content in unknown samples is displayed in the figure.

The concentration of myoglobin in meat samples was calculated based on the equation of the standard curve from the standard myoglobin solution:

$$\text{Myoglobin concentration (mg/ml)} = (abs_{407} - 0.2346)/(0.1344)$$

Average values from the absorbance of meat samples (Table 2) were used in the calculations.

Table 2: Absorbance at 407nm of all meat samples. Values are given for both duplicates and average. Values for calculated myoglobin concentration (mg/ml) based on equation from standard curve are also given.

			Absorbance 407 nm			
Region	Municipality	Cow/ Bull	Duplicate 1	Duplicate 2	Average	Calculated myoglobin concentration (mg/ml)
Rogaland	1	Cow	0.613	0.653	0.633	2.96
Rogaland	1	Young bull	0.545	0.435	0.490	1.9
Rogaland	1	Young bull	0.691	0.816	0.754	3.86
Rogaland	1	Young cow	0.898	0.801	0.850	4.58
Rogaland	2	Young bull	0.613	0.461	0.537	2.25
Rogaland	2	Cow	0.554	0.648	0.601	2.73
Møre og Romsdal	3	Young bull	0.928	0.74	0.834	4.46
Møre og Romsdal	3	Cow	0.674	0.604	0.639	3.01
Møre og Romsdal	4	Young cow	0.609	0.807	0.708	3.52
Møre og Romsdal	4	Young bull	0.529	0.527	0.528	2.18
Sogn og Fjordane	5	Young cow	0.808	0.817	0.813	4.3
Sogn og Fjordane	5	Cow	1.154	0.77	0.962	5.41
Oppland	6	Young bull	0.598	0.555	0.577	2.54
Oppland	6	Young cow	0.627	0.544	0.586	2.61
Nord-Trøndelag	7	Cow	0.447	0.591	0.519	2.12
Nord-Trøndelag	7	Young bull	0.973	0.68	0.827	4.4
Nordland	8	Young bull	0.562	0.61	0.586	2.61
Nordland	8	Young cow	0.541	0.557	0.549	2.28

Appendix 6 – TBARS: Calculating of malonaldehyd (MDA)

The weight and absorption at 532 nm were recorded for all samples as seen in Table 3. From the average absorption the amount of molonaldehyde in mg/kg were calculated based on the extinction coefficient of 1,56 * 105M-1cm-1.

Table 3: Absorbance (532nm) and weight of all meat samples used to calculate malonaldehyde concentrations.

Region	Municipality	Cow/bull	Weight		Absorption (532 nm)		
			Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Average
Rogaland	1	Cow	2.0	2.0	0.088	0.084	0.086
Rogaland	1	Young bull	2.0	2.0	0.188	0.188	0.188
Rogaland	1	Young bull	2.0	2.0	0.093	0.094	0.094
Rogaland	1	Young cow	2.0	2.0	0.079	0.078	0.079
Rogaland	2	Young bull	2.0	2.0	0.050	0.041	0.046
Rogaland	2	Cow	2.0	2.0	0.029	0.035	0.032
Møre og Romsdal	3	Young bull	2.0	2.0	0.178	0.240	0.209
Møre og Romsdal	3	Cow	2.0	2.0	0.040	0.056	0.048
Møre og Romsdal	4	Young cow	2.0	2.0	0.030	0.033	0.032
Møre og Romsdal	4	young bull	2.0	2.0	0.134	0.145	0.140
Sogn og Fjordane	5	young cow	2.0	2.0	0.028	0.042	0.035
Sogn og Fjordane	5	cow	2.0	2.0	0.018	0.021	0.020
Oppland	6	young bull	2.0	2.0	0.086	0.082	0.084
Oppland	6	young cow	2.0	2.0	0.048	0.059	0.054
Nord-Trøndelag	7	cow	2.0	2.0	0.013	0.020	0.017
Nord-Trøndelag	7	young bull	2.0	2.0	0.061	0.078	0.070
Nordland	8	young bull	2.0	1.7	0.019	0.026	0.023
Nordland	8	young cow	2.0	2.0	0.013	0.014	0.014

Table 4: Calculated T-BARS in µM, µmol/kg meat and mg/kg.

Region	Municipality	Cow/bull	Calculated T-bars (µM)	Calculated T-bars (µmol/kg meat)	Calculated T-bars (mg/kg)
Rogaland	1	Cow	0.551	3.286	0.237

Rogaland	1	Young bull	1.205	7.183	0.518(*)
Rogaland	1	Young bull	0.599	3.572	0.258
Rogaland	1	Young cow	0.503	2.999	0.216
Rogaland	2	Young bull	0.292	1.738	0.125
Rogaland	2	Cow	0.205	1.223	0.088
Møre og Romsdal	3	Young bull	1.340	7.985	0.576(*)
Møre og Romsdal	3	Cow	0.308	1.834	0.132
Møre og Romsdal	4	Young cow	0.202	1.203	0.087
Møre og Romsdal	4	Young bull	0.894	5.330	0.384
Sogn og Fjordane	5	Young cow	0.224	1.337	0.096
Sogn og Fjordane	5	Cow	0.125	0.745	0.054
Oppland	6	Young bull	0.538	3.209	0.231
Oppland	6	Young cow	0.343	2.044	0.147
Nord-Trøndelag	7	Cow	0.106	0.630	0.045
Nord-Trøndelag	7	Young bull	0.446	2.655	0.191
Nordland	8	Young bull	0.144	1.011	0.073
Nordland	8	Young cow	0.087	0.516	0.037

Calculation example:

Rogaland cow: Average absorption: 0,086.

The extinction coefficient is 1,56 * 105M-1cm-1.

$$\frac{0.086}{0.156} * 1\mu M = 0.551\ \mu M/L\ TBARS$$

To alter the unit to µmol/kg meat and correlate for sample weight (that should be 2grams) this calculation were performed:

$$\frac{0.551\ \mu M/L\ * (10ml + 1.92ml) * 0.001L}{2g * 0.001kg} = 3.286\ mol/kg\ meat$$

To transfer the unit to mg/kg a last calculation step were made:

$$3.286\ molkg\ meat * 0.0721\ kg/mol\ = 0.237\ mg/kg\ =\ 0.237\ ppm$$

Appendix 7 – Calculated % DPPH scavenging potential

Absorption of all samples with triplicates is presented in Table 5-7. From the mean values, the DPPH scavenging potential was calculated using the formula:

$$\% \text{ DPPH-scavenging} = (A_o - A_t)/(A_o) \times 100$$

Whereas A_0 = the absorption of DPPH working solution, A_t= absorption of meat sample after 1 hour.

Table 5: Absorbance of meat samples, calculated DPPH scavenging potential at a DPPH working soliution absorbance of 0,782.

Trolox:

Trolox blank:	0.75		% DPPH-scavenging
Trolox:	0.408	0.408	45.60

DPPH start abs: 0.782

Reading after 1 hour

Region	Municipality	Cow/bull	Marked	weight (g)	Absorbance (515nm)	Average	% DPPH-scavenging (Ao-At)/(Ao)x100
Rogaland	1	cow	2a	0.491	0.241	0.216	72.4
			2b	0.530	0.216		
			2c	0.515	0.19		
Rogaland	1	young bull	1a	0.498	0.256	0.233	70.2
			1b	0.513	0.22		
			1c	0.545	0.224		
Rogaland	1	Young bull	3a	0.515	0.231	0.231	70.5
			3b	0.514	0.233		
			3c	0.513	0.229		
Møre og Romsdal	4	young cow	9a	0.502	0.282	0.255	67.3
			9b	0.520	0.258		
			9c	0.513	0.226		
Møre og Romsdal	4	young bull	10a	0.533	0.237	0.247	68.4
			10b	0.536	0.235		
			10c	0.490	0.27		
Oppland	6	young cow	14a	0.499	0.261	0.244	68.8
			14b	0.508	0.23		

			14c	0.515	0.242		
Nord-Trøndelag	7	cow	15a	0.503	0.222	0.196	74.9
			15b	0.541	0.187		
			15c	0.519	0.180		
Nord-Trøndelag	7	young bull	16a	0.495	0.21	0.218	72.2
			16b	0.521	0.212		
			16c	0.514	0.231		

Table 6: Absorbance of meat samples, calculated DPPH scavenging potential at a DPPH working soliution absorbance of 0,810

Trolox:

Trolox blank:	0.75		% DPPH-scavenging
Trolox:	0.408	0.408	45.60

DPPH start abs: 0.810

Reading after 1 hour

Region	Municipality	Cow/bull	Marked	weight (g)	Absorbance (515nm)	Average	% DPPH-scavenging (Ao-At)/(Ao)x100
Rogaland	2	Young bull	5a	0.496	0.283	0.255	68.5
			5b	0.557	0.251		
			5c	0.493	0.231		
Oppland	6	young bull	13a	0.537	0.189	0.195	75.9
			13b	0.520	0.205		
			13c	0.544	0.191		
Møre og Romsdal	3	young bull	7a	0.534	0.223	0.220	72.8
			7b	0.496	0.248		
			7c	0.536	0.189		
Rogaland	1	Young cow	4a	0.553	0.224	0.206	74.6
			4b	0.541	0.212		
			4c	0.512	0.181		
Nordland	8	young cow	18a	0.508	0.225	0.209	74.2
			18b	0.545	0.176		
			18c	0.503	0.226		
Sogn og Fjordane	5	young cow	11a	0.562	0.247	0.257	68.3
			11b	0.534	0.253		
			11c	0.500	0.270		

Table 7: Absorbance of meat samples, calculated DPPH scavenging potential at a DPPH working soliution absorbance of 0,736.

Trolox:

Trolox blank:	0.75		% DPPH-scavenging
Trolox:	0.408	0.408	45.60

DPPH start abs: 0.736

Reading after 1 hour

Region	Municipality	Cow/bull	Marked	weight (g)	Absorbance (515nm)	Average	% DPPH-scavenging (Ao-At)/(Ao)x100
Møre og Romsdal	3	cow	8a	0.513	0.246	0.187	74.6
			8b	0.543	0.207		
			8c	0.560	0.108		
Nordland	8	young bull	17a	0.490	0.224	0.186	74.7
			17b	0.547	0.153		
			17c	0.541	0.182		
Roagaland	2	cow	6a	0.485	0.198	0.190	74.2
			6b	0.508	0.172		
			6c	0.534	0.199		
Sogn og fjordane	5	cow	12a	0.513	0.211	0.203	72.4
			12b	0.533	0.172		
			12c	0.487	0.226		

Appendix 8: Protocol for PV measurements

Total muscle hydroperoxide value (PV) measurements by using the ferric-xylenol orange (FOX) method

Rettet 21.05.2014 av Lene R. Lima

When you start a series:

- Try to estimate how much you need of XO and Fe2(III)(SO4)3 solution for a complete series
- Produce the solutions in and put them quickly in Eppendorf Tubes of convenient size so that you can fill them completely and put them in -80°C.
- It is a good idea to prepare all solutions in advance and keep them frozen, but the above is critical – do not risk to change solutions during a series.
- Prepare a 1mmol solution of H2O2 (fresh stock bottle) – keep at -80°C (can be used to check if the assay is stable).

(Antar at vi har maks 0.1 mmol/L i den øverste fasen (70 µL stock og 630 µL Ringers), deretter er dette en standard øvre fase.)

For each meat sample, if you do not incubate with liposomes, then you need 3x samples+1 negative control, which means you need at least 0.4 g meat for each sample's measurement.

Meat from the -800C freezer is homogenized with a blender.

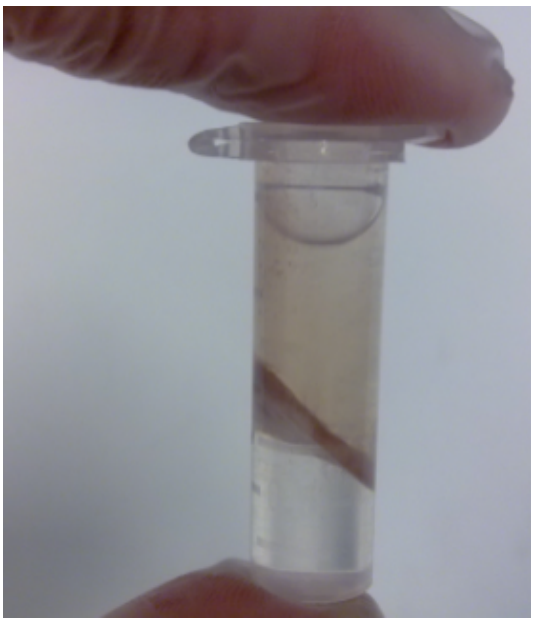
1. Weight out 0.1 g meat powder to each Eppendorf tube.(do not bother to weigh exactly, but record the weight). Use 4 tubes pr. Sample.
2. Add 1ml Ringer's solution to each Eppendorf tube. Add 10 µl 20g/L streptomycin to each Eppendorf tube.

Incubate the Eppendorf tubes in a 70oC water bath for 50 minutes.

3. Meanwhile you need to prepare 8 Eppendorf tubes and mark them, these are prepared for the upper and lower phases measurements.
4. After incubation, add 1 ml Chloroform: Methanol (2:1) solution to each Eppendorf tube, vortex and centrifuge at 16000 rpm, 4oC for 10min.
5. After centrifugation the system separates in three phases which were 1.33 ml polar upper phase (25% methanol+75% Ringer's solution, pH 7), an interphase (the

meat protein aggregate) and 0.67 ml of lower phase (chloroform) containing soluble lipids. Each of the three phases should be removed respectively, for separating hydroperoxide measurements.

It will look like this:



Here you can see that the system has upper, inter and lower phase.

6. Upper phase: carefully transfer 700 µl upper phase to one eppendorf tube and add the following chemicals in this order: 5 µl 4mM BHT; 4 µl 2M H_2SO_4; 40 µl H_2SO_4 at pH 1.8; 30 µl 5 mM XO+ 5M sorbitol mixture at pH 1.8(mix XO and sorbitol 1:1, then take out 30µl from this mixture) and 40 µl 1.67 mM $FeSO_4$ at pH 1.8.

You need a negative control. Add 10 µl 1 M sodium dithionite (DTT) to the last eppendorf tube.(700+5+4+40+30+40=820ul)

Let the Eppendorf tubes stay at room temperature for 1 h to guarantee an entire response. You should cover the samples with something to avoid light.

Centrifuge at 16000 rpm, 4oC for 10min. You need to prepare a solution of 25% Methanol+75% Ringer's for measurement of reference backgroud.

7. Lower phase: Try to remove all the upper phase solution and get 50ul chloroform from the lower phase, PS!! When you try to get the chloroform try to avoid any solution from the upper phase.

Then add chemicals in this order: 200 µl chloroform; 460 µl methanol; 5 µl 4 mM BHT; 12µl 2 M H_2SO_4; 26 µl 10 mM XO at pH 1.8 and 54 µl 1.67 mM $FeSO_4$ at pH 1.8.

You also need a negative control. Add 10 µl 1M triphenylphosphine (TPP) to the last Eppendorf tube.(50+200+460 + 5+12+26+54= 807uL)
Let the Eppendorf tubes stay at room temperature for 1 h to guarantee an entire response. You should cover the samples with something to avoid light. You need to have chlorofrom as reference background.

8. Cake phase: When you finish transferring of the upper and lower phase, carefully pour out all the solution from the effendorf tube to the waste. Wash the protein cake 3 times with Chloroform:Methanol (2:1) solution. Then add 1.7 ml 6 M GuHCl to each eppendorf tube. Let them stand around 30 min.
You then add all the chemicals in this order
12 µl 4mM BHT; 97 µl H_2SO_4 at pH 1.8; 73 µl 5 mM XO + 5M sorbitol mixture at pH 1.8(mix XO and sorbitol 1:1, then take out 75µl from this mixture) and 73 µl 1.67 mM $FeSO_4$ at pH 1.8.
You also need negative control. Add 10 µl 1 M sodium dithionite (DTT) to the last eppendorf tube.(1.7+0.012+0.097+0.073++0.073=1.955)
(1.7 + 0.012+ 0.097+0.073+0.073=1.955) + 0.1 from cake.
Let the Eppendorf tubes stay at room temperature for 1 h to guarantee an entire response. You should cover the samples with something to avoid light. Centrifuge at 16000 rpm, 4oC for 10min. You need to have 6 M GuHCL as backgroud.

9. Read your results at 590nm on the Gen5 96 plate reader.

Be careful when you work. Be aware of the fact that chloroform is hazardous, a possible carcinogen and not recommended to work with if you are pregnant. Use hoods as often as possible.

Tillaging av løsninger:
Her følger noen eksempler på tillaging av løsninger. Du må alltid regne ut hvor mye du trenger til hele serien og så fordele i passende porsjoner i eppendorf rør og fryse ned.

Ringers løsning: 4 tabletter løses i 500ml milliQ vann. Lagres ved 40C.
25%MeOH+75% Ringers: 2,5ml MeOH+7,5ml Ringers løsning. Lagres ved 40C.

Streptomycin 20g/l: 0,04g løses i 2ml milliQ. Lagres ved -800C.
Chloroform:Metanol (2:1) : 334ml kloroform+166ml metanol. Lagres i rom temp.
4mM BHT (Mw 220,36g/mol): 0,008814g BHT løses i 10ml kloroform. Lagres ved -800C.
2M H_2SO_4: 0,56ml 95-98% H_2SO_4 fortynnes til 10ml med milliQ. Lagres ved 40C.
H_2SO_4 pH 1.8: 20ml milli Q tilsettes 2M svovelsyre til pH=1.8 (Bruk pH meter under tilsetting av syra). Lagres ved 40C.
5mM XO (Mw 694,65g/mol): 0,069465g XO løses i 20ml Ringers løsning med pH=1.8. Lagres ved -800C.
10mM XO (Mw 694,65g/mol): 0,027786g XO løses i 4ml Ringers løsning pH 1.8. Lagres ved -800C.
5M Sorbitol pH 1,8 (Mw 182,17g/mol): 18,217g sorbitol løses i 7ml milliQ. Juster pH til 1.8 og fortynn til 20ml i målekolbe. Lagres ved -800C.
1,67mM $FeSO_4$ pH 1.8 (Mw 278,02g/mol): 0,02321467g $FeSO_4$ løses og fortynnes til 50ml med Ringers løsning pH 1.8. Lagres ved -800C.
1M DTT (Mw 174,11g/mol): 0,87055g DTT løses i 5ml milliQ. Lagres ved -800C.
1M TPP (Mw 262,285g/mol): 0,87055g DTT løses i 5ml milliQ. Lagres ved -800C.
6M GuHCl (Mw 95,53): 114,636g GuHCl løses i 1500ml Ringers løsning pH 1.8. Sjekk pH. Juster pH med 1M NaOH. Lagres ved 40C.
Ringers løsning pH 1,8: 300ml Ringers løsning tilsettes 2M svovelsyre til pH=1.8. Lagres ved 40C.

Kontroll av løsninger ved oppstart:

XO – løsning: 0.1 mM XO gir 0.026 ved 560 nm og 0.88 ved 440 nm (er gul)
Ingen endring ved å tilsette 10 µL $FeSO_4$ (om du ikke har H_2O_2 i vannet, og det skal du ikke ha!)
1 ml 0.1 mM XO tilsettes 10µL H_2O_2 (14.7mol /L) og 15 µL av 5 mM Fe (III) SO_4 (blir orange)

Eksempel:

Kontroll av absorb til Fe-XO ved pH 1.8
XO conc er da: (0.005* 0.015)/1.025 = 0.003 dvs 7.32 x 10-5 M, dette ga 0.485 i abs ved 560 nm
ε= 7.32 x 10-5*0.485=6625 M-1 cm-1
Dette er alt for lav verdi skal være mellom 14 000 – 20 000); prøv å finne feilen

Appendix 9 - Expected analytical precision

Expected analytical precision for all analysis performed at Fødevarestyrelsen in Denmark.

Table 8: Expected analytical precision for all analysis performed at Fødevarestyrelsen in Denmark.

Analysis	Expected precision (X ± 2Sr)
Fatty acid analysis profile (in mg/100 gram FA)*	X ± 6.6%
Fatty acids, trans unsaturated (in mg/100 gram FA) *	X ± 9.6%
Cholesterol	X ± 9.8%
Retinol	X ± 8%
β-carotene,	X ± 10.6%
Tocopherols	X ± 9.2%
Vitamin K	N/A
Thiamin -(B1)*	X ± 0,0040 mg/100g X ± 5,6%
Riboflavin -(B2)	X ± 0,0018 mg/100g X ± 6,2%
Pantothenic acid–(B5)	X ± 14%
Pyridoxine– (B6)	X ± 7,4%
Niacin- (B3)	X ± 11.4%
Vitamin- (B12)	X ± 14.6%
Folate- (B9)	4,2%
Fe	8,0%
Se	4,0%
Zinc	3,2%
Magnesium	4,6%
Phosphor	3,0%
Sodium	6,0%
Iodine	4,4%
Manganese	3,4%
Potassium	3,2%
Vitamin D_3, 25-OH-D_3	N/A

Appendix 10 – Calculation of fatty acids

Data from Fødevarestyrelsen in Denmark on fatty acids were given in mg/100 g of fatty acids, but total amount of fatty acids per 100 g edible food where not given. These results where calculated to g/100g edible food by this method:

The average content of total fat in the samples where 13.1 g/100 g. To calculate the total amount of fatty acids per 100 g of food a conversion factor of 0.953 (beef, fat) given by FAO (*Appendix 5: Calculations of fatty acids in 100 g food and 100 g total fatty acids*) where used. The total amount of fatty acids where then: 13.1 g * 0.953 = 12.48 g/100 g edible food.

Then the mean value of all samples where calculated. Thereafter, they where converted to g/100 g of fatty acid by multiplying with 0.001. Then all values where calculated to g per 100 g of food using the formula:

$$Fatty\ acid\ \left(\frac{g}{100g}of\ food\right) = Total\ fatty\ acids\ \left(\frac{g}{100g\ food}\right) * (Fatty\ acid\ (\frac{g}{100g\ fatty\ acids})/100)$$

Appendix 11: Assessment if sample values show a true variation between samples

The criterion to evaluate if there is true variance between samples between animals in the group where based on: $\frac{4* \text{standarddeviation}}{\text{max}-\text{min}} = X$. If X were lower than 1, there is a 95% security that there is true variance. If X is higher than 1, no true variance were detected.

Appendix 12 –PV values and st.dev for all phases

In table 9 the PV values for all phases can be seen, together with the standard deviation. The upper phase (polar peroxides) and the inter phase (protein bound peoxides) variation in min and max values were observed for all animals and samples. For the lower phase, the standard deviation was too high, and thus there was no true variation between samples. In accordance with earlier experience (Gu Yi, personal communication) when the fat content becomes high in meat (here 14%) the sensitivity and the reproducibility for unipolar peroxides are more difficult.

Table 9: Average PV values for all phases, shown as average of three replicates. Samples were measured spectrophotometrically at 590 nm and calculated to mmol/kg mince.

Region	Munici-pality	Cow/ bull	Upper phase		Inter phase		Lower phase	
			Average (mmol/ kg)	St.dev	Average (mmol/ kg)	St.dev	Average (mmol/ kg)	St.dev
Rogaland	1a	Cow	0,455	0.016	0.077	0.018	0.424	0.154
Rogaland	1a	Young bull	0.381	0.027	0.159	0.013	0.178	0.125
Rogaland	1	Young bull	0.160	0.025	0.079	0.010	0.184	0.160
Rogaland	1	Young cow	0.398	0.017	0.100	0.005	0.394	0.107
Rogaland	2	Young bull	0.392	0.038	0.065	0.005	0.146	0.197
Rogaland	2	Cow	0.428	0.020	0.071	0.005	0.076	0.046
Møre og Romsdal	3	Young bull	0.308	0.018	0.085	0.013	0.230	0.062
Møre og Romsdal	3	Cow	0.394	0.011	0.070	0.026	0.127	0.190
Møre og Romsdal	4	Young cow	0.407	0.018	0.090	0.012	0.410	0.168
Møre og Romsdal	4	Young bull	0.368	0.021	0.092	0.007	0.269	0.119
Sogn og Fjordane	5	Young cow	0.423	0.027	0.102	0.018	0.181	0.013
Sogn og Fjordane	5	Cow	0.407	0.010	0.078	0.008	0.333	0.176
Oppland	6	Young bull	0.445	0.017	0.064	0.005	0.297	0.055
Oppland	6	Young cow	0.357	0.020	0.076	0.016	0.251	0.027
Nord-Trøndelag	7	Cow	0.422	0.004	0.076	0.003	0.470	0.072
Nord-Trøndelag	7	Young bull	0.423	0.007	0.040	0.014	0.074	0.121

Nordland	8	Young bull	0.338	0.005	0.103	0.002	0.178	0.079
Nordland	8	Young cow	0.418	0.023	0.092	0.021	0.019	0.044

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