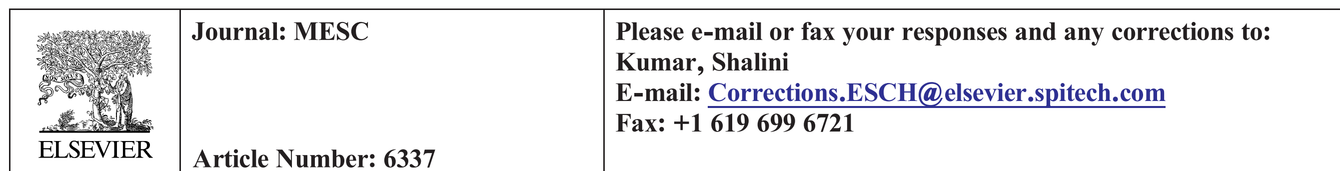
AUTHOR QUERY FORM



ELSEVIER	**Journal: MESC** **Article Number: 6337**	**Please e-mail or fax your responses and any corrections to:** **Kumar, Shalini** **E-mail: Corrections.ESCH@elsevier.spitech.com** **Fax: +1 619 699 6721**

Dear Author,

Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list. Note: if you opt to annotate the file with software other than Adobe Reader then please also highlight the appropriate place in the PDF file. To ensure fast publication of your paper please return your corrections within 48 hours.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Click on the 'Q' link to go to the location in the proof.

Location in article	**Query / Remark: click on the Q link to go** **Please insert your reply or correction at the corresponding line in the proof**
Q1	Please confirm that given names and surnames have been identified correctly.
Q2	Uncited reference: This section comprises references that occur in the reference list but not in the body of the text. Please position each reference in the text or, alternatively, delete it. Thank you.
Q3	Please supply the name of the publisher.
Q4	Medicine, I. o. was captured as the as the surname. Please check if appropriate. Otherwise, kindly provide the specific details. Please check this box if you have no corrections to make to the PDF file. ☐

Thank you for your assistance.

Meat Science xxx (2014) xxx

Contents lists available at ScienceDirect
Meat Science
journal homepage: www.elsevier.com/locate/meatsci

1 # Highlights

2 5 **Vitamin K2 in different bovine muscles and breeds**

Meat Science xxx (2014) xxx–xxx

Rune Rødbotten [a,*], Thomas Gundersen [b], Cees Vermeer [c], Bente Kirkhus [a]

[a] Nofima AS, Norwegian Institute of Food, Fisheries and Aquaculture Research, P.O. Box 210, N-1431 Ås, Norway
[b] AS Vitas, Gaustadalleen 21, N-0349 Oslo, Norway
[c] VitaK BV, Oxfordlaan 70, 6229 EV Maastricht, The Netherlands

- Vitamin K1 and K2 were quantified in three bovine muscles from two different breeds.
- *Longissimus dorsi* contained more vitamin K2 than *Psoas major*.
- Muscles from Jersey had higher levels of vitamin K2 than Norwegian Red.
- Amount of vitamin K2 was not correlated with intramuscular fat or WB shear force.

4

UNCORRECTED PROOF

0309-1740/$ – see front matter © 2014 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.meatsci.2014.01.005

Meat Science xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Vitamin K2 in different bovine muscles and breeds[☆,☆☆]

Rune Rødbotten[a,*], Thomas Gundersen[b], Cees Vermeer[c], Bente Kirkhus[a]

[a] Nofima AS, Norwegian Institute of Food, Fisheries and Aquaculture Research, P.O. Box 210, N-1431 Ås, Norway
[b] AS Vitas, Gaustadalleen 21, N-0349 Oslo, Norway
[c] VitaK BV, Oxfordlaan 70, 6229 EV Maastricht, The Netherlands

ARTICLE INFO

Article history:
Received 5 March 2013
Received in revised form 22 December 2013
Accepted 13 January 2014
Available online xxxx

Keywords:
Beef
Vitamin K2
Muscle
Breed
Menaquinone

ABSTRACT

Meat is a natural source of vitamin K, a vitamin associated with reduced bone loss and prevention of osteoporosis. Whether vitamin K content varies between breeds and muscles in cattle is not known. In the present study, contents of vitamin K1 (phylloquinone) and K2 (menaquinone, MK) were analysed in three different muscles from steers of two different breeds, Norwegian Red and Jersey, respectively. Results showed that MK4 was the most dominant of the vitamin K2 analogues, while only traces were found of MK6 and MK7. Both breeds had higher levels of MK4 in *M. biceps femoris* (BF) and *M. longissimus dorsi* (LD) compared to *M. psoas major* (PM). The results also showed significantly higher MK4 levels in muscles from Jersey compared to Norwegian Red. Furthermore, MK4 was not associated with intramuscular fat, suggesting a physiological role for MK4 in skeletal muscle cells. There were no association between vitamin K content and tenderness.

© 2014 Published by Elsevier Ltd.

UNCORRECTED PROOF

1. Introduction

Recent literature suggests various ways to improve the health profile of meat, e.g. by emphasizing beneficial components and generating new knowledge about physiological functions (Arihara, 2006; Ferguson, 2010; Weiss, Gibis, Schuh, & Salminen, 2010), but there has been little focus on meat as a natural source of vitamin K. Vitamin K (Fig. 1) is the generic name for a family of fat soluble compounds possessing cofactor activity for γ-glutamyl carboxylase, important for blood coagulation, bone formation, soft-tissue calcification, cell growth and apoptosis (Bügel, 2008; Erkkila & Booth, 2008). Dietary intake of vitamin K is obtained from green plants in the form of phylloquinone (vitamin K1), and from animal foods and some fermented legumes in the form of menaquinones (vitamin K2) synthesized by bacteria (Conly & Stein, 1992; Damon, Zhang, Haytowitz, & Booth, 2005; Schurgers & Vermeer, 2000). Menaquinones is a family of structural analogues with side chains composed of a variable number of unsaturated isoprenoid units, designated as MK-*n*, where *n* specifies the number of isoprenoid units. Intake of vitamin K1 is generally higher than K2, but this is equalized by a higher bioavailability of vitamin K2 (Conly & Stein, 1992; Schurgers & Vermeer, 2000). In addition, vitamin K2 has shown better efficiency in reducing coronary calcification than vitamin K1 (Beulens et al., 2009), and it has a vital function in bone metabolism (Bügel, 2008; Shearer & Newman, 2008). Vitamin K2 may also have functions independent of carboxylation. Several studies have demonstrated that intake of vitamin K2 is associated with retardation of bone loss and reduced risk of osteoporosis (Booth & Shea, 2008; Bügel, 2008; Olson, 2000). Beneficial effects on atherosclerosis (Gast et al., 2009; Witteman et al., 2004), cancer (Linseisen, Nimptsch, & Rohrmann, 2008; Ogawa et al., 2007), inflammation (Booth & Shea, 2008) and insulin resistance (Yoshida et al., 2008) have also been suggested, but results are controversial.

Despite all the positive health effects associated with increased consumption of vitamin K2, there are only a few papers reporting its content in meat (Elder, Haytowitz, Howe, Peterson, & Booth, 2006; Koivu-Tikkanen, Ollilainen, & Piironen, 2000). Both these papers report relatively low content of vitamin K in beef, without specifying which muscles or breed the sample material was obtained from. Therefore, the purpose of this study was to investigate several muscles collected from two different breeds to evaluate specific muscle or breed variations.

2. Material and methods

2.1. Animals and muscles

Muscle samples were obtained from 12 Norwegian Red (NRF) and 11 Jersey steers which were reared at the same farm in Rogaland County in Norway. During summer, the animals were grazing at pasture while they were fed indoor during winter with roughage *ad libitum* and concentrate (0.5–3.0 kg per day). Both breed groups were given the same quantity of concentrate. The concentrate (Formel Biff, Felleskjøpet, Norway) contained no forms of vitamin K or menadione. Five Jersey and 6 NRF steers were slaughtered at 18 months of age while the other animals

☆ **Financial support:** The project was supported by the Foundation for Research Levy on Agricultural Products in Norway and Prima Gruppen AS, which is a Norwegian meat processing company.
☆☆ **Conflict of interest**: The authors declare no conflict of interest.
* Corresponding author at: Nofima AS, Norwegian Institute of Food, Fisheries and Aquaculture Research, P.O. Box 210, NO-1431 Ås, Norway. Tel.: +47 64970100; fax: +47 64 97 03 33.
E-mail address: rune.rodbotten@nofima.no (R. Rødbotten).

0309-1740/$ – see front matter © 2014 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.meatsci.2014.01.005

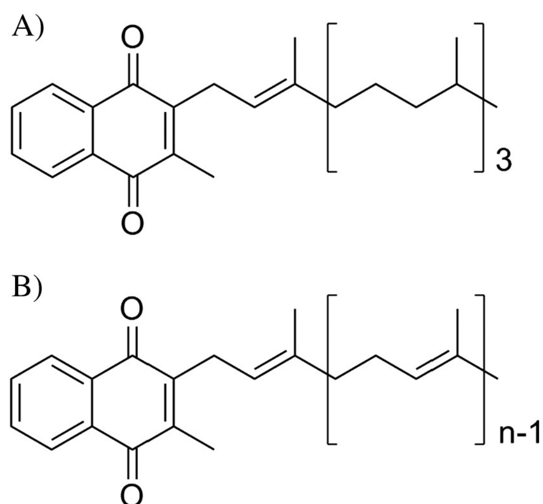


Fig. 1. Molecular structure of A) vitamin K1 (phylloquinone) and B) vitamin K2 (menaquinone). In menaquinone, the side chain is composed of a varying number of isoprenoid residues.

were slaughtered at 23 months of age. Carcass weights and grading results are given in Table 1.

Three muscles, *Longissimus dorsi* (LD), *Psoas major* (PM) and *Biceps femoris* (BF), were excised from the carcasses 2 days post mortem and stored in vacuum bags at 4 °C for 13 days. Then, the muscles were cut into slices for different analyses, including analysis of vitamin K content, intramuscular fat and tenderness. Samples of approximately 100 g were vacuum packed and stored in the dark at minus 20 °C before analysis of vitamin K. The LD and PM samples were analysed by AS Vitas (Oslo, Norway). Unfortunately, one PM sample was lost during transport to the laboratory. The BF samples were analysed by VitaK BV (University of Maastricht, The Netherlands). Two different laboratories were used because the BF samples originally were intended as part of another project. However, because the samples originate from the same animals, the results are reported together. All samples were analysed at one laboratory only, which means data regarding inter-precision between these laboratories was not obtained. Both laboratories perform routine analyses of menaquinones on a commercial basis; therefore, recovery experiments were not performed on the present samples.

2.2. Analytical methods

2.2.1. Vitamin determination for LD and PM muscles

The LD and PM samples were analysed using a modified version of the procedure described by Koivu-Tikkanen et al. (2000), which was set up for detection of phylloquinone and menaquinones MK4–MK13. In short, 40 g of muscle was homogenized with an Ultra-Turrax in phosphate buffer (pH 7.2). An aliquot of this homogenate was transferred to a 50-ml Falcon vial and heated in boiling water for 5 min. After cooling, lipase was added to hydrolyse fats, before extraction with hexane/isopropanol containing vitamin K1(25) as internal standard. The extract was washed with methanol/phosphate buffer, reduced to dryness and re-dissolved in isopropanol before reversed phase analysis by HPLC (Agilent 1200 system). The vitamins were separated using a Zorbax C18 column and reduced electrochemically at −850 mV online. Residual oxygen in the mobile phase was removed using a palladium-based O_2 scrubber to enhance the detector response. PK and MK4 (Sigma, St Louis, MO, USA) and MK5–MK13 (Eisai Co. Ltd., Tokyo, Japan) were used as reference standards. MS/MS detection was used in addition to fluorescence for confirmation of chemical structure.

Table 1
Carcass data, from where the muscle samples were obtained.

Breed	NRF	Jersey
n	12	11
Carcass weight—range (kg)	267–347	176–240
Carcass weight—average (kg)	308	204
EUROP conformation score[a]—range	O–R−	O–P+
EUROP conformation score[a]—average	O+	O−
Fat score[b]—range	3–4	2+–3+
Fat score[b]—average	3+	3

[a] 15 classes, where P− is lowest and E+ is highest.
[b] 15 classes, where 1− is lowest and 5+ is highest.

2.2.2. Vitamin determination for BF muscle

Vitamin contents of the BF samples were quantified using a slightly modified version of the procedure described by Schurgers and Vermeer (2000). In short, 1 g of tissue was transferred to a glass tube and supplemented with 10 μg of vitamin K1(25), which was used as internal standard; distilled water and ethanol were added before homogenization using an Ultra-Turrax. The homogenate was extracted with hexane and pre-purifed on Sep-Pak silica cartridges. The eluate was evaporated to dryness, dissolved in isopropanol and analysed by reversed phase HPLC using a Hypersil C-18 column with fluorescence detection after post-column reduction on a zinc column. Authentic phylloquinone and MK-4 were obtained from Sigma (St Louis, MO, USA) and MK-5 through MK-10 were kind gifts from Roche (Basel. Switzerland). These purified products were used as reference materials.

2.2.3. Intramuscular fat (IMF)

Intramuscular fat (IMF) content was analysed in homogenized meat samples by Low-Field Nuclear Magnetic Resonance (LF-NMR) (Maran Ultra LF-NMR, Resonance Instruments Inc., Witney, UK) as described by Sørland, Larsen, Lundby, Rudi, and Guiheneuf (2004).

2.2.4. Tenderness

Tenderness was measured by Warner–Bratzler (WB) shear force as described by Rødbotten, Lea, and Hildrum (2001). Slices of muscles were vacuum-packed in polyethylene bags, heat-treated in a water bath at 70 °C for 50 min and chilled before analysis in an Intron Materials Testing Machine (model 4202, Instron Engineering Corporation, High Wycombe, UK).

2.3. Data analysis

Because the chemical results were obtained by two different laboratories, the data was analysed independently for each set but also as a combined set. Due to unbalance in the number of samples, the GLM procedure of Minitab (vers. 16.1, Minitab Inc., USA) was used for data analyses where muscle, breed and their interaction were the factors. Tukey's test was used to identify significant differences between the muscles.

3. Results

Levels of phylloquinone and menaquinones in *M. biceps femoris* (BF), *M. longissimus dorsi* (LD) and *M. psoas major* (PM) from steers of Jersey and Norwegian Red are presented in Table 2. Detectable levels were found for vitamin K1, MK4, MK6 and MK7, whereas MK5, MK8, MK9 and MK10 were below the quantification limit. However, MK6 and MK7 were detected in very low quantities, and only in a few samples of BF. Hence, MK4 was the dominant vitamin K2 analogue. Vitamin K2 contents in BF, LD and PM for Jersey compared to Norwegian Red were increased by 53%, 49% and 25%, respectively. In general, the level of MK4 was higher than K1, but the ratio K1:MK4 varied somewhat between muscles, and in PM the levels of K1 and MK4 were similar. The numerically highest value of MK4 was found in a BF sample, and the combined model where all three muscles were analysed together showed significantly ($P = 0.010$) higher MK4 content for the BF muscle compared to the PM (Table 3). In this model, the amount of MK4 found in LD was in between the two other muscles but not significantly different from them. However, when

UNCORRECTED PROOF

Table 2
Levels of vitamin K (μg/100 g) in *M. biceps femoris* (BF), *M. longissimus dorsi* (LD) and *M. psoas major* (PM) from steers of Jersey and Norwegian Red (NFR). Values are presented as mean ± SD.

Muscle	n	K1 (μg/100 g)	MK4 (μg/100 g)	MK6 (μg/100 g)	MK7 (μg/100 g)	Sum Vit. K (μg/100 g)
BF						
Jersey	7	1.37 ± 0.88	4.85 ± 2.15	0.004 ± 0.01	0.006 ± 0.02	6.22 ± 2.45
NRF	7	0.94 ± 0.48	3.02 ± 3.18	0.006 ± 0.01	0.082 ± 0.11	4.06 ± 3.44
LD						
Jersey	11	1.90 ± 0.72	3.39 ± 1.62	nq	nq	5.29 ± 2.12
NRF	12	1.14 ± 0.68	2.43 ± 1.39	nq	nq	3.57 ± 1.79
PM						
Jersey	11	2.19 ± 0.88	2.46 ± 0.89	nq	nq	4.64 ± 1.41
NRF	11	1.89 ± 0.55	1.82 ± 0.58	nq	nq	3.71 ± 0.95

nq: not quantified.

the LD and PM muscles were analysed separately, a significant effect for muscle was shown, Table 4. The content of vitamin K1 was significantly higher ($P = 0.018$) in the PM muscle than the LD. Also, the combined model showed the PM to have a significantly higher ($P = 0.003$) level of K1 than the other two muscles.

The samples obtained from Jersey had a higher content of both K1 ($P \leq 0.018$) and MK4 ($P \leq 0.031$) compared with the samples from Norwegian Red, irrespective of whether there were 2 or 3 muscles in the variance models. Therefore, the average sum of both vitamins (K1 + MK4) was higher ($P \leq 0.009$) for the Jersey compared with Norwegian Red, 5.27 and 3.74 μg/100 g, respectively. However, the separate GLM-model where only the BF samples were analysed did not show a significant effect for Breed.

The carcass weights of the Norwegian Red steers were heavier ($P \leq 0.001$) than the Jersey carcasses (Table 1). Muscle samples obtained from Jersey had higher ($P = 0.004$) contents of intramuscular fat than the Norwegian Red samples (Table 5), 6.8% and 5.5%, respectively. Without respect to breed the BF and LD muscles had similar amount of fat (approximately 5.7%), while the PM samples had an average value of 7.1% intramuscular fat. The content of K1 was highly correlated ($r = 0.44$, $P < 0.001$) with the amount of intramuscular fat, while no such relationship was found for MK4. There was no correlation between tenderness, measured as Warner–Bratzler (WB) shear force, and the content of vitamin K1 or MK4. Tenderness was not different between the breeds, but there was a significant difference ($P < 0.001$) in WB shear force between the muscle groups (Table 5), with highest WB values obtained for LD indicating that this was the toughest muscle in this study. The BF and PM muscles had similar WB values, although there was a tendency for more tender PM muscles.

4. Discussion

The study shows that phylloquinone and MK4 levels in meat from cattle vary between breeds and different muscles. The muscles investigated, *M. biceps femoris* (BF), *M. longissimus dorsi* (LD) and *M. psoas major* (PM), showed varying levels of vitamin K1 and K2, where MK4 was the most dominant vitamin K2 analogue. Only traces were found of MK6 and MK7, while MK5, MK8, MK9 and MK10 were below the detection limit. The results are in agreement with previously reported data (Booth, Sadowski, & Pennington, 1995; Elder et al., 2006; Koivu-Tikkanen et al., 2000). Booth et al. (1995) reported the phylloquinone content of beef steak to be 1.8 μg/100 g, whereas Elder et al. (2006), without specifying muscle group, reported quantities of MK4 in US beef products to be in the range 1.7–8.1 μg/100 g. Koivu-Tikkanen et al. (2000) analysed roast beef, without further muscle description and found MK4 to be in the same range as in the present study. In addition, they reported low concentrations of MK5, MK7 and MK8. In the present study MK6 and MK7 were quantified in some BF samples, but not in the PM or LD samples. Because the analytical method was slightly different for the BF samples compared with PM and LD, the observed difference could be an effect of the method. However, the BF muscle is one of the major muscles in the hind leg of the animal which means this muscle has high activity when the animal walks. The two other muscles PM and LD are less active during movement. Different functions of the muscles may explain why they contain unequal amounts of the menaquinones. It was documented that both bovine rumen and liver contain long-chain menaquinones (MK6–MK13) which probably were synthesized by bacteria (Matschiner, 1970; Matschiner & Amelotti, 1968). In light of the present results, it seems that these long-chain menaquinones, to a very limited extent, are transported into the studied muscles.

Another interesting discovery is that the vitamin K1:K2 ratio varies between different muscle groups, with BF having the lowest ratio and PM the highest. It has previously been shown that the content of vitamin K1 and K2 differs between different tissues and organs in rats (Davidson, Foley, Engelke, & Suttie, 1998; Okano et al., 2008); therefore, it is not surprising that the ratio varies between skeletal muscles also. The present results may indicate that different mechanisms regulate the synthesis of vitamin K2 in various muscles in cattle. For the LD muscle, there was a significant correlation between content of vitamins K1 and K2 ($r = 0.55$, $P = 0.005$), while no relationship was found for the two other muscle groups. There are several studies indicating a role of vitamin K in calcification of smooth muscle cells (Erkkila & Booth, 2008; Saito, Wachi, Sato, Sugitani, & Seyama, 2007; Vermeer & Braam, 2001), but so far there is little reported on a possible function in skeletal muscle cells. Some transmembrane Gla (gamma-carboxyglutamate) proteins have been found in soft tissue, but the function of these is unknown (Ball, 1998).

Table 3
ANOVA results for the combined model where the LD, PM and BF muscles were analysed together with respect to MK4.

Factor	DF	SS	F	p
Breed (B)	1	1599.2	6.56	0.013
Muscle (M)	2	2772.4	5.00	0.010
B × M	2	310.2	0.56	0.575
Error	53	14,687.5		
Total	58	19,369.2		

Table 4
ANOVA results for the model where the LD and PM muscles only were analysed with respect to MK4.

Factor	DF	SS	F	p
Breed (B)	1	689.4	4.99	0.031
Muscle (M)	1	669.9	4.74	0.035
B × M	1	30.2	0.21	0.648
Error	41	5848.6		
Total	44	7238.1		

Table 5
Intramuscular fat (IMF) and Warner–Bratzler (WB) shear force in *M. biceps femoris* (BF), *M. longissimus dorsi* (LD) and *M. psoas major* (PM) from steers of Jersey and Norwegian Red (NFR). Values are presented as mean ± SD.

Muscle	n	IMF (%)	WB (N/cm^2)
BF			
Jersey	7	5.6 ± 1.8^a	41.0 ± 5.7^{abc}
NRF	7	5.8 ± 0.8^a	34.5 ± 3.9^{bc}
LD			
Jersey	11	6.3 ± 1.8^a	42.9 ± 7.0^{ab}
NRF	12	5.1 ± 1.3^a	49.8 ± 12.5^a
PM			
Jersey	11	8.3 ± 1.8^b	31.8 ± 4.2^c
NRF	11	5.8 ± 1.7^a	33.9 ± 7.8^{bc}

a–c within a column means the values are significantly different ($P < 0.05$).

UNCORRECTED PROOF

Please cite this article as: Rødbotten, R., et al., Vitamin K2 in different bovine muscles and breeds, *Meat Science* (2014), http://dx.doi.org/10.1016/j.meatsci.2014.01.005

ARTICLE IN PRESS

From the present results, it seems BF has higher levels of MK4 than the other muscles which may indicate that MK4 is associated with physiological functions of the muscle, maybe through processes involved in muscle activity and energy metabolism. Recent studies have indicated a crucial role of vitamin K2 in mitochrondrial function, helping to maintain ATP production (Vos et al., 2012). However, more research is needed to confirm whether there is a correlation between the amount of MK4 and physiological function of the muscles.

The present study further showed that individual variations in MK4 content were high, in particular for the BF samples, where 0.68 and 9.33 μg/100 g were the lowest and highest quantified levels. Although smaller muscle samples were used during the preparation of the BF samples (1 g) compared to the LD and PM (40 g), it seems unlikely that the analytical methods were the source for this variation, because the variation for vitamin K1 was similar for all three muscles. In addition, most of the muscle samples were analysed once, though one sample was subjected to the analytical procedure three times. The coefficient of variation for these samples was 9.1% compared with approximately 50% for the other samples, which indicated that variation between samples was larger than the analytical variation.

To our knowledge, this is the first time an effect of breed on MK4 levels in muscles of cattle has been reported. Since all animals in the present study were reared at the same farm and given the same feed, the observed difference was most probably due to different genotypes. There was no source of vitamin K or menadione in the concentrate the steers were eating, and both groups were given the same quantity of concentrate. The Jersey breed is a smaller type of cattle than the Norwegian Red which was reflected by lighter carcass weights, Table 1. Differences in MK4 content could theoretically have been a result of unequal consumption of feed but should then have been opposite for the breeds. The steers were slaughtered either at 18 or 23 months age, but no age-effect was observed with respect to vitamin content in the muscles.

The study also showed a highly significant correlation ($r = 0.44$, $P < 0.001$) between intramuscular fat content (IMF) and phylloquinone, whereas no such relationship was found between IMF and the content of MK4. Similar results were reported by Elder et al. (2006) when they analysed ground beef with various contents of fat. The difference between K1 and MK4 supports the novel observation of this study that the content of phylloquinone and MK4 differs between these breeds. If the concentration of both forms of the vitamin had been correlated with IMF, then the breed effect could have been explained by the generally higher fat content found in Jersey. The present samples obtained from Jersey had an average of 6.9% fat which was significantly higher ($P = 0.003$) than the 5.5% in the Norwegian Red samples.

It is well documented that vitamin K1 is a precursor that can be converted to MK4 in most tissues of rodent (Al Rajabi et al., 2012; Okano et al., 2008). It is likely that ruminants have similar enzymes, and therefore the same processes take place. However, menaquinones are also synthesized by microbiota in the rumen (Conly & Stein, 1992). More than 40 years ago, Matschiner (1970) and Matschiner and Amelotti (1968) detected long chain menaquinones (MK10–MK13) in both bovine liver and rumen. In the present study, none of these long chain menaquinones were detected which may indicate that these vitamins have their main function in the liver.

Although the measured contents of vitamin K2 in beef are low compared with other products like green leafy vegetables and natto, it still could make a significant contribution to human nutrition. A typical dinner composed of 200-g beef could give approximately 10 μg of vitamin K. Recommended intakes of 90–120 μg/d are much higher, but the recommendations are based on vitamin contents in foods and do not take into account the bioavailability. Little is known about the bioavailability of vitamin K from foods, but the food matrix, the dietary fat content and the length of the isoprene chain in the vitamin K molecule may all affect the absorption (Ball, 1998). Vitamin K2 present in the lipid fraction of foods is likely to be incorporated into mixed micelles and absorbed, whereas less than 10% of vitamin K1 in green vegetables seems to be absorbed in the digestive tract, probably due to the tight binding to membranes of the chloroplasts. Hence, although vitamin K2 in the diet does not account for more than 10–20% of total vitamin K intake, its contribution to the biological activity may be much higher (Iwamoto, Sato, Takeda, & Matsumoto, 2009).

In conclusion, MK4 levels in different muscles from Norwegian Red and Jersey vary significantly. The results indicate that BF muscle from Jersey has nearly 170% more MK4 than PM muscles from Norwegian Red. However, more research is needed to confirm the present findings and evaluate the potential of other breeds in order to improve vitamin K2 content in meat from cattle.

5. Uncited reference Q2

Medicine, I. o., 2001

Acknowledgments

The financial support from the Foundation for Research Levy on Agricultural Products in Norway and Prima Gruppen AS, which is a Norwegian meat processing company, is highly appreciated. The authors also want to thank Frank Lundby, Nofima, Norway, for his contribution during NMR analyses of fat content.

Contribution of each author to the manuscript

Rune Rødbotten: Design of experiment, collection of samples, analysis and interpretation of data, writing the manuscript.

Bente Kirkhus: Design of experiment, analysis and interpretation of data, writing the manuscript.

Thomas Gundersen: Design of experiment, analysis of vitamin K, revising the manuscript.

Cees Vermeer: Analysis of vitamin K, interpretation of data, revising the manuscript.

References

Al Rajabi, A., Booth, S. L., Peterson, J. W., Choi, S. W., Suttie, J. W., Shea, M. K., Miao, B., Grusak, M.A., & Fu, X. Y. (2012). Deuterium-labeled phylloquinone has tissue-specific conversion to menaquinone-4 among Fischer 344 male rats. *Journal of Nutrition*, *142*(5), 841–845.

Arihara, K. (2006). Strategies for designing novel functional meat products. *Meat Science*, *74*(1), 219–229.

Ball, G. F. M. (1998). Vitamin K. *Bioavailability and analysis of vitamins in foods* (pp. 241–263). UK: Chapman&Hall.

Beulens, J. W. J., Bots, M. L., Atsma, F., Bartelink, M., Prokop, M., Geleijnse, J. M., Witteman, J. C. M., Grobbee, D. E., & van der Schouw, Y. T. (2009). High dietary menaquinone intake is associated with reduced coronary calcification. *Atherosclerosis*, *203*(2), 489–493.

Booth, S. L., Sadowski, J. A., & Pennington, J. A. T. (1995). Phylloquinone (vitamin K-1) content of foods in the US-Food-and Drug-Admininstration total diet study. *Journal of Agricultural and Food Chemistry*, *43*(6), 1574–1579.

Booth, S. L., & Shea, M. K. (2008). Update on the role of vitamin K in skeletal health. *Nutrition Reviews*, *66*(10), 549–557.

Bügel, S. (2008). Vitamin K and bone health in adult humans. *Vitamins and Hormones, Vol. 78*, . : Elsevier.

Conly, J. M., & Stein, K. (1992). The production of menaquinones (vitamin-K2) by intestinal bacteria and their role in maintaining coagulation homeostasis. *Progress in Food and Nutrition Science*, *16*(4), 307–343.

Damon, M., Zhang, N. Z., Haytowitz, D. B., & Booth, S. L. (2005). Phylloquinone (vitamin K-1) content of vegetables. *Journal of Food Composition and Analysis*, *18*(8), 751–758.

Davidson, R. T., Foley, A. L., Engelke, J. A., & Suttie, J. W. (1998). Conversion of dietary phylloquinone to tissue menaquinone-4 in rats is not dependent on gut bacteria. *Journal of Nutrition*, *128*(2), 220–223.

Elder, S. J., Haytowitz, D. B., Howe, J. R., Peterson, J. W., & Booth, S. L. (2006). Vitamin K contents of meat, dairy, and fast food in the US diet. *Journal of Agricultural and Food Chemistry*, *54*(2), 463–467.

Erkkila, A. T., & Booth, S. L. (2008). Vitamin K intake and atherosclerosis. *Current Opinion in Lipidology*, *19*(1), 39–42.

Ferguson, L. R. (2010). Meat and cancer. *Meat Science*, *84*(2), 308–313.

Gast, G. C. M., de Roos, N. M., Sluijs, I., Bots, M. L., Beulens, J. W. J., Geleijnse, J. M., Witteman, J. C., Grobbee, D. E., Peeters, P. H. M., & van der Schouw, Y. T. (2009). A high menaquinone intake reduces the incidence of coronary heart disease. *Nutrition, Metabolism, and Cardiovascular Diseases*, *19*(7), 504–510.

374 Iwamoto, J., Sato, Y., Takeda, T., & Matsumoto, H. (2009). High-dose vitamin K supplementation reduces fracture incidence in postmenopausal women: A review of the literature. *Nutrition Research*, *29*(4), 221–228.
377 Koivu-Tikkanen, T. J., Ollilainen, V., & Piironen, V. I. (2000). Determination of phylloquinone and menaquinones in animal products with fluorescence detection after postcolumn reduction with metallic zinc. *Journal of Agricultural and Food Chemistry*, *48*(12), 6325–6331.
381 Linseisen, J., Nimptsch, K., & Rohrmann, S. (2008). Dietary intake of vitamin K and risk of prostate cancer in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Heidelberg). *American Journal of Clinical Nutrition*, *87*(4), 985–992.
385 Matschiner, J. T. (1970). Characterization of vitamin K from the contents of bovine rumen. *Journal of Nutrition*, *100*(2), 190–192.
387 Matschiner, J. T., & Amelotti, J. M. (1968). Characterization of vitamin K from bovine liver. *Journal of Lipid Research*, *9*(2), 176–179.
Q4 Q3 Medicine, I. o. (2001). *Dietary reference intakes: For vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, manganese, molybdenum, nickel, silicon, vanadium, and zinc* (Washington, DC).
392 Ogawa, M., Nakai, S., Deguchi, A., Nonomura, T., Masaki, T., Uchida, N., Yoshiji, H., & Kuriyama, S. (2007). Vitamins K2, K3 and K5 exert antitumor effects on established colorectal cancer in mice by inducing apoptotic death of tumor cells. *International Journal of Oncology*, *31*(2), 323–331.
396 Okano, T., Shimomura, Y., Yamane, M., Suhara, Y., Kamao, M., Sugiura, M., & Nakagawa, K. (2008). Conversion of phylloquinone (vitamin K-1) into menaquinone-4 (vitamin K-2) in mice. *Journal of Biological Chemistry*, *283*(17), 11270–11279.
399 Olson, R. E. (2000). Osteoporosis and vitamin K intake. *American Journal of Clinical Nutrition*, *71*(5), 1031–1032.
401 Rødbotten, R., Lea, P., & Hildrum, K. I. (2001). Relative influence of low-voltage electrical stimulation, chilling rate and ageing time on tenderness of M. longissimus dorsi of Norwegian cattle. *Acta Agriculturae Scandinavica Section A Animal Science*, *51*(3), 184–191.
405 Saito, E., Wachi, H., Sato, F., Sugitani, H., & Seyama, Y. (2007). Treatment with vitamin K-2 combined with bisphosphonates synergistically inhibits calcification in cultured smooth muscle cells. *Journal of Atherosclerosis and Thrombosis*, *14*(6), 317–324.
408 Schurgers, L. J., & Vermeer, C. (2000). Determination of phylloquinone and menaquinones in food—Effect of food matrix on circulating vitamin K concentrations. *Haemostasis*, *30*(6), 298–307.
411 Shearer, M. J., & Newman, P. (2008). Metabolism and cell biology of vitamin K. *Thrombosis and Haemostasis*, *100*(4), 530–547.
413 Sørland, G. H., Larsen, P.M., Lundby, F., Rudi, A. P., & Guiheneuf, T. (2004). Determination of total fat and moisture content in meat using low field NMR. *Meat Science*, *66*(3), 543–550.
416 Vermeer, C., & Braam, L. (2001). Role of K vitamins in the regulation of tissue calcification. *Journal of Bone and Mineral Metabolism*, *19*(4), 201–206.
418 Vos, M., Esposito, G., Edirisinghe, J. N., Vilain, S., Haddad, D.M., Slabbaert, J. R., Van Meensel, S., Schaap, O., De Strooper, B., Meganathan, R., Morais, V. A., & Verstreken, P. (2012). Vitamin K-2 is a mitochondrial electron carrier that rescues pink1 deficiency. *Science*, *336*(6086), 1306–1310.
422 Weiss, J., Gibis, M., Schuh, V., & Salminen, H. (2010). Advances in ingredient and processing systems for meat and meat products. *Meat Science*, *86*(1), 196–213.
424 Witteman, J. C. M., Geleijnse, J. M., Vermeer, C., Grobbee, D. E., Schurgers, L. J., Knapen, M. H. J., van der Meer, I. M., & Hofman, A. (2004). Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: The Rotterdam Study. *Journal of Nutrition*, *134*(11), 3100–3105.
428 Yoshida, M., Jacques, P. F., Meigs, J. B., Saltzman, E., Shea, M. K., Gundberg, C., Dawson-Hughes, B., Dallal, G., & Booth, S. L. (2008). Effect of vitamin K supplementation on insulin resistance in older men and women. *Diabetes Care*, *31*(11), 2092–2096.

432

UNCORRECTED PROOF

Please cite this article as: Rødbotten, R., et al., Vitamin K2 in different bovine muscles and breeds, *Meat Science* (2014), http://dx.doi.org/10.1016/j.meatsci.2014.01.005