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Feeding potentially health promoting nutrients to finishing bulls changes meat composition and allow for product health claims

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ABSTRACT

Supplementation of feed for bulls with selenium (+50%), vitamin D_3 (+300%), vitamin E (+825%), vitamin K_3 (+325%) and omega-3 fatty acids (+120%) affected beef nutrient composition. Twelve bulls (½ year old) were randomly allocated to two dietary treatments; control (Con) or supplemented (Sup), and fed 170 days pre-slaughter at an amount of 1% of body weight. Daily gain and feed efficiency were equal in the two groups. Homogenate meat from left forequarter in the Sup group contained more selenium (+26%), vitamin MK4 (+123%), D (+197%), E (+318%), and had lower omega-6/omega-3 ratio (−24%) compared to Con meat. Sup meat fulfilled the requirements to be labelled by health claims and nutrient claims as: "A food item containing a significant amount of selenium, vitamin K and vitamin D". We suggest supplementation of cattle rations during the finishing period as a strategy to increase meat content of specific nutrients important to human health.

1. Introduction

Beef is an important dietary source of several essential nutrients such as protein of high biological value, iron, zinc, selenium, vitamins B_{12}, B_6 and K. The focus on the relationship between diet and health has led to increased interest in foods containing compounds that may affect health and diseases. The nutrient content in meat is affected by several factors, with composition of the animal diet as one important component. In Norway, a typical grass-land with only 3% cultivated land and limited potential for cereal and vegetable production, it is important to utilize the resources available to produce high quality food products from non-human food resources. The concerns for greenhouse gasses emitted by ruminants and the awareness for the environment are increasing. Due to this, meat and especially bovine meat, has lately been given much negative attention. Health aspects and environmental concerns of red meat intake has caused authorities in many countries to advice consumers to limit the intake of red meat (Australia - 2013, Brazil - 2014, Netherland - 2015, USA - 2015, Norway - 2016). Most consumers are knowledgeable of possible negative effects of red meat consumption on health such as risk of cancer and cardiovascular diseases (Alexander, Weed, Miller, & Mohamed, 2015; Oostindjer, Alexander, Amdam, Andersen, Bryan, Chen, et al., 2014), but results are conflicting and the subject is debated (Steppeler, Sødring, Egelandsdal, Kirkhus, Oostindjer, Alvseike, et al., 2017).

Supplementation of animal feed can be a cutting edge strategy to increase the intake of selected nutrients for humans. Earlier studies within this topic have primarily focused on the effect of different dietary fat sources on fatty acid composition in meat and eggs from monogastric species (Jiang et al., 2017a, b; Gjerlaug-Enger, Haug, Gaarder, Ljøkjel, Stenseth, Sigfridson, et al., 2015; Haug, Nyquist, Thomassen, Høstmark & Ostbye, 2014; Nyquist, Rodbotten, Thomassen & Haug, 2013; Shapira, Weill & Loewenbach, 2008) and in milk and meat from ruminants (Inglingstad, Skeie, Vegarud, Devold, Chilliard & Eknaes, 2017; Kliem, Humphries, Reynolds, Morgan & Givens, 2017; Bjorklund, Heins, DiCostanzo & Chester-Jones, 2014). Some studies report altered trace element- and vitamin content in products from monogastric- and ruminant animals (Han, Qin, Li, Ma, Ji &, Zhang et al., 2017; Jiang et al., 2017a, b; Burild, Lauridsen, Faqir, Sommer & Jakobsen, 2016; Gjerlaug-Enger et al., 2015; Hayes et al., 2015; Lawler, Taylor, Finley & Caton, 2004). In addition, others have made efforts to increase vitamin D in meat by exposing pigs to UVB light (Barnkob, Argyraki, Petersen & Jakobsen, 2016). However, to our knowledge, there is limited research on how diets supplemented with several different nutrients will affect nutrient composition and quality in bovine meat. When producing bovine meat, all efforts should be taken to obtain products that can benefit the health of meat consumers and offset the carbon footprints to the highest achievable degree.

The objective of the present study was to investigate the effect of

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increasing the level of selenium (Se), vitamin K, vitamin D_3, vitamin E and omega-3 fatty acids in the feed for finishing bulls on nutrient concentration and quality of the meat. Selenium, vitamin K, vitamin D, vitamin E and omega-3 fatty acids all have an impact on human health. Especially for children and youth, and women in childbearing age, an adequate intake of these nutrients are essential. In the Western diet, intakes of Se, vitamin D and omega-3 fatty acids may not be optimal (Rayman, Winther, Pastor-Barriuso, Cold, Thvilum, & Stranges 2018; Odin, 2017; Simopoulos, 2002). It is well known that Se and vitamin E are crucial for defence against oxidative damage and other important biological functions in the body (Comitato, Ambra & Virgili, 2017; Hosnedlova, Kepinska, Skalickova, Fernandez, Ruttkay-Nedecky, Malevu et al., 2017). The influence of adequate intake of long chain n-3 fatty acids and of a low ratio of n-6/n-3 fatty acids on health parameters is well documented (Simopoulos, 2002; Christophersen & Haug, 2011). In Europe there is a gap between the current intake of vitamin D and recommended intake (*NNR* 2012). Insufficient vitamin D status is especially prevalent in children/adolescents in northern latitudes due to lack of adequate sunlight in wintertime (Mortensen et al., 2016; Aspell, Lawlor & O'Sullivan, 2017; Laird, O'Halloran, Carey, Healy, O'Connor, Moore, et al., 2017), and this is also relevant for elderly persons (*NNR* 2012). An established cause and effect relationship exists between the dietary intake of vitamin K and the maintenance of bone structure and blood coagulation (EFSA, European Food Safety Authority, 2009a, b). In addition, vitamin K deficiency has lately been mentioned as a contributing factor in pulmonary and cardiovascular diseases (Janssen & Vermeer, 2017; van Ballegooijen & Beulens, 2017; Cundiff & Agutter, 2016). The vitamin K status, however, has not been thoroughly studied.

Meat is generally rich in important nutrients, but the content varies (Oostindjer, Egelandsdal, Hovland & Haug, 2016). In the present study we have examined whether supplementation of nutrients to feed concentrate during the finishing period of bulls can be a strategy to increase meat content of specific nutrients important to health of many groups such as children, youth, women in childbearing age and elderly.

2. Material and methods

The experiment was managed in accordance with Norwegian legislation controlling experiments with animals (Forskrift om bruk av dyr i forsøk, 2015). The animal experiment complied with the ARRIVE guidelines, and was carried out in accordance to the U.K. Animals Act 1986 and the EU Directive 2010/63/EU for animal experiments. The guidelines were followed throughout the study. The animals were transported and slaughtered at a commercial abattoir (Nortura SA division Rudshøgda, Norway) according to approved procedures from the Norwegian Food Safety Authority.

2.1. Dietary treatments and experimental design

The dietary treatments were two different concentrate mixtures, control (Con) and supplemented (Sup). The concentrates were formulated to be similar with regard to energy- (NEL_{20}), protein- (CP, AAT_{20} and PBV_{20}), starch and NDF content (EAAP publication No. 130, 2011). The Sup concentrate was added extra selenium (Se-yeast), vitamin K_3, vitamin D_3, vitamin E as RRR-alpha-tocopheryl acetate, and Rape seeds and Camelina seeds (*Camelina sativa*) as source of n-3 fatty acids. The ingredient composition and chemical content of the two experimental concentrates are reported in Tables 1, 2 and 3.

All animals were fed the same roughage (silage of grass and clover) ad libitum during the whole experimental period. The dry matter content (DM) of silage was 402 g/kg, crude protein was 112 g/kg DM, neutral detergent fibre (NDF) was 527 g/kg DM, crude fat was 40 g/kg DM, and net energy (NEL_{20}) was 6.64 MJ/kg DM.

Twelve bulls of the Norwegian Red breed (NRF) from Animal Production Experimental Farm at the Norwegian University of Life

Table 1
Formulation of the feed concentrates (%)[1,2].

	Con	Sup
Barley	20.0	24.2
Oats	13.6	8.0
Wheat	5.5	11.5
Wheat bran	12.0	4.7
Rye	10.0	9.0
Corn gluten meal	5.0	5.0
Soybean meal	6.3	0
Rapeseed meal, heat extracted	0	12.5
Faba beans, white	10.0	7.1
Camelina seeds	0	1.8
Rapeseeds	0	1.6
Beet pulp	5.0	5.0
Sugar cane molasses	4.5	4.5
Limestone powder	0.85	0.72
Mono-calcium phosphate	0.70	0.48
Sodium chloride	1.2	1.2
Vitamin/mineral premix 1[3]	2.0	0
Vitamin/mineral premix 2[3]	0	2.0
Magnesium oxide	0.26	0.24
Live yeast premix[4]	0.5	0.5
Soybean oil	2.6	0

[1] Experimental concentrates: Con (Control): Standard concentrate for growing ruminants. Sup (Supplemented): Concentrate for growing ruminants enriched with added extra selenium, vitamin K_3, vitamin D_3, vitamin E as RRR-α-tocoferyl acetate, and n-3 fatty acids from Rape seeds and Camelina seeds.
[2] Produced by Vestfoldmøllene, Norgesfôr, Norway, February 2016.
[3] Produced by Vilomix, Hønefoss, Norway.
[4] *Saccharomyces cerevisiae* I-1077 2×10^9 CFU/kg diet.

Table 2
Composition of the experimental feed concentrates (per kg feed)[1].

		Con	Sup
Calculated from ingredients			
Dry matter	g	870	870
Net energy lactation (NEL_{20})[2]	MJ	6.0	6.0
Crude protein	g	149	149
AAT_{N20}[2]	g	107	104
PBV_{N20}[2]	g	2.0	1.0
Crude fat	g	46.4	44.7
Starch	g	314	321
NDF	g	175	175
Linoleic/α-linolenic acid		9.3	2.0
Calcium	g	11.4	11.2
Phosphorous	g	5.4	5.5
Magnesium	g	3.1	3.2
Sodium	g	4.8	4.9
Additions (per kg feed)			
Vit A	1000 IU	4	4
Vit B_1	mg	0	0
Vit B_2	mg	0	0
Vit B_6	mg	0	0
Vit D_3	1000 IU	1	4
Vit E as all-rac α-tocopheryl acetate	IU	30	30
Vit E as RRR-α-tocopheryl acetate	IU	0	500
Vit K_3	mg	0	10
Selenium as sodium selenite	mg	0.2	0.2
Selenium as Se-yeast[3]	mg	0.0	0.5
Cu	mg	15	15
Mn	mg	30	30
Zn	mg	70	70
I	mg	3.5	3.5
Co	mg	0.4	0.4

[1] Experimental concentrates: Con (Control): Standard concentrate for growing ruminants. Sup (Supplemented): Concentrate for growing ruminants enriched with added extra selenium, vitamin K_3, vitamin D_3, vitamin E as RRR-alpha-tocopheryl acetate, and n-3 fatty acids from Rape seeds and Camelina seeds.
[2] Calculated by using the NorFor system, EAAP, 2011.
[3] Alkosel® R397 from Lallemand Animal Nutrition.

Table 3
Chemical analyses of diets[1] (values per kg feed).

			Con	Sup	Roughage
Dry matter	g		870	870	409
Selenium	µg		980	1460	21.5
Vitamin K	µg				
		K_1	126	108	1995
		MK4	< 1	< 1	< 1
		MK6	3.6	3.6	< 1
		MK7	7.9	8.9	< 1
		MK8	3.8	3.5	< 1
		K_3	54	230	
Vitamin E	mg				
		α-tocopherol	38.0	351.8	17.0
Crude fat	g		52.1	52.0	
		C16:0	6.6	5.0	
		C18:0	1.1	0.77	
		C18:1 n-9	9.4	14.0	
		C18:2 n-6	20.1	14.0	
		C18:3 n-3	1.8	3.9	
		C18:2/C18:3	11	3.6	

[1] Experimental concentrates: Con (Control): Standard concentrate for growing ruminants. Sup (Supplemented): Concentrate for growing ruminants enriched with added extra selenium, vitamin K_3, vitamin D_3, vitamin E as RRR-alpha-tocopheryl acetate, and n-3 fatty acids from Rape seeds and Camelina seeds.

Table 4
Effect of experimental diets[1] on feed intake, growth performance and carcass composition of finishing bulls the last 117 days before slaughter (predicted means ± SE).

Number of animals	Con $n = 6$	Sup n = 6	*P*-value
ADI[2] concentrate mixture (kg DM/day)	3.4 ± 0.03	3.4 ± 0.05	–
ADI[2] roughage (kg DM/day)	5.9 ± 0.32	7.1 ± 0.59	0.127
NE (MJ/day)	59.9 ± 4.6	67.7 ± 5.3	0.148
ADG [3] (kg/day)	1.62 ± 0.03	1.55 ± 0.04	0.196
Live weight at slaughter (kg)	526 ± 9	527 ± 14	0.971
Carcass weight (kg)	257 ± 6	264 ± 7	0.438
Dressing (%)[4]	48.8 ± 0.51	50.1 ± 0.15	0.053
Grading (EUROPE)[5]			
Conformation	4.8 ± 0.2	5.2 ± 0.1	0.397
Fatness	7.8 ± 0.3	7.5 ± 0.3	0.524

[1] Experimental concentrates: Con (Control): Standard concentrate for growing ruminants. Sup (Supplemented): Concentrate for growing ruminants enriched with added extra selenium, vitamin K_3, vitamin D_3, vitamin E as RRR-alpha-tocopheryl acetate, and n-3 fatty acids from Rape seeds and Camelina seeds.
[2] ADI: Average daily feed intake (kg DM).
[3] ADG: Average daily gain (kg BW/day).
[4] Carcass weight expressed as a % of live weight at slaughter.
[5] The classes were replaced by numerical values from 1 to 15. For conformation; higher values indicate higher muscular scores. For fatness; higher values indicate increased fatness.

Sciences, were used in the experiment. The bulls were raised on a diet of commercial concentrate and silage. The animals were randomly assigned into the dietary treatments, Con ($n = 6$) or Sup (n = 6) when they were 199 (± 14) days old. The experiment lasted for 170 days; they were slaughtered at 369 (± 14) days. Average initial body weight (BW) was 251 (± 2) kg and 256 (± 3) kg, and average final BW was 526 (± 2) kg and 527 (± 4) kg in the Con and Sup groups, respectively (Table 4).

The animals were electronically ear tagged, and they were housed in two pens with six animals per pen, three animals from each experimental group per pen. They had free access to water and roughage. The concentrate was fed at an amount of 1% of their BW per day from the start of the experiment at day 199, with a maximum level of 4 kg per day per animal. The electronic ear tag was read every time the bull visited the feeding station, and the right type and daily amount of concentrate was administered automatically over a 24-h period. There was a platform scale in each feeding station, allowing for frequent individual recording of BW. The individual intake of roughage was registered daily for the last 117 days.

2.2. Meat samples

After chill-aging (temperature programmed to be −5, 13, −7 and 2 °C) for 4 days post mortem, the left side of each carcass was quartered between the 12–13 rib, and the left forequarter were sent chilled to Animalia's pilot plant, Oslo. Trained cutters removed the *longissimus thoracis* muscle from each left forequarter ($n = 12$), and samples were taken out from a standardized area of the muscle and vacuumed for further analyses. The entrecote and flat iron steaks (in average 4 kg in each animal) were removed from the left forequarters and not included in the meat mince. The rest of the meat and fat tissue from the left forequarters was cut out and collected for grinding, giving one homogenate sample consisting of muscle and fat tissue of the six left forequarters in the two experimental groups. The tissues were minced to give homogenates containing about 14% fat as assessed by Qvision (Tomra Systems ASA, Asker, Norway). Samples were then taken out from different parts of the meat mince and homogenized further, and the homogenates were vacuumed, frozen at −20 °C and sent for chemical analysis.

2.3. Chemical analyses in meat

Chemical analysis were performed in the accredited laboratory of The Danish Veterinary and Food Administration (Fødevarestyrelsen, FVST, Århus, Danmark). Vitamin K was determined at the National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway. Both laboratories have routines for supplying analyses for Food Composition databases.

2.3.1. Fat and fatty acids

Fat concentration was analysed in 12 *longissimus thoracis* and two forequarter meat homogenates (SBR Fedt, BCR 163, FVST, Århus, Denmark). Fatty acids was determined in duplicate samples by ether/petroleum ether (bp 40–60 °C) extract from 2 g of *longissimus thoracis* samples and homogenate samples, transmethylated with the boron trifluoride-methanol complex (AOCS lipids, n.d). The methyl esters were separated on a capillary column SP2560, 100 m, ID 0.25 µm, film thickness 0.20 µm (Sigma-Aldrich, MO, United States) with a Agilent 6890 with FID detector (Agilent Technologies, Santa Clara, CA 95051, United States). The specified precision (2Sr) was ± 6.6%, and quantities < 0.1 g/100 g fatty acids were not reported.

2.3.2. Cholesterol

One to two grams of meat was used for determination of cholesterol concentration, and samples were taken in duplicate. The samples underwent alkaline hydrolysis leaving cholesterol unsaponificated. Cholesterol were separated on a phenyl column, and UPLC are analysed with an APCI (Atmospheric pressure chemical ionisation) LC-MS/MS Aquity TQD (Triple quadropole detector) from Waters Coporation (Massachusetts, United States). The precision (2Sr) was ± 9.8%.

2.3.3. Vitamin D

Vitamin D_3 (cholecalciferol) and 25(OH) vitamin D_3 (calcidiol); three grams of homogenate meat was used, and samples were taken in duplicate. Alcoholic KOH was used to extract non-saponificated lipids and transferred to pentane. The organic phase was dried under nitrogen, and dissolved in 1% formic acid in acetonitrile. The solution was purified on HybridSPETM (Supelco, Bellefonte, PA Analytical, available from Sigma-Aldrich (MO, USA). Derivatization was performed by 4-phenyl-1, 2, 4,-triazoline-3.5-dione, i.e. PTAD. The solution was

separated by supercritical fluid chromatography (SFC)-MS/MS on Acquity UPC [2] Xevo TQ-S MS/MS (Waters Milford, Massachusetts, United States). The precision (2Sr) was ± 8.2% for vitamin D_3 and ± 6.2% for 25(OH) vitamin D_3.

2.3.4. Vitamin E

α-tocopherol was determined in three to four grams of meat. Following saponification with alcoholic KOH, the un-saponificated part was transferred to pentane: ethyl acetate (80:20). The organic phase was dried under nitrogen, the un-saponificated part was dissolved in the eluent and separated on HPLC (Agilent 1100, Agilent Technologies; Santa Clara, CA 95051, USA). Tocopherols were detected fluorimetrically following excitation at 302 nm and emission at 331 nm. The content of α-tocopherol was quantified by using external standard. The precision (2Sr) was ± 2.5%.

2.3.5. Vitamin K

HPLC analysis was used for determination of vitamin K_1 and K_2 (MK4-MK7). 1 g of meat was analysed in duplicate samples. Fat was removed enzymatically using lipase and vitamin K was extracted. Vitamin K was separated on a C30 column with accompanying reduction of vitamin K hydroquinone in an electrochemical cell after separation in the column. The K vitamins were measured by a fluorescence detector. The method is based on the NS-EN 14148 (2003), Foodstuffs-Determination of vitamin K_1 by HPLC. Vitamin K_3 (Menadion) was determined by reverse phase HPLC- fluorescence detection (Vitamin, n.d, method no 340). The precision (2Sr) was ± 6.6%.

2.3.6. Selenium

Selenium concentration was determined in duplicate samples (Selenium, n.d, CEN 13805, 2014) by a method involving homogenization, destruction with nitric acid in a microwave oven and determination of Se on ICP-MS Agilent 7500cx ICP analysis instrument-MS from Agilent Technologies (Santa Clara, CA 95051, USA). The precision (2Sr) was ± 8.0%.

2.4. Instrumental measurements of meat quality

2.4.1. Tenderness and cooking loss

Twelve *longissimus thoracis*, vacuum-packaged and chill-stored (3 °C) for 3 weeks, were sliced (4 cm thickness) and heated in a hot water (at 75 °C) bath until the internal temperature of the pieces reached 72 °C, then cooled on ice to an internal temperature ~ 20 °C. Muscle samples (1x1x4 cm) were cut parallel to the fibre direction and sheared across the fibre direction. The number of replicates were 10 per animal. Slices were from the same anatomical location. Shear cell HDP/BSK Warner Bratzler (WB), load cell 25 kg and TA-HDi Texture Analyser, Stable Micro Systems (Godalming, UK) were used. The peak of the force (in N) versus distance curve was recorded and is presented as N/cm^2. Cooking loss was measured as the expelled juice from the 12 vacuum-packed *longissimus thoracis* samples. The cooking loss was weighed and given as % cooking loss: g juice lost * 100/ initial weight (g).

2.4.2. Colour stability

Meat colour (L* a* b* coordinates) was measured by spectrophotometer CM 700 d (Konica Minolta Sensing Inc., Osaka, Japan) on 12 *longissimus thoracis* slices chill-stored for 2 weeks with the illuminant D65 and a 2^O observer by L*a*b*. This is a filter instrument that can be used to measure reflectance spectra and thereby estimate surface myoglobin states (AMSA, 2012). The measurements were at 0 (before, B, still vacuum packed) and 40 min blooming (after, A) in individual trays wrapped with oxygen permeable transparent film. Three different places on the meat surface were measured.

2.4.3. Myoglobin equivalent analyses

The analyses were carried out following the analytical method described by Ginevra et al. (2002). Meat supernatants were extracted and the absorbance was measured at 407 nm against a reagent blank. Two repeated measurements and 8 replicates were used for each group (Sup and Con). Myoglobin solutions were used to make a linear standard curve and equivalent myoglobin concentrations were determined from the standard curve. The method determines hemin that originates from myoglobin and proteins like haemoglobin and other haem proteins.

2.5. Statistical analyses

The statistical model that was used was a balanced ANOVA with feed and pen (position in the barn) as main effects and their interactions. Predicted means with standard errors are given in tables. The pen (position in the barn) and the interaction effect with diet were never significant (i.e. $P > .05$, always) and is not referred to below. (The lowest P value for pen was 0.22, for the Warner Bratzler peak force value.) The statistical program used was the open code: The R Project for Statistical Computing (https://www.r-project.org/).

3. Results and discussion

3.1. Animal performance and carcass data

There were no effect of treatment on feed intake, growth performance and carcass grading. The small difference in dressing % between Sup and Con groups, 50.1% and 48.8%, respectively, was close to be significantly different ($P = .053$) (Table 4).

The effects of treatment on tenderness, cooking loss, colour stability and myoglobin equivalents of *longissimus thoracis* are shown in Table 5. The meat from both Con and Sup groups were considered as tender (Hildrum, Rødbotten, Høy, Berg, Narum & Wold, 2009). The small but significant difference in tenderness ($P = .039$) between the experimental groups is difficult to explain. The cooking loss was moderate, and there were no differences between the two experimental groups (Table 5).

The supplemented *longissimus thoracis* were darker (lower L*) while still vacuum-packed, had more redness (a*), and had the nominally highest myoglobin equivalents (hemin) content compared to Con. The a* values of bloomed samples were apparently on the low side. The amount of oxymyoglobin in the surface of the Sup sample was at average 59% with no spot above 81% oxymyoglobin calculated

Table 5
Effect of diet[1] on tenderness (WB peak), cooking loss, colour and myoglobin equivalents of *longissimus thoracis* (predicted means ± SE).

	Bloom- Ing[2]		Con $n = 6$	Sup n = 6	*P*-value
WB peak (N/cm^2)			33.0 ± 2.6	42.5 ± 1.4	*0.039*
Cooking loss %			12.8 ± 1.6	13.5 ± 1.4	0.765
Colour[2]	L*, lightness to	B	38.4 ± 0.5	35.8 ± 0.7	*0.009*
	darkness	A	40.8 ± 0.6	39.0 ± 0.6	0.084
	a*, red (+) to	B	5.4 ± 0.3	7.1 ± 0.2	*0.007*
	green (−)	A	13.1 ± 0.2	15.1 ± 0.6	*0.013*
	b*, yellow (+) to	B	9.3 ± 0.3	8.5 ± 0.3	0.172
	blue (−)	A	14.4 ± 0.3	14.4 ± 0.6	0.937
Myoglobin equiv. (g/kg meat)			3.8 ± 0.3	4.5 ± 0.3	0.120

[1] Experimental concentrates: Con (Control): Standard concentrate for growing ruminants. Sup (Supplemented): Concentrate for growing ruminants enriched with added extra selenium, vitamin K_3, vitamin D_3, vitamin E as RRR-α-tocopheryl acetate, and n-3 fatty acids from Rape seeds and Camelina seeds.

[2] Meat colour is determined by L* a* b* coordinates, by spectrophotometer CM 700 d (Konica Minolta Sensing Inc., Osaka, Japan) on 12 *longissimus thoracis* slices chill-stored for 2 weeks with the illuminant D65 and a 2^O observer. B is before blooming, while still vacuum packed, A is after blooming for 40 min.

Table 6
Effect of diet[1] on nutrient composition in 100 g meat (*longissimus thoracis*) (predicted means ± SE).

			Con	Sup	*P*-value
			n = 6	n = 6	
Selenium	µg		11.7 ± 0.3	15.2 ± 0.5	*< 0.001*
Vitamin K	µg	K_1	0.66 ± 0.07	1.05 ± 0.28	0.219
	µg	MK4	4.6 ± 0.3	11.4 ± 1.4	*0.006*
Vitamin E	µg	α-tocopherol	266 ± 26	677 ± 32	*< 0.001*
Crude fat	g		4.4 ± 0.6	5.4 ± 1.3	0.564
Cholesterol	mg		50.2 ± 0.4	49.5 ± 0.7	0.489
Fatty acids	g				
		C16:0	1.0 ± 0.2	1.2 ± 0.3	0.723
		C18:0	0.8 ± 0.1	1.1 ± 0.3	0.459
		C18:1 n-9	1.4 ± 0.2	1.6 ± 0.4	0.667
		C18:2 n-6	0.11 ± 0.01	0.12 ± 0.02	0.688
		C18:3 n-3	0.02 ± 0.002	0.03 ± 0.008	0.229
		C20:4 n-6	0.02 ± 0.0007	0.02 ± 0.0006	0.327
		C20:5 n-3	0.004 ± 0.0001	0.005 ± 0.0004	*0.039*
		C22:5 n-3	0.009 ± 0.0004	0.011 ± 0.0004	*0.029*
		C20:4n-6/C20:5n-3	5.8 ± 0.2	4.6 ± 0.4	*0.033*
		n-6/n-3	4.2 ± 0.1	3.1 ± 0.1	*< 0.001*
		C20:5n-3 + C22:5n-3	0.013 ± 0.0005	0.016 ± 0.0006	*0.009*

[1] Experimental concentrates: Con (Control): Standard concentrate for growing ruminants. Sup (Supplemented): Concentrate for growing ruminants enriched with added extra selenium, vitamin K_3, vitamin D_3, vitamin E as RRR-α-tocopheryl acetate, and n-3 fatty acids from Rape seeds and Camelina seeds.

according to AMSA (2012). The Con sample had at average 48% oxymyoglobin in the surface. This indicate surface oxidation to metmyoglobin during storage of the slices for 2 weeks. Some influence on the measurements of the underlying thin metmyoglobin layer, where oxygen is 1–2%, cannot be ruled out. Fully oxygenated (100%) oxymyoglobin would give a* ~ 19 for the method used here.

The myoglobin is at average about 3.7 g/L for young lean bull meat in Norway, i.e. we do not expect any national negative consumer response for meat produced here.

The nominally lower b* for Sup combined with nominally higher myoglobin may hint at a better antioxidative capacity for the Sup samples (Table 5), i.e. a larger tendency for an increased fraction of deoxymyoglobin.

3.2. Concentration of selenium, vitamins K, E, D and omega-3 in Sup and Con meat

The effects of treatment on concentration of selenium, vitamin K_1 and MK4, vitamin E (α-tocopherol) and fatty acids in *longissimus thoracis* are shown in Table 6. The concentrations were significantly higher in the Sup meat compared to the Con meat of selenium (+30%), vitamin MK4 (+148%), vitamin E (α-tocopherol) (+155%), C20:5n-3 (+25%) and C22:5n-3 (+22%), and the fatty acid ratio n-6/n-3 was lower (−35%) in the Sup meat compared to Con meat ($P \leq .05$) (Table 6). For the homogenate samples of Sup and Con meat, there were also roughly the same differences between the two experimental groups; the concentration of selenium (+26%), vitamin MK4 (+123%), vitamin D (vit D_3 and 25-OH-D_3) (+197%), vitamin E (α-tocopherol) (+318%) and the n-6/n-3 fatty acid ratio (−24%) (Table 7), but due to the design, statistical analyses could not be done for the homogenate meat.

3.2.1. Selenium

The concentration of Se in *longissimus thoracis* was higher in the Sup than in the Con meats ($P < .001$), (Table 6), and the Se concentration in the Sup homogenate was also higher than in the Con homogenate (Table 7). The dietary level of selenium was 50% higher in the Sup- compared to the Con feed concentrate (Table 3). The increase in Se in Sup meat compared to Con meat were + 30% in longissimus meat and + 26% in homogenate meat, and we consider that the increase in muscle Se was roughly as could be expected from the increase in feed Se.

Table 7
Effect of diet[1] on nutrient composition in 100 g meat homogenate from the forequarter (one homogenate per group).

			Con	Sup
Selenium	µg		10.0	12.6
Vitamin K	µg	K_1	2.1	2.0
	µg	MK4	9.1	20.3
Vitamin D	µg	D_3	< 0.012	0.040
	µg	25-OH-D_3	0.100	0.293
Vitamin E	µg	α-tocopherol	156.5	654.0
Crude fat	g		13.6	14.9
Fatty acids	g			
		C16:0	3.4	3.5
		C16:1 n-7	0.37	0.42
		C18:0	2.6	2.8
		C18:1 n-9	5.0	5.6
		C18:2 n-6	0.27	0.25
		C18:3 n-3	0.06	0.07
		C20:4 n-6	0.03	0.03
		C20:5 n-3	0.006	0.006
		C22:5 n-3	0.02	0.02
		C20:4 n-6/C20:5 n-3	5.2	4.5
		n-6/n-3	3.6	2.9
		C20:5 n-3 + C22:5 n-3	0.023	0.025

[1] Experimental concentrates: Con (Control): Standard concentrate for growing ruminants. Sup (Supplemented): Concentrate for growing ruminants enriched with added extra selenium, vitamin K_3, vitamin D_3, vitamin E as RRR-α-tocopheryl acetate, and n-3 fatty acids from Rape seeds and Camelina seeds.

In the Sup and Con homogenates of left forequarter, the Se concentrations were somewhat lower than the average Se concentrations in *longissimus thoracis*. This could partly be explained by the higher fat content in the homogenate meat that might dilute the Se content in muscle.

The Se concentration in the present study is higher than in grinded meat (with 14% fat) from 70 Norwegian Red bulls and cows that was shown to be 7.9 (± 2.0) µg Se/100 g, with a variation from 3.5 to12.6 µg Se/100 g (Oostindjer et al., 2016). The recommendation for Se intake in humans is 55 µg/day (RDA). 100 g of meat from the Con and Sup- groups in the present study would provide from 18% to 28% of the RDA, respectively, Tables 6, 7 and 8. When a food item contains > 15% of the recommended allowance specified by the Council of the European Communities, (1990), the food can be classified as “A food item containing a significant amount of selenium”, and the meat

Table 8
RDA values for selenium, vitamin K and vitamin D, concentration of selenium, vitamin K and vitamin D in 100 g Con and Sup homogenate meat, and classification* as "A food item containing a significant amount of the nutrient" [1], "A food item being a very good source of the nutrient" [2] and qualifications for health claims of the nutrient.

	Selenium	Vitamin K	Vitamin D[3]
RDA, n.d	55 μg	75 μg	5 μg
100 g Con homogenate meat	10 μg	11.2 μg	0.5 μg
100 g Sup homogenate meat	12.6 μg	22.3 μg	1.5 μg
Con Classified*	Yes[1]	Nearly	No
Sup Classified*	Yes[1]	Yes[1]	Yes[2]
Con Health claim	Yes	Nearly	No
Sup Health claim	Yes	Yes	Yes

[1] When a food item contains > 15% of the RDA, it classifies as "A food item containing a significant amount of the nutrient".
[2] When a food item contains > 30% of the RDA, it classifies as "A very good source of the nutrient".
[3] Vitamin D equivalents in meat is calculated as 25(OH) - vitamin D_3 having 5 times the biological activity of vitamin D.

from both Sup and Con groups in the present study qualified to use this label (Table 8). This meat can also be given a health claim, since the EFSA panel has concluded a cause and effect relationship between the dietary intake of Se and protection of DNA, proteins and lipids from oxidative damage, normal function of the immune system, normal thyroid function and normal spermatogenesis (EFSA, Selenium). Protection of DNA, proteins and lipids is generally linked to prevention of cancers, which is in demand, and good thyroid function and spermatogenesis are also crucial (McCann & Ames, 2011; Skakkebaek 2016). The strategy with Se-fortification of feed has a potential to make a contribution to human Se intake and subsequently to health.

3.2.2. Vitamin K

Vitamin K occurs naturally in two forms. Vitamin K_1 (phylloquinone) is synthesized by plants, while vitamin K_2 (menaquinones, MKs) primarily are produced by bacteria (*NNR*, 2012). Both forms are found in animal tissues. A synthetic, stable variant, vitamin K_3, is used in animal feed. Vitamin K_3 is converted in the body to MK4 (Elder, Haytowitz, Howe, Peterson, Booth 2006; Suttie, 1995; Thijssen & Drittij-Reijnders, 1994).

The content of vitamin K (sum of K_1 and MK4) in *longissimus thoracis* was 12.5 μg/100 g in the Sup group compared to 5.3 μg/100 g in the Con group. In the homogenate meat the concentration was 22.3 and 11.2 μg/100 g, respectively (Tables 6 and 7). The concentration of vitamin K in *longissimus thoracis* in Con is in accordance with the findings by Rødbotten, Gundersen, Vermeer & Kirkhus, (2014), showing 3.6 μg vitamin K/100 g *longissimus thoracis* in NRF steers.

The RDA for vitamin K is 1 μg/kg BW, in average 75 μg (*NNR*, 2012). Thus, meat containing > 11.3 μg vitamin K per 100 g can be classified as a significant source of vitamin K (the Council of the European Communities, 1990). Meat from the Sup group (*longissimus thoracis* and homogenate) was above this value, and could thus be entitled to the classification of being a significant source of vitamin K (Table 8). When a food item contain > 30% of the RDA of vitamin K per 100 g (i.e. 22.5 μg) it can be classified as a very good source of vitamin K (the Council of the European Communities, 1990). In the present study, the homogenate meat from the left quarter front part in the Sup group was very close to this label, containing 22.3 μg/100 g. The control meat homogenate was 11.2 μg/100 g, and was close to be classified as a significant source of vitamin K. According to the EFSA Journal, health claims can be carried by food items that contain > 15% of RDA of vitamin K, i.e. > 11.3 μg/100 g. Therefore, both the *longissimus thoracis* and the homogenate meat from the Sup group are entitled to bear the health claim for vitamin K: "This meat contains enough vitamin K for the maintenance of normal bone and normal blood coagulation" (EFSA, European Food Safety Authority, 2009a, b) (Table 8). Since Norwegian elderly are at high risk for bone fractures and osteoporosis (Grønskag, 2011), this claim is of high relevance to health, and this knowledge should be recognized and consumers should be informed. The present study shows that vitamin K_3 supplementation to feed is a way to increase the vitamin MK4 level in meat. Further studies should be done to establish supplementation doses and feeding practices that with certainty can produce meat with elevated levels of vitamin K.

3.2.3. Vitamin E

Vitamin E activity for humans is confined to α-tocopherol, which is the required nutrient for humans (*NNR, Nordic nutrition recommendations*, 2012). The concentration of α-tocopherol in *longissimus thoracis* was 266 and 677 μg/100 g in the Con and Sup groups, respectively ($P < .001$, Table 6). In the corresponding homogenate meat the levels were 157 and 654 μg/100 g (Table 7). The RDA for vitamin E is 12 mg, (*NNR*, 2012), and the meat cannot be classified as a significant source of vitamin E. However, the stability of colour and drip loss may be affected by the vitamin E concentration in meat (O'Sullivan, Byrne, Stagsted, Andersen & Martens, 2002; Bloomberg, Hilton, Hanger, Richards, Morgan & VanOverbeke, 2011; Liu, Lanari & Schaefer, 1995). There were no differences in cooking loss between the two groups in the present study (Table 5), but the higher redness (a*) observed in the Sup meat (Table 5), has also earlier been linked to vitamin E supplementation (O'Sullivan et al., 2002).

3.2.4. Vitamin D

Vitamin D_3 is synthesized from 7-dehydrocholesterol in the skin by UVB radiation. Vitamin D is also present in plants, feeds and animals. Vitamin D_3 is rapidly converted to 25(OH) - vitamin D_3 (calcidiol) in the liver of animals, both when ingested and formed in the skin. Calcidiol is the storage form of vitamin D_3 in the body, and calcidiol is further activated to 1,25$(OH)_2$ -vitamin D_3 in the kidney. Animal products such as meat and eggs primarily contain calcidiol. There is an ongoing discussion on how the biological activity of the calcidiol should be evaluated when present in human foods. Fødevareinstituttet (DTU, Denmark) states that 1 μg vitamin D = 1 μg vitamin D_3 = 1 μg vitamin D_2 = 0.2 μg 25(OH) vitamin D_3 = 0.2 μg 25(OH) vitamin D_2. This calculation is based on information from several studies (Jetter, Egli, Dawson-Hughes, Staehelin, Stoecklin, Goessl, et al., 2014; Cashman, Seamans, Lucey, Stöcklin, Weber, Kiely, et al., 2012; Jakobsen, 2007; Blunt, Tanaka & DeLuca, 1968), and is also used in the Seventh edition of McCance and Widdowson's "The Composition of Foods" (2015).

The RDA for vitamin D is 5 μg/day. Beef meat has not generally been regarded as a good source of vitamin D even when extra vitamin D is supplemented in the feed. When the 5 times higher biological value of calcidiol is taken into consideration, the homogenate Sup-meat contained 1.5 μg vitamin D equivalents per 100 g meat, and will consequently supply 30% of RDA for vitamin D, and qualify to be classified as a very good source for vitamin D (Table 8). EFSA has concluded that a cause and effect relationship has been established between the dietary intake of vitamin D and contribution to normal development of bones and teeth (EFSA 2014). Thus, the Sup-meat homogenate fulfils the requirements to bear the health claim for vitamin D (Table 8). Actually, the Sup bulls that were given 4000 IU of vitamin E in the feed (upper acceptable EU limit), had a 3 times higher concentration in total vitamin D [(vitamin D_3 + (25-OH vitamin D_3 × 5)] in beef homogenate compared to the controls (Con was given 1000 IU). In a population where both children, young and older adults have low vitamin D intakes and insufficient vitamin D status (*NNR* 2012, Aspell et al., 2017, Laird et al., 2017, McCance and Widdowson's, 2015), this study demonstrates a tool that seems to be simple to implement both for the nutrition authority and agriculture in order to increase the vitamin D intake among people. In the present study vitamin D was only analysed in the meat homogenates of left forequarters, and not in single meat

cuts. The concentration of vitamin D has been shown to vary between meat cuts in beef (Purchas, Zou, Pearce & Jackson, 2007), thus the vitamin D concentrations given in the present study does not represent the vitamin D concentration in total beef meat. Further, more studies could be necessary to validate the €significance of 25(OH) -vitamin D from animal products on vitamin D status in humans.

3.2.5. Fat and fatty acids

The meat fat percent was not different between the two meat groups Sup and Con, and the concentration in the meat of cholesterol and fatty acids with chain length C16 and C18 was not different between the two groups (Tables 6 and 7).

In the present study we aimed at a higher content of n-3 fatty acids- and a lower n-6/n-3 fatty acid ratio in the meat by altering the feed composition by including Rape- and Camelina seeds rich in α-linolenic acid (C18:3 n-3, ALA) in the feed, and reduction of soybean containing linoleic acid (C18:2 n-6, LA). LA and ALA are essential fatty acids that can be elongated and desaturated further in the body (*NNR* 2012). Adequate intake of n-3 fatty acids and a low ratio n-6/n-3 fatty acids are important for health, but is often suboptimal in a typical Western diet (Simopoulos, 2002, Christophersen & Haug, 2011).

The Sup feed contained more than twice as much ALA, and much less LA, compared to the Con feed concentrate, and the n-6/n-3 fatty acid ratio was 3.6 and 11, respectively (Table 3). The fatty acid concentration in *longissimus thoracis* showed higher ALA, C20:5 n-3 and C22:5 n-3 in the Sup- compared to the Con meat, and the ratio between n-6 and n-3 fatty acids was lower in the Sup- compared to the Con meat, being 3.1 and 4.2, respectively, but the differences between the two meat types were not large (Table 6). These results were confirmed by the fatty acid concentration in meat homogenate from forequarter (Table 7). The less than expected differences between the two experimental groups in n-3 fatty acid composition can partly be explained by biohydrogenation of unsaturated fat in the rumen. The effect of feeding different amounts of polyunsaturated fatty acids in the diet may thus be hidden, as reported by Scollan, Dannenberger, Nuernberg, Richardson, MacKintosh, Hocquette, et al. (2014) reviewing beef lipids in bulls with different feeding regimes. Another explanation could be that the Rape- and Camelina seeds were administered as whole seeds in the pelleted concentrate diet, and it is possible that the fat in the seed have not been fully digested since the seed capsule is resistant. The digestibility of the seeds was not determined in the present study.

3.2.6. The extra cost of the supplemented carcass

The extra cost of the carcass when supplementing Se (organic form), vitamin E, vitamin D vitamin K and omega-3 rich seeds to the feed for 170 days prior to slaughter, can only be given as a rough estimate since the market prize for these supplements are varying. Based on prices on feed ingredients in Norway June 2018 an extra cost of 1–2% per carcass, (€ 15–30) can be expected. When supplementing only vitamin D and K the corresponding increase in carcass cost is < 1%.

4. Conclusion

This study showed that supplementation of selenium, vitamin K, vitamin D, vitamin E and omega-3 fatty acids in the diet to finishing bulls resulted in higher content in the meat. The meat from supplemented bulls could be classified and labelled as: “A food item containing a significant amount of selenium, vitamin K and vitamin D" in addition to bear the health claims for selenium, vitamin K and vitamin D. We suggest that supplementation of bull rations during the finishing period is a promising strategy to increase meat concentration of essential nutrients to reach the required levels for classification as a significant source and to give specific health claims. The labelling on meat packages may raise the awareness of red meat as a valuable food ingredient important to health of many, especially to children and youth, women in childbearing age and elderly.

Conflict of interest

The authors confirm that there are no known conflicts of interest associated with this publication.

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