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Immunological effects of feeding macroalgae and various vitamin E supplements in Norwegian white sheep-ewes and their offspring

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ABSTRACT

It is assumed that ewes raised in areas with long indoor winter feeding periods need to be supplemented with vitamins or other substances that help to maintain the health status of the animals. Various supplements are available on the market, but the most widely used supplemental antioxidant and vitamin E source is synthetic *all-rac*-α-tocopheryl acteate. The objective of the present study was to compare potential vitamin E and immune stimulant sources with synthetic vitamin E regarding bioactivity associated with immunological parameters in order to identify alternatives to synthetic vitamin E for small ruminants. Sources tested were meal of the seaweed *Ascophylum nodosum* and natural RRR-α-tocopheryl acetate. Forty pregnant ewes were randomly allocated to four treatment group with two replicates (5 ewes in each replicate). The treatments were supplements containing seaweed (SW: 546 g *Ascophylum nodosum*/kg), natural vitamin E (NatE: 562.5 mg RRR-α-tocopheryl acetate/kg), synthetic vitamin E (SyntE: 1125 mg *all-rac*-α-tocopheryl acetate/kg), or no extra seaweed or vitamin E (control). The supplements were fed at an isoenergetic daily rate, on average 144 g DM/ewe for SW and 114 g DM/ewe for the three other treatments, from mating until start grazing season (200 days). It was assumed that 0.5 mg RRR- α-tocopheryl acetate was equal to 1 IU=1 mg *all-rac*-α-tocopheryl acetate. The planned supplementation in the SyntE and NatE treatments were 140 IU vitamin E/ewe daily, and the daily supplemental rate was on average for the whole experimental period 20, 146, 121 and 4 IU in SW, SyntE, NatE and C treatments, respectively. The ewes and their newborn lambs were monitored throughout the entire indoor feeding period. Supplementing pregnant ewes with natural vitamin E had a positive effect on immunity against *Mycobacterium* sp. in the lambs, whereas supplementing ewes with seaweed interfered with the passive immunity of the offspring resulting in a mortality rate of 35%, compared with 10% in C, 5.6% in NatE and 0% in SyntE. The adaptive immunity of the lambs was not affected by seaweed supplementation. In the

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ewes, it seemed that supplementation with either seaweed, natural vitamin E or synthetic vitamin E had no beneficial health effects, and serum IgG concentrations were reduced in the seaweed treatment group.

1. Introduction

Preserved forages such as hay and silage often have low concentrations of important vitamins. In general, winter-fed sheep on organic farms in Norway have low blood vitamin E (vitE) and selenium (Se) (Govasmark et al., 2005). There is no placental transfer of immunoglobulins (Ig) and fat-soluble vitamins in ruminants, and thus the newborns depend on intestinal uptake of colostral Ig for passive immunization and vitE (Hemmings, 1976; Campbell et al., 1977; Gilbert et al., 1988; Sangild, 2003). Accordingly, it is recommended that ruminants on preserved forage diets be given a vitE supplement, particularly during the peripartum period. In organic production, such supplements should be of natural origin (IFOAM, 2007), and hence it is important to find alternatives to synthetic supplements.

VitE is the most important lipid-soluble antioxidant in animals (Scherf et al., 1996), but Se is a part of the antioxidative enzyme glutathione peroxidase (GSH-PX) and acts similar as vitamin E but at the aqueous phase instead of the lipid layer. Therefore, it is important to supplement feed with both vitE and Se to maintain good immune status in livestock (Finch and Turner, 1996). Dietary supply of both vitE and Se is important for antibody and cytokine production, cell proliferation, prostaglandin metabolism and neutrophil function both in cattle (Smith, 1986) and sheep (Larsen et al., 1988a, 1988b). The vitE exists mainly in two commercially available forms; namely, RRR-α-tocopheryl acetate (natural, derived from vegetable oil) and all-rac-α-tocopheryl acetate (synthetic, α-tocopherol produced by chemical synthesis). It is well established that RRRα-tocopheryl acetate has higher bioavailability than all-rac-α-tocopheryl (Hidiroglou et al., 1992; Jensen and Lauridsen, 2007).

Seaweeds are a rich source of Se and antioxidants such as substituted phenols, polyphenols, vitamins, and vitamin precursors (Allen et al., 2001; Kuda et al., 2005; Devi et al., 2008). Furthermore, seaweeds and seaweed extracts have been shown to stimulate immune function (Liu et al., 1997; Allen et al., 2001; Saker et al., 2004). The brown algea *Ascophyllum nodosum*, which is the most widely utilized seaweed in agriculture, is harvested along the coast of the North Atlantic Ocean and has been used as feed supplement for centuries (Allen et al., 2001; Indergaard, 2010). Supplementation with *Ascophyllum nodosum* meal has shown to have positive effect of dairy cow performance (Jensen et al., 1968), but the effect of long-term supplementation of *Ascophyllum nodosum* on immune status of ewes and their off-springs has not been tested, as far as we know.

The aim of the present study was to compare the effects of supplementing pregnant ewes with seaweed meal or synthetic or natural vitE on transferred and acquired immunity when fed a basal diet of preserved forage during the indoor winter period. One hypothesis tested was that supplementing the diet of ewes with seaweed and vitE would improve the immune status of the offspring. A second hypothesis was that natural vitE would be more efficient than synthetic vitE in improving the immunity of the lambs.

2. Materials and methods

2.1. Animals

A feeding trial was conducted in a commercial sheep herd on the island of Mindland in Northern Norway (65°46°N, 12°28°E). The experiment lasted the entire indoor feeding period from 19 November 2009 to 8 June 2010. Forty 1.5–2.5-year-old ewes of the Norwegian White breed were mated and divided into four feeding treatments by stratified block randomization, accounting for age and sire. Each treatment was allocated randomly to two pens of five animals in each. The animals were housed in an insulated barn on deep straw litter. The ewes lambed indoors from 7 to 26 April 2010. Thereafter, the ewes and lambs were kept together in their pens until the end of the experiment, at which time the lambs were six to nine weeks old.

2.2. Experimental feeding regimens

Four experimental dietary supplements SW, NatE, SyntE, and C were formulated and fed to the ewes the entire indoor feeding period, from mating until turn out to pasture. The SW, containing 570 g/kg DM dried and ground A. nodosum (AlgeaFeed 3.5; Algea AS, Lødingen, Norway), was fed at an average daily rate per ewe of 144 g DM, while NatE, containing 612 mg RRR-α-tocopheryl acetate/kg DM (Vitfoss A/S, DK-6300 Gråsten, Denmark), SyntE containing 1125 mg all-rac-α-tocopheryl acetate/kg DM (Zhejiang Medicine Co. Ltd., Zhejiang, China), and C, with no extra α-tocopherol or seaweed, were all fed at an average daily rate of 110 g DM per ewe. The rest of the components of NatE, SyntE and C diet supplements were a mixture of barley, molasses, minerals, and vitamins (A and D). The SW diet supplement was fortified with corresponding concentrations of the mentioned nutrients (Table 1). The ingredients were blended and pelletized at ForTek, Norwegian University of Life Sciences, Ås, Norway. The vitE in the NatE and SyntE treatments were added in the form of α-tocopheryl acetate assuming that 1 IU was equivalent to 1 mg of *all-rac*-α-tocopheryl acetate and thus also to 0.5 mg RRR- α-tocopheryl acetate (Weiss et al., 2009), and the NatE and SyntE were offered to give approximately 140 IU per ewe daily in order to be fed 5–6

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times more than the NRC (1985) recommendation of 15 IU per kg of feed in dry matter. The SW supplement was offered at a rate to allow a daily intake of 80 g *A. nodosum*. The supplements were formulated to meet the mineral requirements of the ewes, and were given in isoenergetic amounts to compensate for lower energy value of the SW treatment. It was assumed that the animals received enough salt from the diet.

The ewes were given the experimental supplements in the morning before being given access to forage. The animals had free access to forage and water during the rest of the day. Orts of both roughage and supplements were weighed on three consecutive days once a month. The grass silage was produced from the first cut on a three-yr-old field consisting mainly of timothy (*Phleum* pretense), meadow fescue (*Festuca pratensis*), smooth meadow grass (*Poa pratensis*), and white clover (*Trifolium repens*). The grass was harvested, wilted, and wrapped in round bales without addition of preservatives.

From mating until turn out to pasture, each ewe consumed in total on average 2.0 kg DM/d. Over the entire experimental period, the ewes' diet consisted of on average of 84% roughage (silage, hay, and straw), 7.8% barley, and 1.7% fish meal, and the experimental supplements accounted for 7.2% of the diet in the SW group and 5.7% in the SyntE, NatE, and C groups. After mating, individual ewes received 140 g DM/d SW supplement or 110 g DM/d NatE, SyntE, or C supplement. In the SW group, the amount of seaweed meal given to each ewe was 82 g DM/d (4.1% of total DM intake). For a total of 2 weeks during the 4 weeks before expected parturition, the amount of supplements given each ewe was increased to 215 g DM/d in the SW group and to 175 g DM/d in the NatE, SyntE, and C groups to ensure sufficient levels of energy during late gestation and parturition. In the SW group, this increase corresponded to consumption of 116 g DM/d seaweed meal by each ewe. One week before expected parturition, in addition to the initial amounts of diet supplement, each ewe received 100 g/d fishmeal and 480 g/d barley as protein and energy supplements, respectively, and those levels were increased to 160 and 720 g/d after parturition.

The amount of feed offered and the refusal for each pen were weighed daily for three consecutive days, once a month. At the same time, samples of all the dietary components were collected. The monthly collected samples were bulked to one sample of each feed and month and stored frozen (−20 °C). Aliquots of approximately 700 g were oven dried for 48 h at 65 °C and ground using a Tecator Cyclotec grinder (1 mm screen). Diet composition and intake and the diet chemical characteristics and net energy value in the different periods throughout the feeding experiment is presented in Table 2.

Table 1
Ingredient composition of the four diet supplements (g kg $^{-1}$).

	Diet supplement[1]			
	SW	SyntE	NatE	C
Barley	409	879	880	881
Seaweed meal	546	0	0	0
Molasses	30.7	39.6	39.6	39.6
Vitamin E[a]	0	2.25	1.68	0
$CaCO_3$	0	15.2	15.2	15.2
$Ca(H_2PO_4)_2 \times H_2O$	12.8	7.8	7.8	7.8
$MgHPO_4 \times 3H_2O$	0	16.2	16.2	16.2
NaCl	0	36.8	36.8	36.8
Na_2SeO_3	0.05	0.06	0.06	0.06
$ZnSO_4 \times H_2O$	0.90	1.20	1.20	1.20
$MnSO_4 \times H_2O$	0.78	0.99	0.99	0.99
$Ca(IO_3)_2$	0	0.03	0.03	0.03
$2CoCO_3 \times 3Co(OH)_2 \times H_2O$	0.01	0.01	0.01	0.01
Vitamin A, 500000 IU/g	0	0.13	0.13	0.13
Vitamin D3, 500000 IU/g	0	0.04	0.04	0.04

NatE: 365,360 mg of RRR-α-tocopheryl-acetate/kg vitamin E-product.

[1] SW=seaweed meal; SyntE=synthetic vitamin E; NatE=natural vitamin E; C=control.

[a] SyntE: 500,000 mg of *all-rac*-α-tocopheryl-acetate/kg vitamin E-product;

2.3. Immunization of the ewes

The ewes followed standard immunization with 2 mL of Ovivac® P vet (Intervet) given subcutaneously 4 weeks

Table 2
Diet ingredients and chemical composition from mating, during gestation and lambing in the different periods averaged across dietary treatment (week number, year 2009–2010, in brackets).

	Period				
	Mating (47–50)	Gestation (50-7)	Gestation (7–11)	Late gestation (11–14)	Lambing (15–23)
Ingredients (kg DM/d)					
Silage	1.08	1.50	0.64	1.99	2.00
Hay	0.8	0	0	0	0
Straw	0	0	0.48	0	0
Barley	0	0	0	0	0.50
Fish meal	0	0	0	0	0.11
Experimental supplement	0.17	0.12	0.12	0.19	0.08
Total	2.06	1.62	1.24	2.17	2.70
Chemical characteristics					
[a]NEL, MJ/kg DM	5.24	5.22	5.15	5.24	5.62
Crude protein, g/kg DM	139	125	90	125	149
NDF, g/kg DM	612	629	657	623	544

[a] NEL=net energy lactation.

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prior to parturition. Ovivac® P contains toxoids of *Clostridium tetani*, *C. perfringens*, and *C. septicum*, and inactivated cells from *C.* chauvoei, *Mannheimia haemolytica*, and *Pasteurella trehalosi.* The production of antitoxins against different *Clostridium* spp. induced by vaccination represents an important immunoprophylactic means of reducing diseases in sheep production. Antitoxins neutralize the toxins generated in both the intestine and the blood of the animals. Here, we assayed the antibodies against tetanus toxoid as a parameter of antitoxin production. Antibodies against *M. haemolytica* protect the lambs against this important environmental pathogen.

The ewes received an experimental intramuscular immunization with 0.5 mL of Equilis® Reserquin vet (Intervet) vaccine against equine herpes and influenza virus (EIV) given 5 and 2 weeks before expected parturition. This vaccine contains inactivated herpes virus types 1 and 4, and inactivated influenza viruses A/Equi 1 and A/Equi 2. Antibodies against influenza virus hemagglutinin protect cells against infection with such virus, and hence we assayed these antibodies as a measure of disease resistance.

2.4. Immunization of newborn lambs

Two-week-old lambs were vacciNatEd by subcutaneous injection of 0.5 mL of diphtheria (*Corynebacterium diphtheriae*) toxoid vaccine (Difterivaccine, Statens Serum Institut (SSI), Denmark) and intracutaneous injection of live attenuated human tuberculosis vaccine (BCG, *Mycobacterium bovis*) (SSI, Denmark). This was done to study their ability to produce specific antitoxins and the cell-mediated immunity response.

2.5. Immunological parameters in the ewes

The Ig level was monitored by measuring IgG and IgM concentrations (mg/mL) in blood samples at the beginning of the feeding period and two weeks before parturition. This was done using a single radial immunodiffusion assay (Mancini et al., 1965) performed in agarose gel with rabbit polyclonal antibody directed against sheep IgG (Fc) or swine polyclonal antibody to sheep IgM (Fc) (both from Nordic Immunological Laboratories, the Netherlands).

The production of antibodies specific against *M. haemolytica*, tetanus toxoid, and EIV was assayed in blood samples at the beginning of the experiment, at the time of immunization, and at parturition. The antibodies against *M. haemolytica* were assessed using an ELISA technique (Lyche et al., 2006) with *M. haemolytica* A2 and a horseradish peroxidase-linked donkey anti-sheep IgG (Sigma Aldrich, USA). The antibodies against tetanus toxoid were determined by a passive hemagglutination test (Whist et al., 2003), and those against EIV were evaluated by a virus hemagglutination inhibition test (Lyche et al., 2006).

2.6. Transfer of maternal immunity to the new-born lambs

Transfer of the maternal Ig was monitored by measuring IgG and IgM levels in serum samples collected from 1-, 4-, and 6-week-old lambs, as described above. IgG and IgM concentrations were also analyzed in fat-free colostrum collected within 6 h of parturition as far as practically possible. Colostrum was centrifuged for 15 min at 4 °C and RCF 2200*g*, and then placed on ice for 10 min, after which the aqueous phase was transferred to new tubes for further radial immunodiffusion analysis.

Transfer of maternal specific immunity was monitored in 1-, 2-, 4-, and 6-week-old lambs by measuring specific antibodies to *M. haemolytica*, tetanus toxoidin, and EIV as described above.

2.7. Immune status in the lambs

After immunization of the lambs, specific antibodies to *C. diphtheriae* toxoid were measured when the animals were 2, 4, and 6 week-old by passive hemagglutination test (Whist et al., 2003).

The cell-mediated immunity to *M. bovis* was evaluated in 6 week-old lambs 4 week after immunization by analyzing IFN-γ production and antigen-induced lymphocyte proliferation in vitro, and by performing a skin test. The following mitogens and antigens were used: phytohemagglutinin (PHA), poke weed mitogen (PWM), concanavalin A (ConA), and bovine tuberculin purified protein (PPD). Production of IFN-γ in whole-blood cultures stimulated with the PPD antigen was assessed by an ELISA for bovine IFN-γ (Bovigam, Prionics, Switzerland). In short, the cultures were prepared from immunized lambs and them exposed to 20 μL of PPD (final concentration 10 or 40 μg mL^{-1}) or incubated in medium alone as a negative control (Storset et al., 2001). Proliferation of lymphocytes induced by the mitogens and antigens was assayed in vitro by purified lymphocyte culture (Storset et al., 2001) modified for sheep blood. Heparinized (15 IU/mL) blood was centrifuged for 10 min at 900*g*. The buffy coat was collected and mixed with 6–7 mL of Hanks buffered saline solution (HBSS; DAA laboratories, Austria), and then placed on top of 3 mL of Lymfoprep® (Axis-Shield PoC AS, Oslo, Norway; 1.077 g/mL) in siliconized vials. Each vial was centrifuged for 30 min at 700*g*, after which the lymphocyte layer was transferred to a new siliconized vial and washed by centrifugation for 15 min at 700*g* with 10 ml Hanks buffered saline solution and then again for 10 min in the same manner. Thereafter, the supernatant was carefully removed, 9.8 mL of HBSS was added to the pellet (approx. vol. 0.2 mL), and the cells were counted. The cells were subsequently centrifuged for 10 min at 700*g* and then resuspended to 1×10^6 mononucleated cells/mL in RPMI 1640 cell culture medium (RPMI 1640, Gibco Division of Invitrogen, Paisley, UK) containing 10% fetal calf serum (FCS), 2 mM glutamine, 0.15% bicarboNatE, 1% antibiotics (penicillin and streptomycin). A 180 μL aliquot of this cell suspension was immediately dispensed into each 96 flat-bottom wells on a microtiter pate (Nunc, Roskilde, Denmark). The following mitogens and antigen (20 μL/well) were used (final concentration within parentheses): PHA (10 μg/mL; Wellcome, Beckenham, England); PWM (20 μg/mL; Gibco, Renfrewshire, Scotland); ConA (20 μg/mL; Pharmacia) and PPD (15 μg/mL; Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, UK). RPMI 1640 medium was used as diluent and for controls. All assays were performed in

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triplicate cultures, and the plates were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air for 4 days.

Lymphocyte proliferation was evaluated by assessing incorporation of [3H] thymidine added to the cultures. Briefly, after exposure to [3H] thymidine, the cells were collected on a filter plate and counted using a TopCount NXT Gamma Counter (Storset et al., 2001). The lymphocyte response was expressed as the difference in mean optical density (OD) between a stimulated culture and a control culture.

The skin tests were performed on 6- to 7-week-old lambs. The lambs were shaved on the left side of the thorax, and the test was performed by intradermal injection of 0.1 mL of each of the mitogens PHA (50 and 100 μg mL^{-1} in PBS solution), ConA (100 and 200 μg mL^{-1} in PBS solution), and PWM (50 and 100 μg mL^{-1} in PBS solution), and the antigen PPD (2500 and 1250 iu 0.1 mL^{-1}). As a negative control, we used 0.1 mL 0.9% NaCl (Sonne et al., 2006). The skin reaction was measured 24 h after the injections as the diameter (mm) of the localized swelling (palpable raised area), and also according to height in mm (0=negative control to +4) and degree of erythema (0=negative control to +3).

2.8. Analysis of α-tocopherol

The concentration of α-tocopherol was measured in the diet, the colostrum, and plasma samples. Before analysis, the feed samples were freeze-dried and finely milled, and 1 g aliquot was suspended in a solution consisting of 24 mL of ethanol (960 mL/L), 9 mL of methanol, 10 mL of ascorbic acid in water (200 g/L) and 7 mL of KOH:water (1:1 w/v). This suspension was saponified for 30 min at 80 °C (boiling) in the dark and then cooled in cold water. Next, 2 mL of the saponified mixture was diluted with 0.5 mL of water, after which tocopherols were quantitatively extracted with two 5.0 mL portions of heptane. The samples of plasma and colostrum were thawed before analysis. The colostrum samples were also heated at 40 °C for 20 min and mixed thoroughly. To extract tocopherols, 1000 mg of milk or 500 μL of blood plasma was diluted with 0.5 mL of ascorbic acid solution (200 g/L), 0.5 mL of methanol, 2.0 mL of ethanol, and 0.5 mL KOH:water (1:1 w/v). This mixture was adjusted to a final volume of 5.5 mL with water and then subjected to saponification at 80 °C for 20 min. After cooling, tocopherol extraction was performed twice using two 5 mL portions of heptane each time. The two extracts were mixed, and 100 μL was injected into the HPLC system for α-tocopherol analysis. All solvents used were of HPLC grade. The HPLC was carried out on a 4.0 × 125 mm Perkin Elmer HS-5-Silica column using heptane that contained 2-propanol (3.0 mL/L) and was degassed with helium as the mobile phase. The flow rate was 3.0 mL/min, and the column was held at room temperature. Fluorescence detection was performed at excitation and emission wavelengths of 290 and 327 nm, respectively. Identification and quantification of α-tocopherol was achieved by comparison of retention time and peak areas with external standards (Merck, D-64293 Darmstadt, Germany), and an extinction coefficient of, and an extinction coefficient of an 1% solution in an 1 cm cuvette of 71.0 at 294 nm was used for α-tocopherol (Jensen and Nielsen, 1996).

2.9. Feed mineral and quality analysis

Feed and pasture samples were analyzed at the Dairy One, Inc. Forage Testing Laboratory (Ithaca, NY) with wet chemical procedures. Crude protein content was determined using AOAC Method 990.03 (AOAC, 1990). Heat-stable, α-amylase-treated, sodium sulfite NDF was determined using an ANKOM fiber analyzer (ANKOM Technology Corporation, Fairport, NY) based on procedures describe by Van Soest et al. (1991). Digestibility of DM and NDF was determined in vitro after incubation for 48 h using the ANKOM DaisyII Filter Bag Technique, ANKOM Technology, Macedon, NY. The NEL 3 × maintenance was predicted from total digestible nutrients according to the NRC (2001).

The total concentration of Se, As, and I in forage, barley, fishmeal, and the experimental diet supplements was determined by inductively coupled plasma mass spectrometry (ICP-MS; Elan 6000, Perkin Elmer) and Na was determined by ICP-optical emission spectrometry (ICP-OES; Optima 5300 DV, Perkin Elmer).

For the digestion in the analysis of Se, As, and Na, PA-grade nitric acid was purchased from Merck and purified using a sub-boil distillation apparatus to reduce trace contaminants (Milestone SubPur). Approximately 0.25 g of dry sample was digested in an ultraclave (UltraCLAVE 3, Milestone) at 240 °C for 40 min in 3.0 mL of distilled ultrapure HNO_3 (Merck) and 1 mL of deionized water (18 Ω) (Barnstead B-pure). After digestion, each sample was diluted to a volume of 50 mL. Tellur was used as internal standard. As standard reference materials, we used NCS DC 73348 bush twigs and leaves, and NCS ZC 73013 spinach obtained from the China National Analysis Centre for Iron and Steel; 1567a wheat flour and 1570a spinach leaves from the National Institute of Standards and Technology; BCR CRM 129 hay powder from the Community Bureau of References. These materials were digested and analyzed as described for quality assurance and control purposes.

For analysis of I, we used analytical-grade hydrogen peroxide (H_2O_2, 30%) and sodium thiosulfate ($Na_2S_2O_3$) from MERCK, and PA-grade ammonium hydroxide solution (32%) from VWR. Initially, an approximately 0.3 dry sample was digested in 2.5 mL of HNO_3 and 2 mL of H_2O_2 at 240 °C for 40 min in an ultraclave. The digested sample was subsequently cooled to 25 °C and decanted into 50 mL polyethylene tubes, and the pH was adjusted to approximately 8 by adding 3 mL of concentrated NH_3OH. Thereafter, 1.3 mg of thiosulfate in solution was added, and the volume was brought to 50 mL with deionized water. Any precipitate was allowed to form prior to analysis, which was conducted the next day. As internal standard, we used 10 ng of ^{129}I. The standard reference materials BCR CRM 127 hay powder and NCS ZC 730113 spinach were digested and analyzed as described for quality assurance and control purposes.

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2.10. Statistical analysis

The antibody titers were $\log_2$-transformed to normalize the distribution. Sera that showed no inhibition were assigned a titer value of 5 ($\log_2 = 2.3$). The lowest serum dilution assessed by the passive hemagglutination test was 1:8, and sera that showed no hemagglutination at this dilution were entered as 4 ($\log_2 = 2$) in the statistical analysis. For immunization with *C. diphtheriae* toxoid, individual animals with an increase in antibody titer of ≥ 2 were considered to be significant responders. Regarding production of IFN-γ, the results were expressed as equivalents of recombinant bovine IFN-γ by use of a standard curve (ng mL^{-1}). The values noted for control wells were subtracted from those obtained for stimulated wells, and the mean of the calculated values for duplicate samples was used.

The data were analyzed statistically by the mixed model procedure in SAS, 2009. For the ewes, we treated experimental diet supplements (SW, NatE, SyntE, or C) as fixed effect, and pen (1 or 2) and ewe within pen as random effects. The pretreatment level of a parameter, measured at the beginning of the experiment, was included as a covariate. The transfer of maternal immunity to lambs was analyzed by including treatment and age (in weeks) and their interaction as fixed effects, and pen, ewe within pen, and lamb within ewe as random effects. The covariation between lambs was accounted for in an analysis of repeated measure. The parameters for the specific antibody response in the lambs and live weight change was analyzed by including treatment as fixed effect, and pen, ewe within pen, and lamb within ewe as random effects. To discern differences in lamb mortality between treatments, regression was carried out using the proc logistic procedure in SAS (2009). The tables and figures present LSmeans for each treatment and the average group SEM. Means were separated using Tukey's test. Differences of $P < 0.05$ were considered statistically significant.

3. Results

All the experimental diet supplements were well accepted by the ewes, and no orts were observed. Also, the ewes were in good health, and exhibited no particular illness or mortality during the study period. However, mortality and sickness rates were higher among lambs in the SW treatment group (Table 3), and the symptoms exhibited by affected animals were: gurgling sounds when breathing and foam and mucus around the mouth, which correspond to a respiratory infection. Most of the deaths occurred in one of the two SW pens. An autopsy of three of the dead lambs that had displayed the mentioned symptoms (two had died within 24 h of birth and one at the age of 2 weeks) revealed multifocal purulent pneumonia, septicemia, and enteritis. *M. haemolytica* was isolated in abundant numbers from the lungs and livers of those individuals.

No significant differences were found between treatment groups regarding weight change in either the ewes or the lambs (data not shown).

Table 3
Mortality rate among lambs in the four treatment groups.

	Diet supplement[a]			
	SW	SyntE	NatE	C
Number of lambs born	20	17	18	19
Number of lambs dead	7	0	1	2
Mortality rate (%)	35.0	0	5.6	10.5

[a] SW=seaweed meal; SyntE=synthetic vitamin E; NatE=natural vitamin E; C=control.

3.1. α-Tocopherol, selenium, arsenic, sodium and iodine intake

Compared to ewes in the control group C, those in the groups SyntE, NatE, and SW had, respectively, 6.2, 3.1, and 1.3 times higher average daily α-tocopherol intake (Table 4). The concentration of α-tocopherol of the NatE supplement was planned to contain 50% as much as the SyntE product but the actual concentration of a-tocopherol in the NatE product was only 38% of that in the SyntE product. Consequently, the intake of vitamin E, expressed in IU, was lower on NatE than on SyntE. However, calculated per kg body weight, intake of vitamin E was similar on SyntE and NatE, and approximately 3.6 times higher than on the control. The daily intake of the minerals Na, Se, I and As was highest in the SW group (Table 4).

3.2. Immunological response in the ewes

In the ewes, no treatment effect could be discerned with respect to the levels of serum IgM, or specific antibodies against tetanus toxoid, *M. haemolytica*, and EIV. The values were positively correlated with the initial levels in those animals at the start of the feeding period assignment to the respective treatment groups. Also, the serum IgG concentration was the only parameter that was affected by experimental treatment in the ewes, with lower levels found in those in the SW group compared to the other experimental groups ($P < 0.01$; Table 5). No difference was observed in the concentrations of IgM and IgG in the colostrum obtained from the different groups (Table 6).

3.3. Transferred maternal immunity

One-week-old lambs in the SW group had significantly ($P < 0.001$) lower mean serum concentrations of IgG and IgM than the levels found in the other treatment groups (Table 7). However, in serum collected from lambs 4 and 6 weeks after parturition, the IgG and IgM concentrations in the SW group had increased to similar levels observed in the other treatment groups. One week after birth, the lambs in groups SyntE-, NatE- and C-, but not those in the SW, had high concentrations of maternal IgG and IgM; those levels decreased by nearly 50% 4 weeks after parturition. At the age of 6 weeks, the lambs in all groups showed increased serum IgM, whereas the mean concentration of IgG leveled off in all groups, and even showed a decrease in the SyntE group.

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Table 4
α-tocopherol and vitamin E concentration in supplements and in total diet and daily intake of α-tocopherol, vitamin E, Na, Se, As, and I by ewes. Figures are weighted average across the entire experimental period.

	Diet supplement[a]			
	SW	SyntE	NatE	C
Dietary concentration				
Supplement α-tocopherol (mg/kg DM)	68	1293	489	19
Total α-tocopherol (mg/kg DM)	18	86	43	14
Total vitamin E (IU/kg DM)[b]	27	92	65	21
Total vitamin E (IU/kg DM)[c]	36	99	87	28
Daily intake				
Supplemental α-tocopherol (mg)	9.8	146	60	2.0
Total α-tocopherol (mg)	36	173	87	28
Supplemental vitamin E (IU)[b]	15	146	90	3.0
Total vitamin E (IU)[b]	53	186	129	42
Supplemental vitamin E (IU)[c]	20	146	121	4.0
Total vitamin E (IU)[c]	72	199	173	56
Total vitamin E (IU/kg BW)[b]	0.7	2.4	1.9	0.5
Total vitamin E (IU/kg BW)[c]	1.0	2.5	2.5	0.7
Na (g)	5.46	5.19	4.99	4.75
Se (mg)	0.66	0.52	0.51	0.44
As (mg)	3.99	0.83	0.82	0.81
I (mg)	59.7	3.29	3.08	2.70

BW=body weight.
[a] SW=seaweed meal; SyntE=synthetic vitamin E; NatE=natural vitamin E; C=control.
[b] IU of basal vitamin E and in diet supplements SW, NatE and C was calculated as 1.49 x mg of α-tocopherol from feedstuffs.
[c] IU of basal vitamin E and in the diet supplements SW, NatE and C was calculated as 2 x mg of α-tocopherol from feedstuffs.

Table 5
Mean values of the immunological parameters measured at parturition in the ewes in the four treatment groups.

	Diet supplement[1]				SEM[2]	*P*-value
	SW	SyntE	NatE	C		
IgG (mg/mL)	37.2[b]	52.1[a]	56.7[a]	43.1[a]	5.71	0.01
IgM (mg/mL)	2.2	2.5	2.2	2.6	0.46	0.287
Tetanus toxin (titer $\log_2$)	11.8	11.0	11.7	11.4	0.38	0.893
M. haemolytica (titer $\log_2$)	7.1	6.8	6.8	6.4	0.93	0.184
EIV (titer $\log_2$)	4.6	4.4	3.7	4.0	0.45	0.336

[1] SW=seaweed meal; SyntE=synthetic vitamin E; NatE=natural vitamin E; C=control.
[2] SEM=Standard error of the mean.
[a,b] Treatment groups that differed from other treatment groups according to a multiple range test.

Table 6
Mean levels of IgG and IgM (mg/mL) in colostrum collected immediately after lambing from the ewes in the four treatment groups.

	Diet supplement[a]				SEM[b]	*P*-value
	SW	SyntE	NatE	C		
IgG	77.6	61.8	75.1	75.9	9.78	0.719
IgM	5.4	5.2	4.5	5.7	0.46	0.359

[a] SW=seaweed meal; SyntE=synthetic vitamin E; NatE=natural vitamin E; C=control.
[b] SEM=Standard error of the mean.

The transfer of specific antibodies against *M. haemolytica*, tetanus toxoid, and EIV from the vaccinated mothers to the lambs was also significantly reduced in the SW group compared to the other treatments (Table 7). The levels of specific antibodies against *M. haemolytica* in SW remained constant during the first month of life, increasing after 6 weeks as the result of exposure to environmental microbes. The titers of specific antibodies against *M. haemolytica* in the SyntE, NatE and C decreased significantly ($P<0.05$) 2–4 weeks after birth, and seemed to have stabilized when the animals were 6 weeks old. The levels of specific antibodies against EIV and tetanus toxoid in the lambs gradually decreased in all the treatment groups and were consistently lower in the SW groups

3.4. Immune response in the lambs

Considering the specific antibody response, following immunization all of the lambs in the study responded significantly to *C. diphtheriae* toxoid ($P<0.001$; Table 8).

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Table 7
Effect of dietary supplementation of ewes on immunological parameters measured in serum collected from their lambs 1, 2, 4, and 6 weeks after birth.

	Age (weeks)	Diet supplement[a]				SEM[b]	*P*-value[c]		
		SW	SynE	NatE	C		Trtm	Age	Trtm × age
IgG (mg/mL)	1	7.0	48.5	57.4	52.0	4.59	0.03	< 0.001	< 0.001
	4	28.0	33.1	28.6	31.1				
	6	32.9	23.0	28.6	29.2				
IgM (mg/mL)	1	0.4	2.0	1.8	2.3	0.16	0.3	< 0.001	< 0.001
	4	1.3	1.1	1.1	1.4				
	6	1.5	1.3	1.6	1.7				
Tetanus toxin (titer $\log_2$)	1	6.9	10.5	11.7	11.6	0.66	< 0.001	< 0.001	< 0.003
	2	6.2	10.2	11.1	11.4				
	4	5.6	9.9	9.8	10.2				
	6	4.8	8.5	9.1	9.7				
M. haemolytica (titer $\log_2$)	1	4.4	8.3	7.8	7.8	0.79	0.12	< 0.001	< 0.003
	2	4.4	6.6	7.2	7.0				
	4	4.3	6.2	6.1	6.2				
	6	5.5	6.4	6.2	6.3				
EIV (titer $\log_2$)	1	3.6	5.7	6.0	5.9	0.27	< 0.001	< 0.001	< 0.05
	2	3.3	5.3	5.0	5.1				
	4	3.2	4.1	4.0	3.9				
	6	2.7	3.6	3.3	3.8				

[a] SW=seaweed meal; SyntE=synthetic vitamin E; NatE=natural vitamin E; C=control.
[b] SEM=Standard error of the mean for Treatment × Age effect.
[c] *P*-values for dietary group (trtm), age and the interaction dietary group x age (trtmxage) are stated for each parameter.

Table 8
Effect of dietary supplementation of ewes on antibody response to *Corynebacterium diphtheriae* toxoid measured in serum collected from their lambs 2 and 4 weeks after immunization.

Age (weeks)	Diet supplement[a]				SEM[b]	*P*-value
	SW	SynE	NatE	C		trtm × age
2	2.0	2.0	2.0	2.0	0.39	0.426
4	2.9	3.2	3.7	3.9		
6	6.5	6.7	6.4	6.3		

[a] SW=seaweed meal; SyntE=synthetic vitamin E; NatE=natural vitamin E; C=control.
[b] SEM=Standard error of the mean.

However, no difference between treatment groups was noted regarding the levels of specific antibodies to *C. diphtheriae* toxoid in the lambs 2 and 4 weeks after immunization ($P=0.90$; Table 8). Between week 2 and 4 the frequency of significant responders was 8%, 31%, 35% and 47% for the diets SW, SyntE, NatE and C respectively, and 92%, 100%, 100%, and 88% between week 2 and 6.

Evaluation of a cell mediated immune response showed that the production of γIFN in vitro induced by stimulation with PPD was higher for the two groups receiving vitE supplements than for the SW and C treatment groups. Also, at both PPD concentrations tested, the mean level of γIFN produced was higher in samples from the NatE group ($P<0.05$) than in those from the other groups (Fig. 1).

Analysis of mitogen-induced lymphocyte proliferation demonstrated that the cells exhibited a moderate response to PPD and stronger response to the other mitogens. No effect of mitogen treatment was found in either the lymphocyte proliferation test in vitro or the skin test (data not shown).

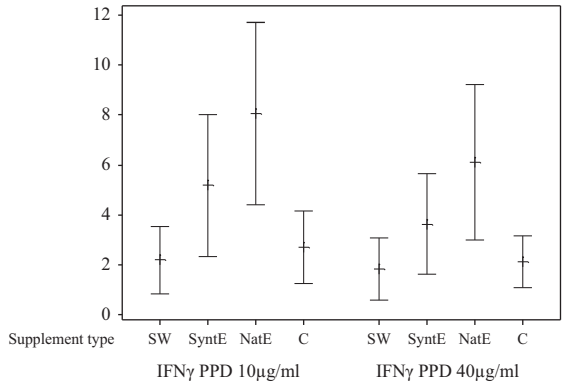

Fig. 1. in vitro IFN_γ production of lymphocytes stimulated with 10 and 40 μg/mL bovine tuberculin purified protein (PPD) in lambs in the different dietary supplement groups (SW=seaweed meal; SyntE=synthetic vitamin E; NatE=natural vitamin E; C=control), 4 weeks after immunization with antigen PPD. The data represent mean IFN_γ levels (μg/mL) with 95% confidence intervals.

3.5. Analysis of α-tocopherol in the ewes and colostrum

The concentration of α-tocopherol in the blood of ewes at parturition was significantly higher in the SyntE group than in the other groups. However, levels of α-tocopherol in the colostrum did not differ between groups (Table 9).

4. Discussion

In the present study, we supplemented the winter-period (7-mo) diet of sheep with seaweed meal or different sources of vitE to determine possible effects on immunological

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Table 9
Mean levels of α-tocopherol (μg/mL) in plasma sampled two weeks before parturition and in the colostrum collected from the ewes in the four treatment groups.

	Diet supplement[1]				SEM	*P*-value
	SW	SyntE	NatE	C		
Plasma	1.70[b]	2.04[ab]	1.71[b]	1.33[c]	0.1	0.0005
Colostrum	9.4	11.1	8.0	9.3	1.1	0.190

[1] SW=seaweed meal; SyntE=synthetic vitamin E; NatE=natural vitamin E; C=control.
[a,b,c] Treatment groups that differed from other treatment groups according to a multiple range test.

parameters in ewes and their offspring, considering the following: production of IgG, IgM, specific antibodies, and antitoxins; cell-mediated immunity; transfer of maternal immunity to the lambs. All diets had a concentration of vitamin E higher than the NRC (1985) recommended standard of 15 IU per kg of feed DM. The SyntE and NatE diets had a total content of vitE that were 7 and 6 times higher, respectively, than 15 IU/kg DM (Table 4). The levels in NatE and SynE are close to the planned levels of 5–6 times 15 IU per kg DM in feed (Table 4). However, compared to the NRC (2007) recommended intake level of about 5.3 IU vitamin E per kg BW for pregnant ewes, the intake in the current study were deficient; C 13%, SW 19%, NatE 47% and SyntE 47% of 5.3 IU/kg BW (calculations done using values for Total vitamin E (IU/kg BW)[3] in Table 4).

The processing of the supplements that were offered to the ewes might have influenced the content of α-tocopherol, explaining that the content of α-tocopherol in NatE was 38% of the SyntE and not 50% as planned. Difference in intake explains the higher concentration of α-tocopherol in plasma in ewes fed SyntE than NatE. However, when the differences in plasma concentrations are adjusted to account for the differences in α-tocopherol intake [(plasma concentration from NatE treatment/plasma concentration from SyntE treatment)/(α-tocopherol intake by NatE treatment/α-tocopherol intake by SyntE treatment)], the concentrations of α-tocopherol in plasma per unit of α-tocopherol consumed were 17% higher in ewes that fed NatE compared to SyntE. This finding is in agreement with results from sheep reported by Hidiroglou et al. (1988) indicating that the natural form of vitamin E resulted in higher serum α-tocopherol concentrations than the synthetic form. The explanation for this effect may lay in a more favorable secretion from the liver in blood lipoproteins of the natural form (Burton and Traber, 1990).

Activation by γIFN stimulates macrophages to kill intracellular-bacteria and thus plays an important role in controlling infections (Tufariello et al., 2003; Gomez and McKinney, 2004). The effect of the NatE treatment on IFN-γ secretion in the BCG-vaccinated lambs in our study suggests that dietary supplementation with natural vitE can be beneficial means of fighting mycobacterium infections in animals and may also aid treatment of tuberculosis in humans. Moreover, the stimulatory effect on cell-mediated immunity in lambs indicates that supplementation with natural vitE may have a similar impact on immunity to infections with other intracellular microbes in ruminants (sheep).

Like Hatfield et al. (2002), we found that experimental dietary treatments had no effect on antibody levels after vaccination of ewes, which supports other findings reviewed by Nockels (1986) reporting that much larger doses of vitE (20 times and more than the recommendations given by NRC (1985)) are required to elicit immune responses. Research on pregnant mares has shown that supplementation with natural vitE prior to parturition increases the transfer of both vitE and Ig to milk and foals (Bondo and Jensen, 2011). Supplementation did not influence the concentrations of α-tocopherol or IgG in colostrum in the present study, and the correlation between α-tocopherol concentration in plasma and colostrum was not significant ($r=0.11$, $P=0.54$). Our results agree with those of Daniels et al. (2000), who did not find effect of supplementation with 400 IU vitE on IgG titers in the ewes. According to Puls (1994) blood α-tocopherol concentrations of 1.5–4.0 μg/mL are considered to be adequate. Other publications state that serum α-tocopherol concentrations of <2.0 μg/mL are considered deficient (Maas et al., 1984; MacMurray and Rice, 1982). This means that the plasma α-tocopherol values measured in our study were at the lower end of the adequate level, which suggests that we might have not have fed enough α-tocopherol. On the other hand, relying on the plasma α-tocopherol concentrations as the sole indicator of vitE status in the ewes might not be an adequate method since α-tocopherol is stored in many other tissues. We don't have measurements of α-tocopherol in other tissues and therefore we cannot explain the α-tocopherol concentrations found in the colostrum samples.

The transfer of maternal immunity was severely impaired in the SW treatment, but this did not influence the adaptive immune responses in the lambs, as indicated by an increase in their production of IgG, IgM, and specific antibodies against *M. haemolytica*. The higher incidence of deaths and symptoms related to *M. haemolytica* infections among the offspring in the SW group can be explained by the low immune status of those animals during the first weeks of life, and probably also by increased exposure to *M. haemolytica* due to the environmental conditions. In addition, the lambs in the SW treatment were poorly protected against clostridium intoxication, as demonstrated by a low titer of transferred maternal antibodies. Such lambs will be at increased risk of developing clostridium-related diseases when they are released to pasture. Inadequate levels of ingested or absorbed antibodies could explain the low concentrations of the passive immunity parameters measured in the lambs in the SW treatment group. The amount of colostrum consumed by the lambs was not measured, and hence it is not possible to establish the exact level of Ig intake by the offspring. On the other hand, *A. nodosum* contains relatively high concentrations of polyphenols (Ragan and Jensen, 1978). Polyphenols excreted in the milk interact strongly with proteins (Broderick et al., 1991), and in this way may have contributed to rendering the Ig in the colostrum unavailable for absorption by the lambs. Seaweeds are also rich in minerals (MacArtain et al., 2007), and some studies have shown that supplementing ewes with I and other minerals in the final weeks before parturition reduces the

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absorption of IgG in lambs (Crosby et al., 2004; Boland et al., 2005; Boland et al., 2006, 2008; Rose et al., 2007). I is transported through milk ingestion in large quantities (Caumette et al., 2007). The potential effect of As on IgG is uncertain. The daily intake of As in our study was not high. Other reports have described much higher intake under both experimental and natural conditions (Herbert and Wiener, 1978; Hansen et al., 2003; Lu et al., 2006). However, arsenic is transported through the placenta, though poorly with milk (Caumette et al., 2007). There are human studies indicating increased fetal and infant mortality when the mother is exposed to arsenic during pregnancy, but this depends on the species of arsenic (Vahter, 2009).

Se intake in our ewes was higher for those fed SW, but we don't have records of the Se status in our animals. The Se compounds consumed will have a major effect but Se in seaweed is mainly organically bound (Maher, 1985), and may be more available than the inorganic Se added to the other supplements. However, As in the seaweed meal can affect the Se status negatively (Zeng et al., 2005). In such situations, recommended vitE doses may be inadequate (ARC, 1980). This could have been affected the immunological function in the ewes resulting in reduced IgG concentrations in the SW group.

5. Conclusion

In conclusion, we found that supplementation of ewes with natural vitE had a positive impact on γIFN expression in lambs that were immunized with BCG. The consumption of *A. nodosum* seaweed meal by the ewes at the levels applied in our study decreased the serum IgG concentrations in the ewes and also lowered the passive immunity of the lambs during their first week of life. Therefore, supplementation with seaweed meal should be avoided during late gestation and early lactation. The seaweeds supplementation we used had no influence on adaptive immunity. Further research is warranted to assess seaweed components and the mechanisms responsible for their effects, and best practice models need to be established for inclusion of whole seaweed products in animal feed.

Conflict of interest

The authors report that there is no conflict of interest relevant to this publicaton.

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References

AOAC, 1990. Official Methods of Analysis, 15th edition Association of Official Analytical Chemists, Arlington,VA.

ARC (Agricultural Research Council), 1980. The Nutrient Requirements of Ruminant Livestock. Commonwealth Agricultural Bureaux, Slough, UK.

Allen, V.G., Potin, P., Saker, K.E., Fontenot, J.P., Bagley, C.P., Ivy, R.L., Evans, R.R., Schmidt, R.E., Fike, J.H., Zhang, X., Ayad, J.Y., Brown, C.P., Miller, M.F., Montgomery, J.L., Mahan, J., Wester, D.B., Melton, C., 2001. Tasco: Influence of a brown seaweed on antioxidants in forages and livestock – a review. J. Anim. Sci. 79, 21–31.

Boland, T.M., Brophy, P.O., Callan, J.J., Quinn, P.J., Nowakowski, P., Crosby, T.F., 2005. The effects of mineral supplementation to ewes in late pregnancy on colostrum yield and immunoglobulin G absorption in their lambs. Livest. Prod.Sci. 97, 141–150.

Boland, T.M., Callan, J.J., Brophy, P.O., Quinn, P.J., Crosby, T.F., 2006. Lamb serum vitamin E and immunoglobulin G concentrations at 24 h post partum in response to various maternal mineral and iodine supplementation regimes. Anim. Sci. 82, 319–326.

Boland, T.M., Hayes, L., Callan, J.J., Baird, A.W., Keely, S., Crosby, T.F., 2008. The effcts of cobalt and iodine supplementation of the pregnant ewe diet on immunoglobulin G, vitamin E, T3 and T4 levls in the progeny. Animal 2, 197–206.

Bondo, T., Jensen, S.K., 2011. Administration of RRR-alpha-tocopherol to pregnant mares stimulates maternal IgG and IgM production in colostrum and enhances vitamin E and IgM status in foals. J. Anim. Phisiol. Anim. Nutr. 95, 214–222.

Broderick, G.A., Wallace, R.J., Ørskov, E.R., 1991. Control of rate and extent of protein degradation. In: Tsuda, T., Sasaki, Y., Kawashima, R. (Eds.), Proceedings of the 7th International Symposium on Ruminant Physiology, Academic Press Inc., San Diego, CA, pp. 541–592.

Burton, G.W., Traber, M.G., 1990. Vitamin E: antioxidant activity, biokinetics and bioavailability. Annu. Rev. Nutr. 10, 357.

Caumette, G., Ouypornkochagorn, S., Scrimgeour, C.M., Raab, A., Feldmann, J., 2007. Monitoring the arsenic and iodine exposure of seaweed-eating North Ronaldsay Sheep from the gestational and suckling periods to adulthood by using horns as a dietary archive. Environ. Sci. Technol. 41 (8), 2673–2679.

Campbell, S.G., Siegel, M.J., Knowlton, B.J., 1977. Sheep immunoglobulins and their transmission to the neonatal lamb. New Zeal. Vet. J. 25, 361–365.

Crosby, T.F., Boland, T.M., Brophy, P.O., Quinn, P.J., Callan, J.J., Joyce, D., 2004. The effects of offering mineral blocks to ewes pre-mating and in late pregnancyon block intake, pregnant ewe performance and immunoglobulin statusof progeny. Anim. Sci. 79, 493–504.

Daniels, J.T., Hatfield, P.G., Burgess, D.E., Kottt, R.W., Bowman, J.G., 2000. Evaluation of ewe and lamb immune response when ewes were supplemented with vitamin E. J. Anim. 78, 2731–2736.

Devi, K.P., Suganthy, N., Kesika, P., Pandian, S.K., 2008. Bioprotective properties of seaweeds: in vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. BMC Complement Altern. Med. 8, 38.

Finch, J.M., Turner, R.J., 1996. Effects of selenium and vitamin E on the immune responses of domestic animals. Res. Vet. Sci. 60, 97–106.

Gilbert, R.P., Gaskins, C.T., Hillers, J.K., Parker, C.F., McGuire, T.C., 1988. Genetic and environmental factors affecting immunoglobulin G1 concentrations in ewe colostrum and lamb serum. J. Anim. Sci. 66, 855–863.

Gomez, J.E., McKinney, J.D., 2004. *M. tuberculosis* persistence, latency, and drug tolerance. Tuberculosis (Edinb) 84, 29–44.

Govasmark, E., Steen, A., Strom, T., Hansen, S., Singh, B.R., Bernhoft, A., 2005. Status of selenium and vitamin E on Norwegian organic sheep and dairy cattle farms. Acta Agric. Scand. A-Anim. Sci. 55, 40–46.

Hansen, H.R., Hector, B.L., Feldmann, J., 2003. A qualitative and quantitative evaluation of the seaweed diet of North Ronaldsay sheep. Anim. Feed Sci. Technol. 105, 21–28.

Hatfield, P.G., Robinson, B.L., Minikhiem, D.L., Kott, R.W., Roth, N.I., Daniels, J.T., Swenson, C.K., 2002. Serum alpha-tocopherol and immune function in yearling ewes supplemented with zinc and vitamin E. J. Anim. Sci. 80, 1329–1334.

Hemmings, W.A., 1976. Maternofoetal Transmission of Immunoglobulins. Cambridge University Press, New York.

Herbert, J.G., Wiener, G., 1978. The effect of breed and of dried seaweed meal in the diet on the levels of copper in liver, kidney and plasma of sheep fed on a high copper diet. Anim. Product. 26, 193–201.

Hidiroglou, N., McDowell, L.R., Papas, A.M., Antapli, M., Wilkinson, N.S., 1992. Bioavailability of vitamin E compounds in lambs. J. Anim. Sci. 70, 2556–2561.

Hidiroglou, N., McDowell, L., Pastrana, R., 1988. Bioavailability of various vitamin E compounds in sheep. Int. J. Vitam. Nutr. Res. 58, 18.

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IFOAM, 2007. The IFOAM norms for organic production and processing version 2005.
Indergaard, M., 2010. Tang og tare – i hovedsak norske brunalger: Forekomster, forskning og anvendelse. NTNU, Trondheim.
Jensen, A., Nebb, H., Sæter, E.A., 1968. The value of Norwegian seaweed meal, as amineral supplement for dairy cows. Norwegian Institute of Seaweed Research, Oslo, 1–35.
Jensen, S.K., Lauridsen, C., 2007. Alpha-tocopherol stereoisomers. Vitam. Horm. 76, 281–308.
Jensen, S.K., Nielsen, K.N., 1996. Tocopherols, retinol, beta-carotene and fatty acids in fat globule membrane and fat globule core in cows' milk. J. Dairy Res. 63, 565–574.
Kuda, T., Tsunekawa, M., Goto, H., Araki, Y., 2005. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. J. Food Compos. Anal. 18, 625–633.
Larsen, H.J., Moksnes, K., Øvernes, G., 1988a. Influence of selenium on antibody production in sheep. Res. Vet. Sci. 45, 4–10.
Larsen, H.J., Øvernes, G., Moksnes, K., 1988b. Effect of selenium on sheep lymphocyte responses to mitogens. Res. Vet. Sci. 45, 11–15.
Liu, J.N., Yoshida, Y., Wang, M.Q., Okai, Y., Yamashita, U., 1997. B cell stimulating activity of seaweed extracts. Int. J. Immunopharmacol. 19, 135–142.
Lu, Y., Suliman, S., Hansen, H.R., Feldmann, J., 2006. Iodine excretion and accumulation in seaweed-eating sheep from Orkney, Scotland. Environ. Chem. 3, 338–344.
Lyche, J.L., Larsen, H.J.S., Skaare, J.U., Tverdal, A., Johansen, G.M., Ropstad, E., 2006. Perinatal exposure to low doses of PCB 153 and PCB 126 affects maternal and neonatal immunity in goat kids. J. Toxicol. Environ. Health A 69, 139–158.
Maas, J., Bulgin, M.S., Anderson, B.C., Frye, T.M., 1984. Nutritional myodegeneration associated with vitamin E deficiency and normal selenium status in lambs. J. Am. Vet. Med. Assoc. 184, 201–204.
MacArtain, P., Gill, C.I.R., Brooks, M., Campbell, R., Rowland, I.R., 2007. Nutritional value of edible seaweeds. Nutr. Rev. 65, 535–543.
MacMurray, C.H., Rice, D.A., 1982. Vitamin E and selenium deficiency disease. Ir. Vet. J. 36, 57–65.
Maher, W.A., 1985. Selenium in macroalgae. Bot. Marin. 28, 269–273.
Mancini, G., Carbonara, A.O., Heremans, J.F., 1965. Immunochemical quantitation of antigens by single radial immunodiffusion 2, 235–254 Immunochemistry 2, 235–254.
NRC (National Research Council), 1985. Nutrient Requirements of Sheep, Sixth Revised Edition, 1985 The National Academies Press, Washington, DC (1985).
NRC (National Research Council), 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. The National Academies Press, Washington, DC.
NRC (National Research Council), 2001. Nutrient Requirements of Dairy Cattle, Seventh Revised Edition National Academy Press, Washington, D.C.
Nockels, C.F., 1986. Nutrient modulation of the immune system. In: Haresign, W., Cole, D.J.A. (Eds.), Recent Advances in Animal Nutrition, Butterworths, London, pp. 177–192.
Puls, R., 1994. Vitamin Levels in Animal Health: Diagnostic Data and Bibliographies, 1st ed. Sherpa International, Clearbrook, BC, Canada.
Ragan, M.A., Jensen, A., 1978. Quantification studies on brown algal phenols. II. Seasonal variation in polyphenol content of *Ascophyllum nodosum* (L.) Le Jol. and *Fucus vesiculosus* (L.). J. Exp. Mar. Biol. Ecol. 34, 245–258.
Rose, M.T., Wolf, B.T., Haresign, W., 2007. Effect of the level of iodine in the diet of pregnant ewes on the concentration of immunoglobulin G in the plasma of neonatal lambs following the consumption of colostrum. Br. J. Nutr. 97, 315–320.
Saker, K.E., Fike, J.H., Veit, H., Ward, D.L., 2004. Brown seaweed- (Tasco) treated conserved forage enhances antioxidant status and immune function in heat-stressed wether lambs. J. Anim. Physiol. Anim. Nutr. 88, 122–130.
Sangild, P.T., 2003. Uptake of colostral immunoglobulins by the compromised newborn farm animal. Acta Vet. .Scand. Suppl. 44 (1), 105–122.
Scherf, H., Machlin, L.J., Frye, T.M., Krautmann, B.A., Williams, S.N., 1996. Vitamin E biopotency: comparison of various "natural-derived" and chemically synthesized alfa-tocopherols. Anim. Feed Sci. Technol., 115–126.
Smith, K.L., 1986. Vitamin E-enhancement of immune response and effects on mastitis in dairy cows., Roche Symposium "The Value of Vitamins in Animal Nutrition", F-Hoffmann-La Roche Animal Nutrition and Health, London, England, p. 1.
Sonne, C., Dietz, R., Larsen, H.J.S., Loft, K.E., Kirkegaard, M., Letcher, R.J., Shahmiri, S., Moller, P., 2006. Impairment of cellular immunity in west Greenland sledge dogs (*Canis familiaris*) dietary exposed to polluted minke whale (*Balaenoptera acutorostrata*) blubber. Environ. Sci. Technol. 40, 2056–2062.
Storset, A.K., Hasvold, H.J., Valheim, M., Brun-Hansen, H., Berntsen, G., Whist, S.K., Djonne, B., Press, C.M., Holstad, G., Larsen, H.J.S., 2001. Subclinical paratuberculosis in goats following experimental infection. An immunological and microbiological study. Vet. Immunol. Immunopathol. 80, 271–287.
Tufariello, J.M., Chan, J., Flynn, J.F., 2003. Latent tuberculosis: mechanisms of host and bacillus that contribute to persistent infection. Lancent Infect. Dis. 3, 578–590.
Vahter, M., 2009. Effects of arsenic on maternal and fetal health. Annu. Rev. Nutr. 29, 381–399.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Weiss, W.P., Hogan, J.S., Wyatt, D.J., 2009. Relative bioavailability of all-rac and RRR vitamin E based on neutrophil function and total alpha-tocopherol and isomer concentrations in periparturient dairy cows and their calves. J. Dairy Sci. 92, 720–731.
Whist, S.K., Storset, A.K., Johansen, G.M., Larsen, H.J.S., 2003. Modulation of leukocyte populations and immune responses in sheep experimentally infected with Anaplasma (formerly Ehrlichia) phagocytophilum. Vet. Immunol. Immunopathol. 94, 163–175.
Zeng, H., Uthus, E.O., Combs Jr, G.F., 2005. Mechanistic aspects of the interaction between selenium and arsenic. J. Inorg. Biochem. 99, 1269–1274.

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