Effect of vitamin E supplementation of sheep and goats fed diets supplemented with polyunsaturated fatty acids and low in Se

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Abstract

Vitamin E (VitE) and selenium (Se) are an essential part of the antioxidative functions of metabolism. There are situations of low supply of both micronutrients. As VitE is involved in ruminal biohydrogenation of polyunsaturated fatty acids (PUFA) and their protection against oxidation in metabolism, diets supplemented with PUFA may challenge VitE to an extent making recommended supplies insufficient. Twelve goats and sheep each were fed a diet supplemented with PUFA and characterised by low Se and limited VitE contents during the last 2 months of gestation and the first 2 months of lactation. The basal diet consisted of hay and concentrate. Six goats and sheep received extra VitE, while the control groups received no extra VitE. Blood and milk samples were taken. In addition, liver, heart muscle and spleen samples were obtained from the offspring after slaughtering at an age of 8 weeks. No significant changes were observed in serum Se and VitE. A significant increase in serum VitE concentrations between 2 and 4 weeks postpartum (pp) was evident in the supplemented kids. In 4, 6 and 8 weeks pp, the serum concentrations of VitE in the supplemented kids were significantly higher compared to the unsupplemented group. In the kids, VitE was higher in liver of the supplemented groups. There were no significant differences in response to extra VitE between sheep and goat. The kids responded to serum VitE different from that of lambs, as a significant difference was observed between supplemented and unsupplemented animals in the goat kids, but not the lambs. In conclusion, goats and sheep have to be viewed differently and may not be considered alike relating to VitE/Se metabolism and requirements, especially in young animals.
Effect of vitamin E supplementation of sheep and goats fed diets supplemented with polyunsaturated fatty acids and low in selenium

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Keywords

small ruminants, vitamin E, selenium, polyunsaturated fatty acids

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Summary

Vitamin E (VitE) and selenium (Se) are an essential part of the antioxidative functions of metabolism. There are situations of low supply of both micronutrients. As VitE is involved in ruminal biohydrogenation of polyunsaturated fatty acids (PUFA) and their protection against oxidation in metabolism, diets supplemented with PUFA may challenge VitE to an extent making recommended supplies may not be sufficient.

Twelve goats and sheep each were fed a diet supplemented with PUFA, characterised by low Se and limited VitE contents during the last two months of gestation and the first two months of lactation. The basal diet consisted of hay and concentrate. Six goats and sheep received extra VitE, while the control groups received no extra VitE. Blood and milk samples were taken. In addition, liver, heart muscle and spleen samples were obtained from the offspring after slaughtering at an age of eight weeks. No significant changes were observed in serum Se and VitE. A significant increase in serum VitE concentrations between two and four weeks pp was evident in the supplemented kids. Four, six and eight weeks pp, the serum concentrations of VitE in the supplemented kids were significantly higher compared to the unsupplemented group. In the kids, VitE was higher in livers of the supplemented groups. There were no significant differences in the response to extra VitE between sheep and goat. The kids responded in serum VitE different from the lambs, since a significant difference was observed in between supplemented and unsupplemented animals in the goat kids, but not the lambs. In conclusion, goats and sheep have to be looked at differently and may not be considered alike relating to VitE/Selen metabolism and requirements, especially in young animals.
Introduction

Two micronutrients are of great importance in maintaining the antioxidative status of the metabolism of animals, vitamin E (VitE) and selenium (Se). The primary function of VitE is to maintain the functional integrity of cellular and subcellular membranes, by preventing lipid peroxidation of unsaturated fatty acids (Hoekstra, 1975). Nutritional muscular dystrophy, liver necrosis in pigs and rats, fetal resorption in rats, and encephalomalacia in poultry are possible signs of VitE deficiency (Blood and Radostits, 1989). Deficiency of VitE has been observed in virtually all taxa of herbivores. VitE deficiency can develop in herbivores when they are fed hay with a low VitE content, due to late cutting, leaching or excessive storage, leading to oxidation of VitE (Rice et al., 1985). Reductions in VitE of 80 to 90% during the maturation of grasses are common.

Selenium is of particular interest in animal nutrition because, in many areas of the world, plants contain concentrations that are either deficient in or toxic to the animal metabolism. One of the major functions of Se is as a component of the enzyme glutathione peroxidase, which reduces peroxides and hydroperoxides. When dietary selenium is insufficient to prevent this oxidation, muscle membranes rupture and leak cellular enzymes into the extracellular circulation (Feldmann et al., 1998). These damaged muscles become non-functional and turn white, hence the name ‘white muscle disease’. Animals differ in their requirements for selenium and their susceptibility to white muscle disease. Some of these species variations (Neumann-Mumme and Bronsch, 1991) may be due to differences in the activity of the nonselenium-dependent glutathione peroxidase.

There are various interactions between VitE and Se (Kessler, 1999; Larsen et al., 1988; Smith et al., 1997) and it may be possible that there are situations where both
micronutrients are at or below requirements. For instance, low dietary Se may be a contributing factor to VitE deficiency developing in hay-fed animals (Rice et al., 1985). The situation may become critical when either of the two micronutrients becomes deficient. One such situation, not yet accounted for in recommendations given for supply of VitE and Se, may be the degradation of VitE by gastrointestinal microflora when animals consume unsupplemented high-grain diets. With an increasing corn content the destruction of VitE was found to increase from 8 to 42%, probably as a consequence of the associated increase in the amount of polyunsaturated fatty acids (PUFA) (Shin and Owens, 1990, Alderson et al., 1971). This observation could not be confirmed by Leedle et al. (1993) in an in-vitro experiment, where a high corn diet was used. Depending on the composition and the ingredients of the concentrates (more carbohydrates or more fat), the response in VitE may vary. Dietary PUFA are extensively biohydrogenated in the rumen resulting in the absorption of a higher proportion of saturated fatty acids at the small intestine as that present in the diet (Wachira et al., 2000). Despite the high levels of degradation of dietary PUFA, feeding high PUFA diets has been demonstrated to substantially increase concentrations of these PUFA within the polar and neutral lipids in sheep (Cooper et al., 2004). Therefore, although supplementation of PUFA may have positive effects on offspring, it may also increase the oxidative challenge to the animal, with effects on the antioxidant status (Capper et al., 2002) and this in the situation where a substantial part of VitE has been already spent in the rumen.

In the present study, the hypothesis was tested that a PUFA-supplemented diet, at simultaneously low Se supply, challenges VitE to an extent which requires dietary supply of VitE beyond recommended minimum levels. As this may differ between ruminant species, the same diets with and without extra VitE were tested in sheep
and goat. Soybean cake (residue from pressing with relatively high residual oil content) and pure soybean oil, lipid sources rich in mainly linolenic acid, and ground full-fat linseed, rich in linolenic acid, were employed to supplement the diet with PUFA. Focus in terms of effects was put on variables describing VitE and Se status of organs and blood, and pregnant and lactating ewes and goats as well as their offspring were investigated.

Material and Methods

Animals and diets

Twelve pregnant goats and sheep each, and their offspring, were employed in the present study (12 5-years old Saanen goats, 12 5-years old Ostfriesian dairy sheep). The animals were fed a diet high in polyunsaturated fatty acids (16.3 g/kg) and low in selenium (0.06 mg/kg; supply: 0.05-1 mg/kg DM; RAP, 1999) during the last two months of gestation and the first two months of lactation. The diet consisted of hay (2 kg/day) and concentrate (depending on gestation or lactation period 200 g up to 500 g/day) (Table 1). The dams were fed according to nutrient requirements (RAP, 1999), except for VitE in half of the sheep and goat (high VitE groups). These groups received 2 g/day of extra VitE (1 g = 44 IU; Vitamin E Streuli, G. Streuli&Co. AG, Uznach, Switzerland) by mixing this amount into the concentrate. The supplementation added up to a total of 72 mg/kg DM; groups GE, SE), while the control animals received the basal diet only with a concentration of 35mg VitE/kg DM (groups GC, SC) (minimal requirement: 15 mg/kg DM, RAP, 1999). The animals ate the whole ration, were kept in single pens and had free access to water. The offspring was kept with their mothers until an age of 6 weeks. After weaning, the lambs and kids were kept in groups until culling at the age of 8 weeks. The lambs
and kids from unsupplemented dams remained unsupplemented (LC = lambs, KC = kids) and the lambs and kids from supplemented dams were supplemented with extra VitE (LE = lambs; KE = kids). This was accomplished by feeding the same diets as that for the dams from week 6 onwards. Already from week 3, the kids and lambs received small volumes of the diets fed from 6 weeks onwards to adapt them to hay and concentrate.

Collection of milk, blood and organ samples

Milk samples were taken at parturition, 1 week postpartum (pp) and 4 weeks pp. 50-100 ml of milk were sampled and stored at -20°C. Blood samples were collected from the jugular vein 3, 2 and 1 week ante partum (ap), at parturition, 1, 2, 3, 4 and 6 weeks pp in the adult animals. In the offspring, blood was sampled at 1, 2, 4, 6 and 8 weeks of age. Blood was centrifuged (1,500 × g, 10 min) within 30 min of collection, and serum and plasma were stored at -20 °C until analyses were performed. The erythrocytes were washed with physiologic sodium chloride (0.9%) solution and stored at -20°C until analyses. Samples were analyzed for VitE, Selenium (Se) and glutathion peroxidase (GSH-Px). In addition, samples of heart, liver, muscle and spleen (an organ rich in Se; Lawler et al., 2004) were taken from the lambs and kids after slaughtering. Liver and spleen were stored at -20°C. The heart samples were first put into 4% formaldehyde, then for 24 h into water, and finally stored in 70% ethanol solution.

Analysis of samples

Vitamin E determination in plasma, milk and liver
Vitamin E was analysed by HPLC following solvent extraction. Plasma VitE samples were determined by the method of McMurray and Blanchflower (1979) employing ethanol and hexane. Milk, colostrum and liver VitE concentrations were determined according to a modification of this method (Burton et al., 1985). The latter samples had been subjected to hydrolysis by a solution of KOH before extraction of VitE by ethanol and hexane. Elutants from colostrum, milk and liver samples were passed through a normal phase column packed with silica (125 × 4mm with 5µm particle size; Macherrey-Nagel, Oensingen, Switzerland) with a mobile phase of 96% n-hexane-methanol and 4% 1,4 dioxane within an HPLC apparatus (Varian, Varian AG, Zug, Switzerland). Fluorescence detection of VitE was at an excitation of 297 nm and emission of 330 nm.

Selenium determination in plasma and spleen

Selenium was determined by a FIA-GF-AAS system (flow injection analysis, graphite furnace atomic absorption spectrometry) coupled with a hydride generation (FIAS 400 Perkin-Elmer, GF-AAS Analyst 600, Perkin-Elmer AG, Schwerzenbach, Switzerland). Plasma samples (1 ml) or 1 g of lyophilised spleen tissue were dissolved in 20 ml of HNO₃ and 4 g Mg nitrate and vortexed. Thereafter pre-digestion was performed overnight. After pre-digestion, the samples were heated gradually and totally 6 ml of H₂O₂ was added. Then, the samples were ashed at 420°C. The leftover was dissolved in 5 ml demineralised water and 11 ml HCl (37%) and then heated again. After cooling to room temperature, the samples were flushed in HCl (3%) and volume was filled up to 50 ml and subjected to measurement thereafter.

Histology of the heart
After storing the samples in 70% ethanol, the samples were dehydrated in graded ethanol (70, 96 and 100%), methyl benzoate, xylene and paraffin (60 °C) for 2x4 h each and finally embedding in paraffin (histowax®, Leica). Transverse sections were cut at 5 µm, mounted on Superfrost® plus adhesive slides (Menzel-Gläser®, Braunschweig) and dried at 60 °C for 30 min. Hematoxilin and eosin (Böck, 1989) stained sections were performed to verify physiological state of the tissues and exclude animals possibly exhibiting pathological modifications of the heart.

Statistical analysis
All data were reported as mean ± standard error. Differences between time periods for sample collection were analysed by a multivariate ANOVA for repeated measurements as calculated by use of a statistical software program (Systat version 8.0, SPSS Inc, Chicago, USA). The factor group (=treatment within species, i.e. SE vs. SC and GE vs. GC) was included in the model, which was applied separately for each species. In addition, the species were compared (goats vs. sheep). Differences were considered significant at a p-level of ≤ 0.05.

Results
Vitamin E concentrations in serum of dams and kids
The VitE concentrations in serum of the dams at the beginning of the experiment were 0.6 ± 0.1, 0.8 ± 0.1, 0.5 ± 0.1 and 0.7 ± 0.1 µg/ml for SC, SE, GC and GE, respectively (Fig. 1a, 1b). Three to four weeks ante partum the concentrations increased significantly to 1.0 ± 0.1 µg/ml for SC (p = 0.046), 1.3 ± 0.1 µ/ml for SE (p = 0.028), 1.8 ± 0.1 µg/ml for GC (p = 0.028) and 1.7 ± 0.2 µg/ml for GE (p = 0.028). Until the end of the experiment the concentrations stayed more or less at the same
level in all groups, despite a significant decrease \( (p = 0.046) \) at parturition as well as a significant increase until 4 weeks pp \( (p = 0.046) \) that was observed for GE. Differences among VitE groups were not significant. The goats had significantly higher VitE concentrations throughout the whole experiment compared to the sheep \( (p = 0.004) \).

At parturition the serum VitE concentrations were 0.1 ± 0.0 µg/ml for the lambs (LC and LE) and 0.5 ± 0.1 µg/ml for the goat kids (KC and KE). Until one week of age a significant increase was observed in all groups reaching 0.9 ± 0.1 µg/ml (LC, \( p = 0.001 \)), 1.2 ± 0.2 µg/ml (LE, \( p = 0.005 \)), 1.1 ± 0.1 µg/ml (KC, \( p = 0.019 \)) and 1.3 ± 0.1 µg/ml (KE, \( p = 0.005 \)) (Fig. 2 a, 2b). In addition, a significant increase was shown for the lambs of the control group (LC) until two weeks of age \( (p = 0.019) \). Thereafter the concentrations decreased until four weeks of age (lambs with additional VitE (LE) \( p = 0.047 \)) and started to increase again until the end of the experiment. The increase between six and eight weeks of age to concentrations of 1.5 ± 0.1 and 2.1 ± 0.3 µg/ml in LC and LE, respectively, was significant for both lamb groups (LC \( p = 0.010 \), LE \( p = 0.047 \)). In the control goat kid group (KC) a decrease was observed until six weeks of age. Thereafter a significant increase was found until week 8 \( (1.2 ± 0.1 \mu g/ml; \ p = 0.041) \). In contrast, the kids with extra VitE in the diet expressed a significant increase from two weeks of age until the end of the experiment (eight weeks of age; \( 1.7 ± 0.1 \mu g/ml \)). In addition, a significant increase was shown between weeks 2 and 4 of age \( (p = 0.012) \). At four \( (p = 0.009) \), six \( (p = 0.001) \) and eight \( (p = 0.009) \) weeks of age, the concentrations of vitamin E in the supplemented group of kids (KE) were significantly higher compared to the unsupplemented (KC) group. Between the corresponding lamb groups no significant difference was found.
Vitamin E concentrations in the milk of mothers and liver of the kids

In Table 2 the VitE concentrations of colostrum and milk are shown. The VitE content of colostrum was about twice as high (p = 0.023) in sheep compared to goats, while a significant effect of VitE within the species occurred in sheep and goats only 4 weeks pp (p = 0.026 and 0.031). The VitE concentrations in milk one and four weeks pp in milk were significantly lower compared to the colostrum (p = 0.008, 0.002, 0.028; table 2).

VitE concentrations in the liver of the lambs and goat kids significantly increased with extra VitE given to the dams and kids, respectively (p=0.005 for the lambs, p<0.001 for the goat kids; Table 3).

Plasma and spleen selenium concentrations

The mean plasma Se concentrations in the dams were 1.5-fold higher in the sheep than in the goats at the beginning of the experiment (Table 4). Until one week ap the Se concentrations decreased in all four groups, but significantly only in three groups with p-values of 0.028, 0.028 and 0.046 for SC, GC and GE, respectively. In the sheep this decrease was observed until the end of the experiment. In contrast, the goats expressed an increase in that period. No significant differences were observed between the SC/SE group and the GC/GE group. Across the entire period, sheep had significantly higher plasma Se concentrations compared to the goats (p = 0.004). The mean plasma Se concentrations of the kids at parturition were similar between species (Table 5). The lambs expressed a significant increase in plasma Se until six weeks of age (p = 0.046 for LE). In contrast, the Se concentrations in the goat kids first decreased and then increased again. No significant VitE effect within species
was observed, but from two weeks of age onwards the lambs always showed significantly higher Se concentrations compared to the goat kids.

The Se concentration in the spleens of the kids, as shown in Table 6, was not significantly affected by VitE supplementation.

Histology of the heart

No degeneration of the heart muscle was observed in any lamb or goat kid.

Discussion

In the present study, no significant differences in VitE concentration in serum of the dams were observed between the control groups (SC, GC) and the VitE supplemented (SE, GE) groups. One explanation might be that VitE can be stored in the liver for two to three months (Kolb et al., 1997; Bickhardt et al., 1999) which is not much shorter than the duration of the experiment. This assumption could have been tested when liver biopsies of the dams would have been taken at the beginning and the end of the present study, but this was not possible due to animal welfare regulations. It is, however, likely that the body of the control animals would have been depleted of VitE to a certain extent at the end the experiment while the VitE stores of the supplemented animals would have been rather high. Another factor, which has to be kept in mind, was the unexpectedly high VitE supply via the hay (38.60 mg vitamin E/kg DM), which alone theoretically covered the VitE requirements as stated by various sources (Vila, 1975; Ferrando and Barlet, 1979; Völker and Steinberg, 1981; Jordan et al., 1985; Kolb et al., 1997; Kessler, 1999; Kessler, 2004). Also in other studies (Finch and Turner, 1996, Hakkarainen et al., 1983), the investigators had problems to design a diet which was low in VitE. Lannek and Lindberg (1975) described an influence of VitE, Se and fatty acids on VitE-Se-
deficiency, where one system compensated for the other system. However, it seems
that the challenging the VitE-/Se-system by a high PUFA/low Se diet still did not
result in a situation of deficiency. When comparing the serum concentrations of the
present study with those from the literature (Camas et al., 1986; Gubler, 1986;
Bickhardt et al., 1999), it may be concluded that the dietary VitE concentrations
administered to the dams in all groups were sufficient at least for sheep. Doncon and
Steele (1988) also stated that measuring VitE only in the serum may lead to wrong
conclusions and the determination of VitE in the liver would give much more
information about the VitE status of the animals.

The low VitE concentrations in serum of the lambs and the goat kids at parturition
and the increase with the uptake of colostrum is in accordance to other studies; this
is in line with the known correlation between the serum VitE concentrations of the
mother, their colostrums and the VitE concentration of the lambs after colostrum
consumption (Bostedt and Schramel, 1978; Camas et al., 1986; Watson et al., 1988;
Pehrson et al., 1990; Hermülheim et al., 1992, Njeru et al., 1994, Capper et al.,
2005). In serum VitE there was one of the few species-specific differences in
response to extra VitE since goat kids responded and lambs not. These species
differences may have resulted from the lower VitE concentrations in the colostrum of
the goats (non-significantly higher in the VitE supplemented goats) thus creating a
situation of deficient VitE without extra VitE in the goat kids. Bickhardt et al. (1999)
claimed that concentration below 1.0 µg VitE/ml plasma indicates deficiency in
lambs. In the present study, the concentrations were below this value only at
parturition which means that from then on VitE supply also returned to sufficient
levels without extra VitE. The VitE concentrations determined by Gubler (1986) in
plasma of unsupplemented goat kids were consistent with the concentrations found in the present study in the unsupplemented group.

In accordance to the literature (Pehrson et al., 1990; Bickhardt et al., 1999), colostrum contains higher VitE concentrations compared to milk. Since VitE is transferred to the foetus only in small amounts, this micronutrient is enriched in colostrum in order to support an adequate supply for the offspring (Njeru et al., 1994; Kolb et al., 1997). Also Gubler (1986) described VitE concentrations in milk which were comparable to those found in the present study. The high colostral VitE concentrations and the lower milk VitE concentrations appears to be in contrast with the postpartal increased serum VitE concentrations of the lambs and kids after parturition, but this is also a function of concentration times intake. Also in the literature such observations are reported (Bostedt and Schramel, 1978; Camas et al., 1986; Watson et al., 1988; Pehrson et al., 1990; Hermülheim et al., 1992, Njeru et al., 1994).

In the present study the VitE supplemented lambs and goat kids expressed significantly higher concentrations of VitE in the liver compared to the unsupplemented groups. In contrast to the present observations, Agag et al. (1995) found much higher VitE concentrations in the liver of supplemented lambs (without giving data on overall diet supplementation). Also Buchanan-Smith et al. (1969) and Doncon and Steele (1988) described higher concentrations but in older animals. Green et al. (1995) considered concentrations of 25 µg vitamin E/g liver as to be in the normal range. In the present study, the concentrations in liver were often lower than the concentrations described in the literature even though the animals were either supplemented or had received high native VitE concentrations via feeds. It is likely, therefore, that there was a significant VitE expenditure due to actions in rumen
and metabolism related to dietary PUFA. Since no clinical or histological symptoms of white muscle disease were found (e.g. via heart histology), the concentrations of VitE in serum were in the normal range, and the animals were younger than most described in literature, it still can not be concluded that the lambs and kids were actually deficient in VitE. Additionally, Steele et al. (1980) defined that only VitE concentration of less than 1 µg/g liver of weaner sheep as to be deficient. The observation that VitE concentrations in the liver, but not in serum, in offspring of both species was increased by supplementary VitE, suggests that during phases of sufficient VitE supply the liver reservoir is first filled before differences in serum VitE concentrations can be observed where levels are homeostatically controlled anyway. The liver is the main reservoir for VitE in sheep (Hidiroglou, 1987). Obviously the only group reaching the borderline of deficiency under the experimental dietary conditions without extra Vit E were the goat kids thus indicating requirements higher than those assumed in most recommendations. However, it cannot be totally excluded that the reservoir in the supplemented goat kid group was excessively filled and therefore the VitE concentrations in the blood were unusually high. Despite its important task in the body’s antioxidative systems, only species-specific differences in Se concentrations in plasma and spleen (one of the major Se stores in the body; Lawler et al., 2004)) of the offspring were observed in the present study. Buchanan-Smith et al. (1971) also found no effect of VitE supplementation on Se concentrations in different organs. In contrast, Sharp et al. (1970) found higher Se concentrations in the kidneys of pigs after VitE supplementation of 5.5 IU/kg of diet. They described that the minimal reference value of 80 µg Se/l plasma (Bickhardt et al., 1999) is probably a good indicator for the Se status of an animal. According to
this standard, the plasma Se concentrations of the animals in the present study seem
to have been adequate.

In the literature not many data on Se contents in spleen are found. Oelschläger and
Menke (1969) described mean Se in spleen of pigs of unknown age of 330 µg/kg
DM. This is three times less than the values analysed in the offspring in the present
study. In humans, children had higher Se concentrations in spleen (370 µg/kg DM)
compared to adults (270 µg/kg DM) (Dickson and Tomlinson, 1967). Lawler et al.
(2004) determined concentrations of 2000 µg Se/kg wet weight of spleen in beef
cattle with adequate Se supply. This is in accordance to the concentrations found in
the present study. Haenlein (1999) mentioned Se concentrations of 1648 µg/kg DM
in spleen in adequately supplied and of 838 µg/kg DM in insufficiently supplied adult
goats. In the present study, sheep of all age groups exhibited higher Se
concentrations in spleen than those of Hartley (1976). As concentrations of Se in the
spleen of eight weeks old lambs and kids are described for the first time in literature
to the authors’ knowledge in the present study, and because these are relatively high
compared to young animals of other species, they might be used as reference values
for lambs and goat kids with a borderline sufficient supply of Se. This may lead to the
assumption that either the requirements for goat and sheep found in literature are
rather on the upper level even in the situation of high supply of polyunsaturated fatty
acids particularly susceptible to oxidation or that there is no extra usage of Se due to
oxidation of these fatty acids.

In conclusion, from the present results it appears that the level of VitE supply by the
basal diet was likely to be sufficient (sheep) or at the borderline of sufficiency (goats)
when feeding diets with elevated PUFA and borderline Se. No signs of white muscle
disease were evident which would have indicated a serious deficiency. A likely
explanation for these findings is the ability of the liver of the dams to store vitamin E over two to three months. Apart from VitE content of the basal diet, which may be reduced when using forages after extended storage, the VitE feeding history therefore seems decisive for the expression of VitE deficiency in dams and offspring when challenged by high PUFA intakes. There were some species differences in the response to extra VitE. These might have resulted from the different baseline values in serum VitE and plasma Se between the two species making them differently susceptible to low supply of either VitE or Se or stressors of VitE such as PUFA. Accordingly, goat kids seem to be more susceptible to variations compared to lambs. Further detailed studies are necessary to identify the mechanisms responsible for such species differences.

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Table 1 Raw materials of the concentrate and chemical composition of the diet components (g/kg fresh weight). CP=crude protein, CF=crude fiber; EE=ether extract, Ca=Calcium, P=Phosphorus.

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<tr>
<td>CP</td>
<td>g/kg</td>
<td>138.7</td>
</tr>
<tr>
<td>CF</td>
<td>g/kg</td>
<td>255.3</td>
</tr>
<tr>
<td>EE</td>
<td>g/kg</td>
<td>31.6</td>
</tr>
<tr>
<td>HCl-insoluble ash</td>
<td>g/kg</td>
<td>17.2</td>
</tr>
<tr>
<td>Ca</td>
<td>g/kg</td>
<td>7.0</td>
</tr>
<tr>
<td>P</td>
<td>g/kg</td>
<td>2.7</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>mg/kg</td>
<td>38.60</td>
</tr>
<tr>
<td>Selenium</td>
<td>mg/kg</td>
<td>0.04</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>g/kg</td>
<td>28.50</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>g/kg</td>
<td>3.40</td>
</tr>
<tr>
<td>Linolenic acid (C18:3)</td>
<td>g/kg</td>
<td>9.10</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>g/kg</td>
<td>12.50</td>
</tr>
</tbody>
</table>
Table 2 Vitamin E concentrations (µg/ml) of colostrum and milk (mean and standard error).

P-value indicates time difference $t_i-t_j$, ns = not significant. SC = control sheep, SE = Vitamin E supplemented sheep, GC = control goats, GE = Vitamin E supplemented goats. Different letters indicate significant differences within species between groups.

<table>
<thead>
<tr>
<th>Time</th>
<th>SC</th>
<th>p</th>
<th>SE</th>
<th>p</th>
<th>GC</th>
<th>p</th>
<th>GE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parturition (colostrum) $t_1$</td>
<td>9.0 ± 1.6</td>
<td>10.7 ± 3.2</td>
<td>4.7 ± 0.5</td>
<td>6.2 ± 1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1w pp (milk) $t_2$</td>
<td>0.3 ± 0.1</td>
<td>&lt;0.05</td>
<td>0.5 ± 0.2</td>
<td>&lt;0.05</td>
<td>0.2 ± 0.2</td>
<td>&lt;0.05</td>
<td>0.7 ± 0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4w pp (milk) $t_3$</td>
<td>1.0 ± 0.2</td>
<td>&lt;0.05</td>
<td>0.3 ± 0.1</td>
<td>ns</td>
<td>0.04 ± 0.0$^a$</td>
<td>ns</td>
<td>0.3 ± 0.1$^b$</td>
<td>ns</td>
</tr>
</tbody>
</table>
Table 3 Vitamin E concentrations (µg/g) in liver (mean and standard error). LC = control lambs, LE = lambs supplemented with VitE, KC = control goat kids, KE = goat kids supplemented with VitE; p-value indicates group effect within species.

<table>
<thead>
<tr>
<th></th>
<th>LC</th>
<th>LE</th>
<th>p LC vs. LE</th>
<th>KC</th>
<th>KE</th>
<th>p KC vs. KE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit E</td>
<td>2.2 ± 0.3</td>
<td>3.8 ± 0.3</td>
<td>0.005</td>
<td>1.5 ± 0.2</td>
<td>3.4 ± 0.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 4 Mean Se concentrations (µg/l) in plasma of goats and sheep (mean and standard error). P-value, time pattern t₁-t₄, ns = not significant. BS0 = blood sample before experiment, SC = control sheep, SE = Vitamin E supplemented sheep, GC = control goats, GE = Vitamin E supplemented goats.

<table>
<thead>
<tr>
<th>Time</th>
<th>SC</th>
<th>p</th>
<th>SE</th>
<th>p</th>
<th>GC</th>
<th>p</th>
<th>GE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP0 t₀</td>
<td>206.3 ± 11.7</td>
<td></td>
<td>206.7 ± 13.9</td>
<td></td>
<td>141.8 ± 6.3</td>
<td></td>
<td>130.8 ± 8.3</td>
<td></td>
</tr>
<tr>
<td>1w ap t₁</td>
<td>186.1 ± 7.9</td>
<td>&lt;0.05</td>
<td>188.0 ± 5.8</td>
<td>ns</td>
<td>115.6 ± 3.5</td>
<td>&lt;0.05</td>
<td>110.6 ± 1.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2w pp t₂</td>
<td>133.2 ± 6.7</td>
<td>&lt;0.05</td>
<td>147.4 ± 11.1</td>
<td>ns</td>
<td>134.0 ± 5.2</td>
<td>&lt;0.05</td>
<td>122.5 ± 6.2</td>
<td>ns</td>
</tr>
</tbody>
</table>
Table 5 Mean Se concentrations (µg/l) in plasma of lambs and goat kids (mean and standard error). P-value, time pattern t_i-t_j, ns = not significant. LC = control lambs, LE = Vitamin E supplemented lambs, KC = control goat kids, GE = Vitamin E supplemented goat kids.

Supplementation via concentrate and hay from week 3 onwards.

<table>
<thead>
<tr>
<th>Time</th>
<th>LC</th>
<th>LE</th>
<th>KC</th>
<th>KE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parturition t_1</td>
<td>87.0 ± 2.8</td>
<td>77.4 ± 9.1</td>
<td>81.3 ± 5.1</td>
<td>72.7 ± 3.1</td>
</tr>
<tr>
<td>2w pp t_2</td>
<td>95.1 ± 5.5</td>
<td>ns</td>
<td>92.8 ± 2.7</td>
<td>0.046</td>
</tr>
<tr>
<td>6w pp t_3</td>
<td>97.6 ± 6.7</td>
<td>ns</td>
<td>99.0 ± 6.2</td>
<td>ns</td>
</tr>
</tbody>
</table>
Table 6  Se concentration in the spleen of lambs and goat kids (µg/kg DM) (mean ± standard error). ns = not significant. LC = control lambs, LE = Vitamin E supplemented lambs, KC = control goat kids, GE= Vitamin E supplemented goat kids.

<table>
<thead>
<tr>
<th></th>
<th>LC</th>
<th>LE</th>
<th>p LC vs. LE</th>
<th>KC</th>
<th>KE</th>
<th>p KC vs. KE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se</td>
<td>1176 ± 72.1</td>
<td>1197 ± 64.7</td>
<td>ns</td>
<td>1203 ± 50.1</td>
<td>1328 ± 45.7</td>
<td>ns</td>
</tr>
</tbody>
</table>
Legends of the figures:

Fig. 1 a,b Serum Vitamin E concentrations (Mean ± SE) before parturition and during lactation (n=6 for each group; SC: unsupplemented sheep; SE: Vitamin E supplemented sheep; GC: unsupplemented goat; GE: Vitamin E supplemented goat); blood samples were taken on day 0, 3-4 weeks ante partum (ap), 2 weeks ap, 1 week ap, at parturition, 1 day post partum (pp), 1 week pp, 2 weeks pp and 4 weeks pp. Values with * differ significantly (p ≤ 0.05) within time (time effect within a group).

Fig. 2 a,b Serum Vitamin E concentrations (Mean ± SE) at birth until 8 weeks of age (LC: unsupplemented lambs; LE: Vitamin E supplemented lambs; KC: unsupplemented goat kids; KE: Vitamin E supplemented goat kids); blood samples were taken at birth, 1 week post partum (pp), 2 weeks pp 4 weeks pp and 8 weeks pp. Values with * differ significantly (p ≤ 0.05) within time (time effect within a group). Values with different letters differ significantly (p ≤ 0.05) within a group (group effect at timepoint). Supplementation via concentrate and hay from week 3 onwards.