Blood serum Vitamin A and E concentrations and distribution into lipoprotein fractions of pregnant sheep and newborn lambs

NEZIR Y. Toker*

Istanbul University, Faculty of Veterinary Medicine, Department of Biochemistry, 34320 Avciel-Istanbul TURKEY
Telephone: 0090 212 4737070/17125 - Fax: 0090 212 4737241

* Corresponding author: E-mail: nytoker@superonline.com

SUMMARY

This study was supported by the Istanbul University research fund. Project No.: 1447/05052000

In this study, the vitamin A and E concentrations in plasma and their distribution in VLDL/LDL fractions were determined by HPLC over a period of 5 weeks after parturition in ewes (10 crossbred Sakiz sheep) and their progeny. In order to test the vitamin A and E supply by colostrums, newborns were divided into 2 equal groups (n = 10): the SL groups (new-born suckling their mothers) and the AFL group (new-borns were separated from their mothers immediately after birth and were fed with full fat milk). All lambs were progressively adapted to food based on dry grass and concentrates.

The plasma vitamin A and E concentrations markedly fell in ewes at parturition and 3 days after (p < 0.01), thereafter gradually increased for significantly exceeding the initial values (10 days before parturition). The effects of feeding art (FA) and day on vitamin A level were significant (p<0.001). The effect of FA x Day interaction on vitamin A level was also significant (p<0.01). While the effect of FA on vitamin E level was highly significant (p<0.001), the effect of day was significant at p<0.01 level. On the other hand the effect of FA x Day interaction on vitamin E level was not significant (p>0.05). Moreover, mortality and delayed growth were also recorded in this group. The vitamin A bound to lipoproteins was not detected nor in ewes, neither in new-borns. These results show that colostrum is the main source of fat-soluble vitamins (A and E) for new-borns otherwise a vitamin deficiency may occur and that an early food diversification may restore fat-soluble vitamin supply and limit the severity of the deficiency.

Keywrods : Vitamin A, Vitamin E, VLDL/LDL, ewe, lamb.

RÉSUMÉ

Concentrations sériques des vitamines A et E et leur distribution dans les lipoprotéines chez les brebis gestantes et leurs agneaux

Au cours de cette étude, les concentrations plasmatiques en vitamines A et E et leurs proportions dans les VLDL et LDL ont été déterminées par CLHP pendant une période de 5 semaines après les agnelages chez 10 brebis croisées Sakiz ainsi que sur leur progéniture. Afin de tester les apports par le colostrum en vitamines A et E, les nouveau-nés ont été divisés en 2 groupes égaux (n = 10) : dans le groupe SL, les agneaux ont tété leur mère, et dans le groupe AFL, les agneaux ont été séparés de leur mère immédiatement après la naissance et ont été artificiellement nourris par du lait entier. Tous les agneaux ont progressivement été habitués à une nourriture à base d’herbes et de concentrés. Les concentrations plasmatiques en vitamines A et E ont nettement diminué chez les brebis lors de l’agnelage et 3 jours plus tard (p<0.01) puis progressivement augmenté pour dépasser significativement les valeurs initiales obtenues 10 jours avant le part (p<0.01). Chez les agneaux qui ont tété leur mère, elles ont augmenté dès le 3ème jour (p<0.01) jusqu’à la fin de l’expérimentation. Les effets de l’art de alimentation (FA) et du jour (Day) au niveau de vitamine A étaient significatifs (p<0.001). L’effet de l’interaction de FA x Day au niveau de vitamine A était également significatif (p<0.01). Tandis que l’effet du FA au niveau de la vitamine E était fortement significatif (p<0.001). L’effet du jour était significatif au niveau p<0.01. Sur la main d’oher l’effet de l’interaction de FA x Day au niveau de la vitamine E n’était pas significatif (p>0.03). Ces résultats montrent que, d’une part, le colostrum est la principale source de vitamines liposolubles (A et E) chez les nouveau-nés, sinon une carence peut apparaître et que, d’autre part, une diversification précocée de l’alimentation peut restaurer l’apport vitaminique et limiter la sévérité de la carence.

by the small intestine by a facilitated diffusion with a ratio of about 20-25% [12]. The quality and the quantity of dietary fat influence the absorption ratio. Retinol is esterified with dietary fatty acids to form retinyl esters in enterocytes and then is released into bloodstream in chylomicrons. But, retinol is also directly bound to plasma proteins such as albumin and specific proteins like Retinol Binding Protein (RBP) [26] and can be stored in the liver in a great amount for a long time [8, 13]. Vitamins E (tocopherols) are also absorbed by the small intestine with a ratio of 20 to 40% and are transported into the bloodstream, mainly by lipoproteins (VLDL, LDL) [26, 32].

The main objective of sheep-breeding today is to acquire a high reproduction rate and a rapid growth of healthy young animal. The concentrations of vitamins A and E in blood depend on factors such as feed, climate and gender, and have an influence particularly on the pregnancy rate and on the growth [1, 2, 5, 6, 30]. The amount of vitamin A in sheep milk and the amount of vitamin E in blood [21, 22] depends on food variation. Due to the type of placenta in mammals, only a very small quantity of fat-soluble vitamins pass from mother to foetus while a high amount is discharged after birth into the colostrum [8]. OZPINAR et al [24] has reported that the vitamin A and E concentrations in cows were the highest in colostrum at birth and then they rapidly decreases. Symptoms and diseases related to vitamin deficiency are encountered very often in pre-partum [30, 31] and in some particular species and breeds [13, 19, 23]. The effects of vitamins A and E in new-borns, fed with the colostrum, are not yet full explained and the metabolism is not yet fully understood [20]. It is not clear whether the immunity of lamb is due to the immunoglobulins taken up with the colostrum or to the vitamins. In animal breeds with high birth rates, new-born deaths are often observed and are related to deficiency in fat-soluble vitamins. No report on the capacity of the mother to supply this deficiency is available. Furthermore, investigations on vitamins and vitamin deficiency in sheep are based on applications of parenterally applied injections [1, 2, 8, 13, 15, 19, 26].

The aim of this study were 1) to measure the vitamin A and E concentrations in blood plasma and in lipoprotein fractions in pregnant ewes and their progeny, and 2) to show that the colostrums is an essential source of vitamins for new-borns.

Materials and Methods

ANIMAL AND PROTOCOL DESIGN

In this study 10 cross-breed Sakiz sheep highly prolific from the scientific farm of the Istanbul University Faculty of Veterinary Medicine (Istanbul University) were used. The animals became pregnant with synchronization: sponges with 60 mg medroxyprogesterone acetate were intravaginally applied to the ewes during two weeks, then 600 IU of PM56 (Pregnant mare serum gonadotropin (PMSG) synchroject 6000 IU, Vetimex Bladel/ Holland) per ewe was injected (IM). The ewes were placed with 1 Sakiz ram for mating, 48 hours after. Pregnancies were controlled by ultra sound examination (Dynamic Imaging Co. Linear Prop 5 MHz). Immediately after birth, the new-borns were separated into 2 equal groups (n = 10). In the first groups (group SL), the lambs were fed by their mothers, whereas the others were artificially fed with full fat bovine milk (group AFL). The respective vitamin A and E concentrations found in mother milk were of $1310 \pm 378$ and $145 \pm 43$ whereas vitamin A and E concentrations found in bovine milk (full fat, commercial) were of $498 \pm 79$ and $51 \pm 18$.

The ewes received high quality grass ad libitum and were supplemented every day with 300g of concentrates (88% dry matter, 15% crude protein, 12% crude cellulose and with a calorific value of 2800 kcal/kg).

Blood samples were collected on EDTA tubes from V. *Jugularis* puncture from ewes 10 days before parturition, then at birth and 1, 2, 3, and 5 weeks after parturition. New-borns were sampled by V. *Jugularis* puncture immediately after the birth and 1, 2, 3 and 5 weeks after. Their growth performance (body weight gain) was recorded. Blood samples were centrifuged (5000 g for 15 minutes, at 4° C) and plasma were at stored – 40° C until analysis.

METHODS

To determine the concentrations of vitamin A and E in the serum and VLDL/LDL extracts, the samples were mixed with an equal amount of ethanol. They were then washed twice with 3 parts of n-Hexane (BHT, Fluka HPLC grade) and finally dried under nitrogen atmosphere in a vacuum extraction unit. The extracts obtained were dissolved in a mixture of methanol and ethanol 8/2 and the vitamin A and E content determined with HPLC [instrument: Lutegrater (HP-3394 A), equipped with latek-pump (400 P model), with a 150 x 4.6 mm, RCM 100 RP18 reverse phase column filled with Hypersil ODS (51m, Supelco Inc. Supelco Park Bellofanta. PA16823-0048) using a fluorescence detector (Jascoo 820-FP) [4, 7, 10, 11, 16, 17, 18, 27]. The vitamin concentrations were calculated from a standard curve obtained by dilution of stock vitamin solutions (vitamin A: 3.84 mg/l – vitamin E: 9.47g/l). The HPLC method gives a linear relation between the signal detection and the vitamin A and E concentrations found in mother milk were of $1310 \pm 378$ and $145 \pm 43$ whereas vitamin A and E concentrations found in bovine milk (full fat, commercial) were of $498 \pm 79$ and $51 \pm 18$.

The ewes received high quality grass ad libitum and were supplemented every day with 300g of concentrates (88% dry matter, 15% crude protein, 12% crude cellulose and with a calorific value of 2800 kcal/kg).

Blood samples were collected on EDTA tubes from V. *Jugularis* puncture from ewes 10 days before parturition, then at birth and 1, 2, 3, and 5 weeks after parturition. New-borns were sampled by V. *Jugularis* puncture immediately after the birth and 1, 2, 3 and 5 weeks after. Their growth performance (body weight gain) was recorded. Blood samples were centrifuged (5000 g for 15 minutes, at 4° C) and plasma were at stored – 40° C until analysis.

METHODS

To determine the concentrations of vitamin A and E in the serum and VLDL/LDL extracts, the samples were mixed with an equal amount of ethanol. They were then washed twice with 3 parts of n-Hexane (BHT, Fluka HPLC grade) and finally dried under nitrogen atmosphere in a vacuum extraction unit. The extracts obtained were dissolved in a mixture of methanol and ethanol 8/2 and the vitamin A and E content determined with HPLC [instrument: Lutegrater (HP-3394 A), equipped with latek-pump (400 P model), with a 150 x 4.6 mm, RCM 100 RP18 reverse phase column filled with Hypersil ODS (51m, Supelco Inc. Supelco Park Bellofanta. PA16823-0048) using a fluorescence detector (Jascoo 820-FP) [4, 7, 10, 11, 16, 17, 18, 27]. The vitamin concentrations were calculated from a standard curve obtained by dilution of stock vitamin solutions (vitamin A: 3.84 mg/l – vitamin E: 9.47g/l). The HPLC method gives a linear relation between the signal detection and the vitamin A and E concentrations found in mother milk were of $1310 \pm 378$ and $145 \pm 43$ whereas vitamin A and E concentrations found in bovine milk (full fat, commercial) were of $498 \pm 79$ and $51 \pm 18$.

The ewes received high quality grass ad libitum and were supplemented every day with 300g of concentrates (88% dry matter, 15% crude protein, 12% crude cellulose and with a calorific value of 2800 kcal/kg).

Blood samples were collected on EDTA tubes from V. *Jugularis* puncture from ewes 10 days before parturition, then at birth and 1, 2, 3, and 5 weeks after parturition. New-borns were sampled by V. *Jugularis* puncture immediately after the birth and 1, 2, 3 and 5 weeks after. Their growth performance (body weight gain) was recorded. Blood samples were centrifuged (5000 g for 15 minutes, at 4° C) and plasma were at stored – 40° C until analysis.

METHODS

To determine the concentrations of vitamin A and E in the serum and VLDL/LDL extracts, the samples were mixed with an equal amount of ethanol. They were then washed twice with 3 parts of n-Hexane (BHT, Fluka HPLC grade) and finally dried under nitrogen atmosphere in a vacuum extraction unit. The extracts obtained were dissolved in a mixture of methanol and ethanol 8/2 and the vitamin A and E content determined with HPLC [instrument: Lutegrater (HP-3394 A), equipped with latek-pump (400 P model), with a 150 x 4.6 mm, RCM 100 RP18 reverse phase column filled with Hypersil ODS (51m, Supelco Inc. Supelco Park Bellofanta. PA16823-0048) using a fluorescence detector (Jascoo 820-FP) [4, 7, 10, 11, 16, 17, 18, 27]. The vitamin concentrations were calculated from a standard curve obtained by dilution of stock vitamin solutions (vitamin A: 3.84 mg/l – vitamin E: 9.47g/l). The HPLC method gives a linear relation between the signal detection and the vitamin A and E concentrations found in mother milk were of $1310 \pm 378$ and $145 \pm 43$ whereas vitamin A and E concentrations found in bovine milk (full fat, commercial) were of $498 \pm 79$ and $51 \pm 18$.

For the tests of lipoprotein fractions, 1 part of 10% dextran sulfate (Sigma) and 5 parts of 1 M CaCl$_2$ (Riedel De Haen) were added to 2 ml serum. 280µl of this mixture were taken, stirred and left to incubate at room temperature for 1hr. After centrifugation (40 min, 4°C, 2 000g) the precipitate was dissolved in 500µl of 1 M NaCl and the VLDL/LDL obtained were treated like the serum for the determination of vitamin A, vitamin E and cholesterol [20, 29].

STATISTICAL ANALYSIS

To compare of Vitamin A and E concentrations of ewes in the different periods of study, One-way ANOVA and Duncan
test were used. Two-way ANOVA method was used to determine the effects of feeding art and day on Vitamin A and E levels. The differences were considered as significant when p was less than 0.05. Pearson Correlation method was used to investigate the relationship between ewes and lambs in respect of vitamin A and E levels.

Results

In the AFL group, 2 newborns died on the 3rd day and the body weight gains were lower in this group compared to suckling lambs, particularly after 3rd day (Table 1).

As shown Table 2, the plasma concentrations of the vitamins A and E were low 10 days before parturition, markedly decreased 3 days after parturition (p<0.01), then gradually increased for reaching highest values 5 weeks after (p<0.01) in ewes.

By contrast Table 3, the effects of feeding art (FA) and day on vitamin A level were significant (p<0.001). The effect of FA x Day interaction on vitamin A level was also significant (p<0.01). While the effect of FA on vitamin E level was highly significant (p<0.001). The effect of day was significant at p<0.01 level. On the other hand the effect of FA x Day interaction on vitamin E level was not significant (p>0.05).

However, vitamin A was not detected in the VLDL/LDL fractions in ewes and new-borns. In ewes, the vitamin E proportions in lipoproteins were around 11 to 13% and remained relatively constant throughout the experiment. The amount of vitamin E associated to lipoproteins (14-22%) was slightly elevated in lambs (SL and AFL groups), but not significantly compared to mothers. However, weak decreases of this parameter were noticed when plasma tocopherol concentrations began to rise: at 3 and 7 days after birth in the SL group, and at 2 and 5 weeks after birth in the AFL group. But no significant difference between lamb groups was evidence.

### Table 1: Mortality and growth performance (body weight gains) in lambs suckling their mother (group SL) or artificially fed (group AFL) according to time after birth. Results are expressed as means ± standard deviations.

<table>
<thead>
<tr>
<th>Group</th>
<th>at birth</th>
<th>3 days after birth</th>
<th>1 week after birth</th>
<th>2 weeks after birth</th>
<th>5 weeks after birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL (n=10)</td>
<td>3 205 ± 667 (10)</td>
<td>3 506 ± 648 (10)</td>
<td>4 070 ± 554 (10)</td>
<td>6 245 ± 267 (10)</td>
<td>11 150 ± 1 289 (10)</td>
</tr>
<tr>
<td>AFL (n=10)</td>
<td>3 120 ± 518 (10)</td>
<td>3 250 ± 646 (8)</td>
<td>3 838 ± 616 (8)</td>
<td>5 513 ± 1 003 (8)</td>
<td>9 348 ± 1 111 (8)</td>
</tr>
</tbody>
</table>

### Table 2: Amounts of vitamin A and E in mother sheep and their ratios in VLDL (n:10, X ±SD).

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>µg/l</th>
<th>% in VLDL/LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>1) 219,80±23,44c 2) 172,00±26,21b 3) 136,10±25,22a 4) 238,70±37,81 5) 247,90±27,25a 6) 253,80±25,52a</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1) 1580,50±298,96 2) 1522,40±252,58 3) 1303,10±233,72 4) 1453,60±171,53 5) 1749,00±278,43 6) 1889,60±243,75b</td>
<td>12,98±1,96</td>
</tr>
</tbody>
</table>

The differences between rows with different letters for the values of vitamin A and E are significant (p<0.005).

### Table 3: Effects of feeding art (FA) and day (D) on vitamin A and E levels in lambs (values least square means).

<table>
<thead>
<tr>
<th>Trait</th>
<th>SL</th>
<th>AFL</th>
<th>AB</th>
<th>D3</th>
<th>W1</th>
<th>W2</th>
<th>W5</th>
<th>FA</th>
<th>D</th>
<th>FAxD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>73,08a</td>
<td>50,98b</td>
<td>36,10d</td>
<td>43,61cd</td>
<td>50,33c</td>
<td>78,53b</td>
<td>101,59a</td>
<td>1,258</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>576,72c</td>
<td>501,73b</td>
<td>118,00c</td>
<td>247,58c</td>
<td>439,96b</td>
<td>930,14c</td>
<td>960,45b</td>
<td>13,466</td>
<td>***</td>
<td>**</td>
</tr>
</tbody>
</table>

NS (not significant) = p>0.05
aynı satırda farklı fartlı harf farklı ortalamalar arası fark önemlidir, (***=p<0,001, **=p<0,01)
SEM: Standart Error Mean
FA: Feeding Art, D: day, AB: at birth, D3: third day, W1: first week, W2: second week, W5: fifth week

Revue Méd. Vét., 2007, 158, 8-9, 413-417
The correlations obtained between ewes and lambs on the 3rd day for the vitamin E concentrations were significantly negative (between ewes and SL group: \( r = -0.731, p < 0.05 \) and between ewes and AFL group \( r = -0.637, p < 0.05 \)). For vitamin A, the correlations obtained on the 1st week after birth were significantly positive (between ewes and SL group: \( r = 0.660, p < 0.05 \) and between ewes and AFL group \( r = 0.650, p < 0.05 \)). On the other hand, between SL and AFL groups of lambs, vitamin E was positively correlated on the 3rd day after birth (\( r = 0.884, p < 0.01 \)), and negatively the 1st week (\( r = -0.999, p < 0.001 \)), and vitamin A concentrations were highly positively correlated (on the 1st week: \( r = 0.999, p < 0.001 \) and on the 5th week: \( r = 0.935, p < 0.001 \)). In the ewes 2 vitamins were negatively correlated (\( r = -0.633, p < 0.05 \)).

**Discussion**

In our study, marked decrease of plasma vitamin A and E concentrations were evidence in ewes on the 3rd day after parturition whereas in the same time, the plasma fat soluble vitamin concentrations gradually increased in suckling lambs. By contrast, the fat-soluble vitamin status establishment of artificially fed lambs was obviously delayed compared to the SL group. Besides, the lower body weight gains and the mortality on the 3rd day observed in the AFL group confirmed the occurrence of the vitamin deficiency and the impairment of the immune system according to [7, 12, 19, 20, 22, 24, 25]. Moreover, the calculated correlations between plasma vitamin A and E concentrations in ewes and in lambs show that these 2 fat-soluble vitamins were supplied to lambs by the same source, i.e. colostrum, and that mothers transferred high amounts of fat and fat-soluble compounds to new-borns via the colostrums.

In ruminants, the type of placenta does not allow passage of high molecular weight substances towards foetus. Because vitamin A is transported in sheep as RBP-vitamin A complex with a high rate, and at a lesser extend in VLDL/IDL fractions, the circulating forms are too big to cross the placenta. The fact that we have not succeeded in detecting vitamin A in lipoprotein fractions confirms that retinol is mainly associated to RBP, and/or rapidly stored into liver. On the other hand, it is well known that a great amount of tocopherols (15 - 20%) are transported by lipoproteins (VLDL/IDL) [1, 8, 25] and consequently, vitamin E cannot be delivered to foetuses in this form [14, 25]. However, the proportions of vitamin E included into lipoproteins observed in pregnant ewes were lower (11 to 13%) than expected, suggesting that free and diffusible tocopherol fraction would be enhanced or that other transport proteins would be implicated [20].

In ewes, plasma vitamin A and E concentrations gradually increased from 1 week to 5 weeks after parturition to significantly exceed on the 5th week the initial values observed 10 days before parturition and probably to reach pre-pregnancy values. These significant differences could be related to the deviation of the maternal metabolism in favour to the foetus. In this way, the hypothesis that minor fractions of fat-soluble compounds including vitamins would cross over the placenta during pregnancy cannot be exclude. Besides, some previous studies [1, 8, 24] have reported that multiple births could affect fat-soluble vitamin concentrations in mothers. In agreement with this point, in ordinary ewes which give usually single birth, the blood vitamin A and E concentrations observed during pregnancy seemed to be higher (vitamin E: 2 000 – 4 000 µg/l and vitamin A: 350- 500 µg/l) than in super-ovulating ewes used in this study.

The effects of feeding art on plasma vitamin A and E showing that other fat soluble vitamin sources were available for lambs. Indeed, they were adapted to dry grass and concentrates during the experiment: \( \beta \)-carotens found in grass and cereals were rapidly converted into retinol by enterocytes [8, 26] and furthermore, iron plays an important role in the intestine absorption of vitamins A and E [29]. Consequently, an early food diversification improves supply and absorption of fat-soluble vitamins and would be out of interest for limiting vitamin deficiency in not suckling lambs.

**References**

15. - NEZIR Y. TOKER: The effects of feeding art on plasma vitamin A and E showing that other fat soluble vitamin sources were available for lambs. Indeed, they were adapted to dry grass and concentrates during the experiment: \( \beta \)-carotens found in grass and cereals were rapidly converted into retinol by enterocytes [8, 26] and furthermore, iron plays an important role in the intestine absorption of vitamins A and E [29]. Consequently, an early food diversification improves supply and absorption of fat-soluble vitamins and would be out of interest for limiting vitamin deficiency in not suckling lambs.


**Nezir Y. Toker**  
BLOOD SERUM VITAMIN A AND E CONCENTRATIONS AND DISTRIBUTION INTO LIPOPROTEIN FRACTIONS OF PREGNANT SHEEP AND NEWBORN LAMBS


