Bioavailability of Vitamin E Compounds in Lambs\textsuperscript{1,2}

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ABSTRACT: A study was carried out to assess the bioavailabilities of several forms of vitamin E in lambs. A total of 40 lambs was allotted to eight dietary groups of five each and supplemented or not daily for 60 d with equimolar amounts of different vitamin E compounds as follows: 1) control, no supplemental vitamin E, 2) DL-\textalpha-tocopheryl acetate, 3) D-\textalpha-tocopheryl acetate, 4) D-\textalpha-tocopheryl succinate, 5) D-\textalpha-tocopheryl polyethylene glycol 1,000 succinate (TPGS), 6) DL-\textalpha-tocopheryl nicotinate, 7) DL-\textalpha-tocopheryl nicotinate + TPGS, or 8) D-\textalpha-tocopheryl acetate + TPGS. During these 60 d, serum \textalpha-tocopherol concentrations in the control lambs remained constant and lower ($P < .05$) than in lambs that received all treatments. Various indices of bioavailability, including $C_{\text{max}} - C_i$ (concentration maximum - concentration initial), $C_t - C_i$ (concentration terminal - concentration initial), areas under the serum concentrations profiles, and pooled increment were higher ($P < .05$) with D-\textalpha-tocopheryl acetate + TPGS than in the other groups, suggesting a synergism between these forms. No such effect was observed between nicotinate and TPGS. For the TPGS, a water-soluble form of vitamin E, the indices of bioavailability were lower ($P < .05$) than for the other groups. D-\textalpha-tocopheryl acetate resulted in a bioavailability that outranked all the other forms of vitamin E, except those of D-\textalpha-tocopheryl acetate + TPGS. A slightly higher bioavailability index was observed for D-\textalpha-tocopheryl succinate than for DL-\textalpha-tocopheryl nicotinate. Dosing daily with 300 mg of various vitamin E compounds in lambs resulted in significant differences in serum \textalpha-tocopherol concentrations.

Key Words: Vitamin E, Bioavailability, Sheep, Sources

Introduction

Biological potencies of various vitamin E compounds have been established primarily using the rat fetal resorption assay with the following relationships: 1 mg of DL-\textalpha-tocopheryl acetate = 1.00 IU; 1 mg of D-\textalpha-tocopheryl acetate = 1.36 IU; 1 mg of D-\textalpha-tocopheryl succinate = 1.21 IU; and 1 mg of DL-\textalpha-tocopheryl succinate = .89 IU.

Recent vitamin E bioavailability studies across animal species raise questions on the reliability of these established biological potencies (Ingold et al., 1987). In rats, according to Ames (1979), the unit/weight relationships for the various forms of vitamin E currently evaluated by the National Formulary have not been validated using a bioassay based on biological functions. In humans, it was reported that D-\textalpha-tocopheryl acetate was 2.16 times more potent in elevating plasma \textalpha-tocopherol levels than was DL-\textalpha-tocopheryl acetate after a single oral dose (Horwitt, 1980; Horwitt et al., 1984). A recent study using a multiple oral dosing regimen of deuterium-labeled vitamin E acetate in humans showed a preference for D-\textalpha-tocopherol over DL-\textalpha-tocopherol ranging from 1.68 to 2.38 in plasma (Acuff et al., 1991). Cattle and sheep provided orally with 1,000 and 400 IU/d, respectively, of various vitamin E sources had higher ($P < .05$) serum \textalpha-tocopherol levels from D-\textalpha-tocoph-

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erol (alcohol or acetate moiety) than from the corresponding DL-α-tocopherol forms (Hidiroglou et al., 1988a,b). Similar trends were observed in the tissues of these studies. Currently accepted biopotencies of D- and DL-α-tocopheryl acetate esters may be invalid as a result of findings with various species (Papas et al., 1991a,b). Further research on other esters is required, because such information is lacking in ruminants. The predominant source of vitamin E supplementation in animal feeds has been DL-α-tocopheryl acetate. Although other commercial preparations of vitamin E are available, including the succinates and polyethylene glycol-1,000 succinate, very little information is available concerning their bioavailability in ruminants (Hidiroglou and Charmley, 1991). Recently, data were published on the use of D-α-tocopheryl polyethylene glycol-1,000 succinate (TPGS), a water-soluble form of vitamin E, as a therapeutic agent for preventing vitamin E deficiency in humans (Traber et al., 1986; Sokol et al., 1987). However, only one paper has been published on TPGS bioavailability in ruminants (Hidiroglou and Ivan, 1991). The objectives of this study were 1) to assess the bioavailability of various forms of vitamin E in lambs in a relatively long-term study and 2) to estimate the enhancement of absorption of tocopherol sources in combination with TPGS.

Materials and Methods

Animals. Forty crossbred wether lambs averaging 35 kg were provided with water ad libitum and fed a diet (Table 1) calculated to be adequate in protein, energy, vitamins, and minerals for this class of animal. Protocol for animal care had been approved by the University Animal Use Committee. Sheep were placed in individual pens (1.4 m²) for a 10-d adjustment period before beginning the 60-d experiment. The standard diet, which contained 25 IU/kg of vitamin E, was offered at 1 kg/d during the experiment. Supplemental forms of vitamin E were provided to be equivalent to 300 mg·lamb⁻¹·d⁻¹ of DL-α-tocopheryl acetate. Other forms were administered to be equimolar to this amount. The lambs were randomly allotted to eight dietary groups of five each as follows: 1) control (no vitamin E supplementation), 2) DL-α-tocopheryl acetate, 3) D-α-tocopheryl acetate, 4) D-α-tocopheryl succinate, 5) TPGS, 6) DL-α-tocopheryl nicotinate, 7) DL-α-tocopheryl nicotinate + TPGS (3:1 molar ratio α-tocopheryl basis), and 8) D-α-tocopheryl acetate + TPGS (3:1 molar ratio α-tocopheryl basis) to be mixed with the diet.

Blood samples were taken at various intervals by jugular venipuncture on d -1, 0 (baseline α-tocopherol), 1, 2, and 3 and then twice weekly for the remainder of the experiment. Blood was centrifuged immediately and serum was removed and stored at −70°C until it was analyzed for α-tocopherol.

Analytical Methods. Serum samples were prepared for vitamin E determination according to the method of Traber and Kayden (1989). Quantification of serum α-tocopherol was performed by HPLC (Hewlett Packard 1090, Hewlett Packard, Kennett Square, PA) with a hypersil octadeckysilane column (4.6 mm × 100 mm, 5 µm particle size) (Altech Assoc., Deerfield, IL) equipped with a programmable fluorescent detector (HP1046P, Hewlett Packard). Wavelength settings were 295 and 332 nm for excitation and emission, respectively. The mobile phase was a solvent system (HPLC grade) consisting of methanol and water (99:1) with a flow rate of 1.0 mL/min. Identification and quantification of α-tocopherol were by comparison of retention times and peak areas with tocopherol standards.

Analysis of Data. Methods of estimating bioavailability of tocopherol forms included comparisons of initial ($C_i$) to terminal ($C_t$) and maximum ($C_{max}$) serum concentrations, and areas under the serum concentration profile (AUC), respectively, as well as pooled concentrations adjusted for initial concentrations (Koch-Weser, 1974). Bioavailability methods used in this experiment have been previously described (Hidiroglou et al., 1988a,b, 1990).

Treatment means were calculated by the least squares method (SAS, 1985). Analysis of variance, as well as Duncan’s multiple range test, were used to compare serum α-tocopherol pooled increments among similar-time blood sampling between treatments. Statistical models used in this experiment were as follows: $Y_{ij} = \mu + g_i + e_{ij}$, where $g_i$ is the effect of the $i$th group (treatment), and $Y_{ij} = \mu + d_i + e_{ij}$, where $d_i$ is the effect of the $i$th day.

Analysis of variance was also carried out for $C_{max} - C_i$ and for $C_t - C_i$. Similar analysis of

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**Table 1. Dry matter content of basal diet administered to lambs**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage (DM basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground yellow corn</td>
<td>59.00</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>21.00</td>
</tr>
<tr>
<td>Soybean meal (44% CP)</td>
<td>12.00</td>
</tr>
<tr>
<td>Alfalfa meal (14% CP)</td>
<td>3.00</td>
</tr>
<tr>
<td>Corn oil</td>
<td>3.00</td>
</tr>
<tr>
<td>Trace minerals and salt</td>
<td>1.00</td>
</tr>
<tr>
<td>Ground limestone</td>
<td>1.00</td>
</tr>
<tr>
<td>Vitamin A and D₃</td>
<td>25</td>
</tr>
</tbody>
</table>

*a* Diet contained 25 IU of vitamin E/kg of feed.

*b* Provided, per kilogram of diet: 2.1 g of NaCl, 22 mg of I, 0.68 mg of Co, 9 mg of Fe, 725 mg of Cu, 2.625 mg of Mn, and 8.75 mg of Zn.

*c* Provided, per kilogram of diet: 2.000 IU of vitamin A and 550 IU of vitamin D₃.
variance was carried out for $C_{\text{max}} - C_i$, $C_t - C_i$, and $\text{AUC}$ among treatments.

**Results**

Data on serum $\alpha$-tocopherol concentrations are summarized in Figures 1 through 5. Serum $\alpha$-tocopherol concentration was almost constant in control sheep (Figure 1) but increased in sheep on other treatments continuously, with some fluctuation over the 2-mo period. During the first 2 wk of supplementation, time affected ($P < .05$) the rates of increase in serum $\alpha$-tocopherol. This result was primarily due to treatments, D-\(\alpha\)-tocopheryl acetate + TPGS and D-\(\alpha\)-tocopheryl acetate. Peak concentrations in serum tocopherol for all treatments were observed between 15 and 21 d, which was followed by a plateau.

In lambs supplemented with D-\(\alpha\)-tocopheryl acetate, serum $\alpha$-tocopherol concentration in all samples was higher ($P < .05$) than with DL-\(\alpha\)-tocopheryl acetate. Sheep given D-\(\alpha\)-tocopheryl acetate + TPGS had higher serum $\alpha$-tocopherol concentration ($P < .01$) for all times than did sheep given TPGS (Figure 2). Indeed, a scanning of the figures (2 and 5) show that this combination of D-\(\alpha\)-tocopheryl acetate with TPGS outranked all the other forms of vitamin E in serum $\alpha$-tocopherol concentrations. This occurred despite the fact that all the forms given contained equal amounts of tocopherol. Across all the treatments, the TPGS-dosed sheep contained the lowest $\alpha$-tocopherol serum concentrations. A detailed comparison of bioavailability is illustrated in Table 2. The difference in serum $\alpha$-tocopherol concentration between d 60 and 0 ($C_t - C_i$) was higher ($P < .05$) in the D-\(\alpha\)-tocopheryl acetate + TPGS group than in all others, whereas TPGS alone was the lowest except for controls. The $C_{\text{max}} - C_i$ was lower ($P < .05$) in the controls than in other treatments. For $C_{\text{max}} -$
C_l, D-α-tocopheryl acetate, DL-α-tocopheryl acetate, DL-α-tocopheryl nicotinate, and D-α-tocopheryl acetate + TPGS were higher than TPGS.

The greatest AUC was observed in the D-α-tocopheryl acetate + TPGS treatment, which outranked (P < .05) all the other treatments. The AUC in the D-α-tocopheryl acetate was second-highest (P < .05), and the lowest AUC was observed for the control animals. The AUC in the TPGS treatment was lower (P < .05) than in the DL-α-tocopheryl acetate treatment.

The pooled serum increment in α-tocopherol concentration during the 2-mo ingestion of various treatment vitamin E preparations is shown in Table 3. The greatest rises in serum α-tocopherol concentrations occurred after D-α-tocopheryl acetate + TPGS supplementation. This was followed by the D-α-tocopheryl acetate preparation, and the difference between these two treatments was significant (P < .01). Both preparations outranked the other vitamin E preparations in their effect on serum α-tocopherol increases.

### Discussion

Elevation of serum tocopherol levels in sheep has been used in this experiment to estimate vitamin E response to the various vitamin E supplements in sheep (Baker et al., 1986). Indeed, according to Horwitt et al. (1984), the biological activity of vitamin E compounds is related to its concentrations and the time it remains in the blood. This is the basis for measuring plasma concentrations of α-tocopherol as functions of time after administration of vitamin E compounds. On the basis of α-tocopherol concentrations in serum, the various tocopherol preparations given orally to sheep were not absorbed with equal effectiveness. Previous studies with sheep and cattle have indicated that the natural form (D) resulted in higher serum α-tocopherol concentrations than the synthetic form (DL) and that the alcohol form resulted in higher concentrations than did the acetate ester (Hidiroglou et al., 1988a,b).

Physiological differences were reflected in the overall increases in serum α-tocopherol concentrations, which show that after hydrolysis (Blomstrand and Forsgren, 1988) D-α-tocopheryl acetate was more effective in raising the circulating blood levels than was the DL form of vitamin E. Similar beneficial responses in plasma α-tocopherol levels were reported in calves supplemented with either D-α-tocopheryl acetate + TPGS or D-α-tocopheryl acetate (Roquet et al., 1991). It was reported that in humans and monkeys D and DL-α-tocopheryl

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum α-tocopherol, µg/mL</th>
<th>C terminal - initial (C_t - C_i)</th>
<th>C maximum - initial (C_max - C_i)</th>
<th>Area under serum curve, µg/(mL·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.19^d</td>
<td></td>
<td>62.92^b</td>
</tr>
<tr>
<td>DL-α-tocopheryl acetate</td>
<td>3.04^c</td>
<td>4.29^ab</td>
<td></td>
<td>175.39^c</td>
</tr>
<tr>
<td>D-α-tocopheryl acetate</td>
<td>4.02^b</td>
<td>5.11^a</td>
<td></td>
<td>231.84^b</td>
</tr>
<tr>
<td>D-α-tocopheryl succinate</td>
<td>3.19^bc</td>
<td>3.84^bc</td>
<td></td>
<td>167.94^cd</td>
</tr>
<tr>
<td>D-α-tocopheryl polyethylene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycol 1,000 succinate (TPGS)</td>
<td>2.06^d</td>
<td>2.91^c</td>
<td></td>
<td>115.11^e</td>
</tr>
<tr>
<td>DL-α-tocopheryl nicotinate</td>
<td>2.78^cd</td>
<td>4.12^b</td>
<td></td>
<td>152.90^d_e</td>
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<tr>
<td>DL-α-tocopheryl nicotinate + TPGS</td>
<td>3.04^c</td>
<td>3.54^abc</td>
<td></td>
<td>150.00^d_e</td>
</tr>
<tr>
<td>D-α-tocopheryl acetate + TPGS</td>
<td>5.34^a</td>
<td>5.55^a</td>
<td></td>
<td>282.23^a</td>
</tr>
<tr>
<td>SE</td>
<td>.30</td>
<td>4.0</td>
<td></td>
<td>14.78</td>
</tr>
</tbody>
</table>

^a,b,c,d,e Values in a column not sharing a common superscript are significantly different by analysis of variance for \(C_t - C_i\), \(C_{\text{max}} - C_i\), and area under the serum curve at \(P < .05\).
Table 3. Sheep serum α-tocopherol (μ/mL) measurement (for all days pooled) adjusted (by subtraction) for initial concentration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of samples</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>114</td>
<td>3.91f</td>
</tr>
<tr>
<td>DL-α-tocopheryl acetate</td>
<td>114</td>
<td>2.06c</td>
</tr>
<tr>
<td>D-α-tocopheryl acetate</td>
<td>114</td>
<td>2.92b</td>
</tr>
<tr>
<td>D-α-tocopheryl succinate</td>
<td>114</td>
<td>1.85d</td>
</tr>
<tr>
<td>D-α-tocopheryl polyethylene glycol 1,000 succinate (TPGS)</td>
<td>114</td>
<td>1.24a</td>
</tr>
<tr>
<td>DL-α-tocopheryl nicotinate</td>
<td>114</td>
<td>1.63c</td>
</tr>
<tr>
<td>DL-α-tocopheryl nicotinate + TPGS</td>
<td>114</td>
<td>1.69c</td>
</tr>
<tr>
<td>D-α-tocopheryl acetate + TPGS</td>
<td>76</td>
<td>3.52a</td>
</tr>
</tbody>
</table>

SE

a, b, c, d, e, f Means with the same letter do not significantly differ (P < .05).

Treatment


higher C1 - C1 was influenced by the form of vitamin E given, with the lowest (P < .05) value for the control and highest (P < .05) for D-α-tocopheryl acetate + TPGS.

Paucity of bioavailability data for various forms of tocopherol esters in sheep limits comparison with the present data. Recent studies by Hidiroglou and Charmley (1991) found a higher index of bioavailability (P < .05) in sheep given a single dose of DL-α-tocopheryl acetate than DL-α-tocopheryl nicotinate during 180 h after administration. Hidiroglou and Singh (1991) observed significantly higher plasma α-tocopherol concentrations in sheep supplemented weekly with DL-α-tocopheryl acetate than in those supplemented with D-α-tocopheryl succinate over 3 wk. Recently, Hidiroglou and Ivan (1991) reported that the bioavailability, based on α-tocopherol determined in plasma, of TPGS in sheep is lower than that of DL-α-tocopheryl acetate. The present data are in agreement with the results of these workers, indicating a lower bioavailability of the nicotinate and succinate esters of α-tocopherol compared with the acetate form. The foregoing results suggest a need of more data bearing on the relative efficacy of TPGS in combination with other forms of vitamin E in ruminants.

Implications

Bioavailability of α-tocopherol is dependent on the form administered. D-α-tocopheryl acetate is a highly available form, the bioavailability of which is increased further when combined with D-α-tocopheryl polyethylene glycol succinate.

Literature Cited


