Kinetic behavior of three preparations of α-tocopherol after oral administration to postpubertal heifers

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Objective—To assess the kinetic behavior of 3 preparations of α-tocopherol (vitamin E) after oral administration to heifers.

Animals—8 postpubertal Friesian heifers.

Procedure—A single oral bolus of 5,000 U of α-tocopherol in oil or encapsulated in liposomes or cyclodextrin was administered to each cow, using a 4 × 4 design with 8 days between treatments. Blood samples for kinetic analyses were obtained at various times for 168 hours after treatment.

Results—Mean (± SEM) maximal plasma concentrations of α-tocopherol were 4.86 ± 0.49 µg/ml, 5.03 ± 0.39 µg/ml, and 5.08 ± 0.56 µg/ml after administration of oil, liposomal, and cyclodextrin preparations, respectively. Plasma concentrations peaked 21 to 34 hours after administration. The disappearance rate constant (K_d) was less after administration of α-tocopherol encapsulated in liposomes, compared with the other 2 preparations. Area under the concentration versus time curve was greater after administration of either encapsulated form of α-tocopherol, compared with α-tocopherol in oil, but these differences were not significant.

Conclusions and Clinical Relevance—The lower K_d determined for α-tocopherol encapsulated in liposomes suggests that this formulation may result in longer persistance of the vitamin in plasma than the other 2 preparations. Dietary supplementation with α-tocopherol encapsulated in liposomes may enhance plasma availability of this vitamin in cattle and could be useful during periods of increased vitamin E requirements, such as parturition and early stages of life. (*Am J Vet Res* 2000;61:589–593)

Natural vitamin E sources in feedstuffs are unstable in the presence of heat, oxygen, and moisture because these conditions promote oxidation. Activity of vitamin E decreases during processing and storage of feedstuffs as well as during the manufacture and storage of finished animal feeds.[1] The rate of oxidation of vitamin E in finished feeds increases noticeably as a result of pelleting or after the addition of trace minerals and fat. Consequently, dietary supplementation with vitamin E is recommended, particularly for high producing or rapidly growing animals or those in stressful environmental conditions.

Serum and tissue concentrations of vitamin E are dependent on the method of dietary supplementation and the chemical formulation of the vitamin given; optimization of these variables may, therefore, allow reduction in the dose necessary to maintain a desired tissue concentration.[2-7] Because of the poor stability of vitamin E in animal feeds, chemically modified forms (principally acetate and nicotinate esters of α-tocopherol) that are resistant to oxidation have been introduced. More recently, encapsulation techniques developed by the pharmaceutical industry have been adopted by the feed industry to protect and extend the storage life of vitamin E and other feed additives.

Encapsulation may also increase the bioavailability of vitamins administered to ruminants by protecting vitamins from degradation within the rumen. Encapsulation may be done by use of chemical or mechanical means, depending on the properties of the active ingredient.[8] Numerous polymers, including alginates, cellulose, gelatin, gum arabic, synthetic polymers, starches and maltodextrins, and waxes and lipids have been tested as vehicles for encapsulated products. The choice of vehicle depends on the processing method used and the desired properties of the final product. In addition, the physical form and chemical characteristics of the final product are important considerations when adding encapsulated products to animal feed.

Liposomes and cyclodextrins have been proposed for use in human and veterinary medicine as encapsulating agents for the protection of particularly labile additives.[9,10] Liposomes are spherical vesicles formed typically of phospholipid bilayers, which can entrap both water- and lipid-soluble compounds. They are prepared by converting an initial proliposome preparation into a liposome dispersion by dilution with an aqueous phase under strictly controlled conditions.[11] Lipid-soluble substances are retained within hydrophobic bilayers, and the amount that can be retained is directly related to the solubility of that substance in nonpolar solvents. Cyclodextrins are water-soluble cyclic glucans of approximately 6 to 8 glucose residues.[10] Drugs are generally entrapped within 2 molecules of cyclodextrin

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in such a way as to establish maximum contact between hydrophobic regions of the drug and nonpolar regions of the cyclodextrin molecules.

Encapsulation of vitamin E in liposomes or cyclodextrins can be accomplished without chemical modification. However, although the encapsulating membrane enhances the stability of vitamin E during manufacturing and storage of animal feed, it may also modify absorption and kinetic properties (eg, half-life and bioavailability). The purpose of the study presented here was to assess the relative bioavailability of 1 nonencapsulated and 2 encapsulated preparations of α-tocopherol by means of a plasma kinetics study following oral administration to heifers.[12-14]

Material and Methods

Animals—Eight clinically normal postpubertal Friesian heifers with a mean body weight of 350 kg were acclimatized for 2 weeks to a complete mixed diet (**Table 1**). The diet was available ad libitum and contained adequate protein, energy, minerals, and vitamins, but no added vitamin E.

Experimental design—After the acclimatization period, heifers were assigned to 1 of the following 3 treatment groups: α-tocopherol dissolved in oil (n = 2), α-tocopherol encapsulated in liposomes (2), and α-tocopherol encapsulated in cyclodextrin (2). Two heifers did not receive α-tocopherol; these served as controls. Heifers were assigned to new groups at 8-day intervals, according to a replicated 4 × 4 design.

Each vitamin preparation contained 5000 U of α-tocopherol, and preparations were administered orally as a single bolus in pure cellulose.[a] Cellulose was used, because it is highly degraded in the rumen.[15] Boluses were freshly prepared and filled with the appropriate preparation of α-tocopherol immediately before administration. Blood samples for determination of plasma α-tocopherol concentration were obtained from the jugular vein immediately before (time 0) and 1, 10, 11, 21, 30, 48, 72, 96, and 168 hours after administration of each α-tocopherol preparation.

Encapsulation of α-tocopherol—Liposomes containing α-tocopherol were produced, using a patented manufacturing system.[b] Briefly, a mix of phospholipids in a minimal quantity of aqueous medium was formed into a series of hydrated lipid bilayers with a lamellar structure. α-Tocopherol was introduced and the mixture converted to liposomes by addition of water at room temperature (20 to 25 C). Liposomes ranged in size from 225 to 250 nm and contained 3% phospholipids.

Cyclodextrins were produced by the hydrolysis of starch, using the microbial enzyme cyclodextrin glucosyl transferase, according to the method of Citernesi et al.[16] The hydrolysis product was β-cyclodextrin and consisted of 7 glucopyranose residues. α-Tocopherol was introduced into this cyclic structure and stabilized by van deer Waals interactions between the hydrophobic region of the vitamin molecule and the apolar internal cavity of the cyclodextrin molecule.

Before the encapsulated preparations were administered to heifers, α-tocopherol concentration was determined. Preparations were saponified in an alkaline solution (ie, 40% KOH), and α-tocopherol concentration was measured by use of **high pressure liquid chromatography (HPLC)**.[17]

Determination of plasma α-tocopherol concentrations—Blood samples were centrifuged immediately after collection at 2000 × g for 10 minutes. Plasma was decanted, frozen, and stored at –20 C pending analysis. Hydroxytoluene butylate (10%) was added as a preservative to plasma before extraction. Plasma concentrations of α-tocopherol were determined by use of HPLC, according to the method of McMurray and Blanchflower.[18] The analytical precision for determining α-tocopherol values in a pooled plasma sample on different days (n = 10; mean [± SD] concentration, 2.8 mg/ml ± 0.11; coefficient of variation, 5%) was evaluated. The pooled samples were kept under the same storage conditions as the experimental samples. In such conditions, α-tocopherol concentrations do not change significantly for as long as 60 days.[19] Recovery was routinely estimated by adding α-tocopherol dissolved in ethanol to bovine plasma, and was consistently close to 97%.

Kinetic analysis—Concentration versus time curves determined from data obtained for each heifer were analyzed, using iteratively reweighted least squares regression analysis[20] and a nonlinear curve-fitting software program.[c] The Minimum Aikaike's Information Criterion Estimation test[21] was applied to select the model that best described the distribution kinetics. The program provided estimates of the **appearance and disappearance rate constants (K_a and K_d**, respectively), **area under the curve (AUC)**, and the **disappearance half-life ($t_{1/2[K_d]}$)**. Plasma α-tocopherol concentration versus time curves after oral administration of vitamin fit the following equation:

$$C(t) = A(e^{-K_d \times t} - e^{-K_a \times t})$$

where C(t) = plasma concentration at time t, and A = intercept term.

Initial estimates of the kinetic variables were obtained, using the method of residuals.[22] **Peak concentrations (C_{max})** and **time to peak concentration (T_{max})** were reported as experimental values.

Statistical analysis—Data were analyzed by use of ANOVA[d] after first testing data for normal distribution. The statistical model included heifer, period, and treatment as independent variables. Significance was determined when $P \leq 0.05$.

Table 1—Ingredients and chemical composition of diet fed to 8 healthy prepubertal Friesian heifers

Ingredient	% DM basis
Hay*	38.7
Maize silage	34.7
Soybean meal 44%	13.6
Ground corn	11.1
Mineral premix†	1.4
Vitamin A and D‡	0.5
Chemical composition	**% of DM**
Dry matter	52.3
Crude protein	15.2
Crude fat	2.9
Neutral detergent fiber	43.5
Ash	9
Calcium	0.7
Phosphorus	0.5
Magnesium	0.17
Vitamin E	10 U/kg of DM
NE_L§	0.79 Mcal/kg of DM

*Mixed grass and legumes. †Contained 10.0% calcium, 10.0% phosphorus, 10.2% sodium, 3.4% magnesium, 2,778 parts per million (ppm) of manganese, 445 ppm of copper, 8,350 ppm of zinc, 2800 ppm of iron, 14.5 ppm of cobalt, and 9 ppm of selenium. ‡Provided 500,000 U of vitamin A/kg of DM and 50,000 U of vitamin D/kg of DM. §Estimated.
DM = Dry matter. NE_L = Net energy lactation.

Results

Plasma α-tocopherol concentration prior to administration of the 3 vitamin preparations did not vary significantly among groups; mean (± SEM) concentration for all cows was 2.4 ± 1.07 μg/ml. Plasma concentrations

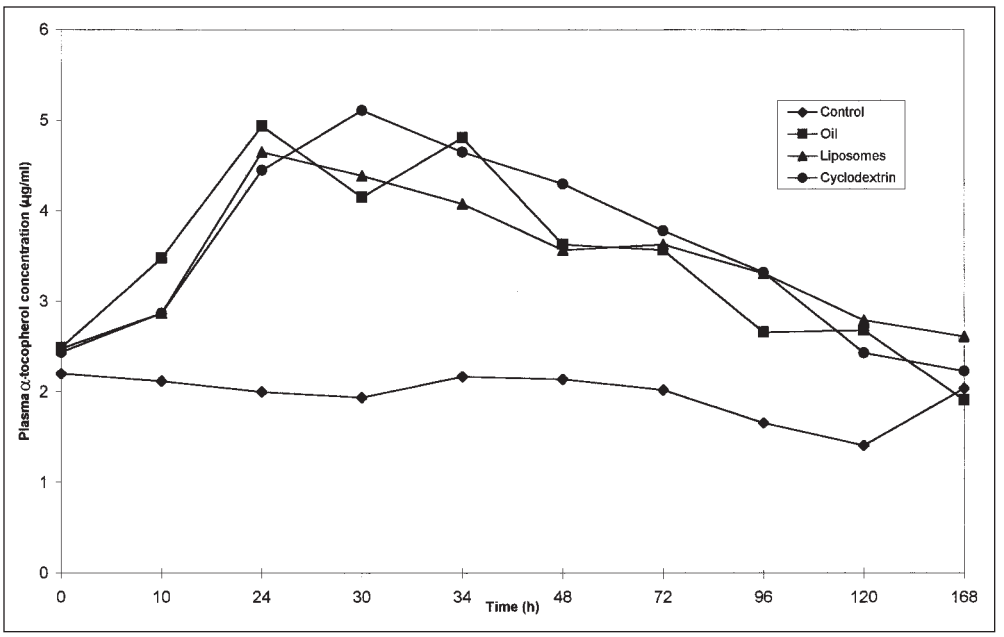

Figure 1—Plasma α-tocopherol concentration versus time curves determined from data collected after oral administration of 5,000 U of α-tocopherol in oil or encapsulated in liposomes or cyclodextrin to 8 healthy prepubertal Friesian heifers. The control group did not receive α-tocopherol.

Table 2—Pharmacokinetic variables (mean ± SEM) of α-tocopherol in plasma after oral administration of 5,000 U of α-tocopherol in oil, liposomes, or cyclodextrin to 8 healthy prepubertal Friesian heifers

	α-Tocopherol preparation			
Pharmacokinetic variables	**Oil**	**Liposomes**	**Cyclodextrin**	***P* value***
Initial concentration (µg/ml)	2.58 ± 0.35	2.48 ± 0.28	2.21 ± 0.32	NS
Maximum concentration (µg/ml)	4.86 ± 0.49	5.03 ± 0.39	5.08 ± 0.56	NS
Time to peak concentration (h)	22.7 ± 2.7	33.7 ± 6.2	31.6 ± 1.0	NS
Appearance rate constant (K_a)	0.028 ± 0.005	0.028 ± 0.004	0.022 ± 0.004	NS
Disappearance rate constant (K_d)	–0.007 ± 0.001[a]	–0.004 ± 0.001[b]	–0.006 ± 0.001[a]	< 0.05
Final concentration (µg/ml)	1.97 ± 0.47	2.61 ± 0.33	2.29 ± 0.54	NS
Disappearance half life (h)	49 ± 11.13	68 ± 8.88	60 ± 10.07	NS
Area under curve (µg/ml/h)	496 ± 54.0	547 ± 26.7	544 ± 37.6	NS

*Values among groups compared by use of ANOVA.
NS = Not significant.
[a,b]Within a row, values with different superscripts are significantly different.

of α-tocopherol increased over the first 24 hours after administration of the oil and liposome preparations or 30 hours after administration of the cyclodextrin preparation (**Fig 1**). In the cows that received the liposome and cyclodextrin preparations, α-tocopherol concentrations decreased steadily after the initial peak, whereas a second peak of plasma α-tocopherol concentration was observed 34 hours after administration of the oil preparation.

Maximum plasma concentration and T_{max} were similar following administration of all α-tocopherol preparations (**Table 2**). The K_d was lower after administration of α-tocopherol encapsulated in liposomes, compared with the other 2 preparations. Final plasma α-tocopherol concentrations (ie, concentration at 168 hours after treatment), AUC, and $t_{1/2(K_d)}$ did not differ significantly after administration of the 3 preparations.

Discussion

The vitamin E status of animals reflects the vitamin content of feed.[23] The cows used in the present study did not receive dietary vitamin E supplementation for at least 2 weeks before the study, and this would explain the low initial plasma concentrations of α-tocopherol, compared with values reported for healthy dairy cattle.[24,25] The relative bioavailability of α-tocopherol is dependent on the chemical form in which it is administered.[26-28] A recent report suggests that administration of the natural D form may increase serum and tissue α-tocopherol concentrations more than the synthetic DL form.[29] However, because the free alcohol is easily oxidized, more stable esters of α-tocopherol (eg, acetate, succinate, and nicotinate) are increasingly used for dietary supplementation. In sheep, α-tocopherol availability is greater after administration of the DL-acetate form than after the nicotinate or succinate forms.[4,30]

The quantity of α-tocopherol absorbed and, hence, its bioavailability, may also be influenced by the physical form of the supplement.[31] Shin and Owens[28] found that DL-α-tocopheryl acetate was degraded in the

rumen of steers at a faster rate after administration of an oil preparation, compared with a silica or spray preparation. Furthermore, in another study that evaluated different preparations of DL-α-tocopheryl acetate administered to dairy cows,[12] the authors found that silica-adsorbed and liposome-encapsulated α-tocopherol had different pharmacokinetic profiles and greater bioavailability than an oil-based preparation.

In the present study, we used the free alcohol form of α-tocopherol. This form of the vitamin has higher biological activity than DL-α-tocopheryl acetate, although it is less stable than the synthetic acetate form. We found that the mean C_{max} and T_{max} determined after administration of all α-tocopherol preparations were in the range indicated by results of similar studies.[12,14] After plasma concentration reached its maximum, a decrease in concentration was detected. This is in contrast to results of 2 other studies,[12,30] in which a plateau of plasma α-tocopherol concentration similar to C_{max} was detected after intraruminal administration of DL-α-tocopheryl acetate. The slower appearance of α-tocopherol in plasma after intraruminal administration of DL-α-tocopheryl acetate may be attributable to hydrolysis of the acetate before absorption can take place.[32, 27]

The plasma α-tocopherol concentration versus time curve obtained after administration of the oil-based α-tocopherol preparation was biphasic during the initial postadministration hours. This was similar to results of another study,[32] in which the authors suggested that vitamin E undergoes a mixing process in the rumen before reaching its site of absorption in the small intestine.

Following administration of the liposome and cyclodextrin preparations, we did not observe a biphasic plasma α-tocopherol concentration versus time curve. This suggests that there was a more gradual release of α-tocopherol from the rumen following administration of the encapsulated forms, and formulation may have an important effect on the kinetic behavior of the vitamin.

The second phase of the plasma α-tocopherol concentration versus time curves, which were characterized by a linear decrease in plasma α-tocopherol concentrations, represents elimination or tissue uptake.[5] We did not investigate whether the decrease was a result of tissue absorption or elimination from the body. However, from the significantly lower K_d that was determined after administration of the liposomal preparation, it is reasonable to conclude that this preparation may result in greater availability of the vitamin than the other 2 preparations.

The AUC that we calculated from the concentration versus time curves after administration of each of the preparations were in agreement with those reported by others for steers[28] and lactating cows,[12] and indicates a slightly greater availability when α-tocopherol is administered in encapsulated forms.

Our results indicated that α-tocopherol persists longer in blood when administered as an encapsulated preparation, compared with an oil-based preparation. We also found that α-tocopherol availability was greater after administration of the liposomal preparation than after administration of the cyclodextrin preparation. Thus, encapsulation in liposomes may provide better protection for the alcohol form of vitamin E (ie, α-tocopherol). This form has greater biological activity than ester derivatives, but it is also more easily oxidized. However, stability of the encapsulating medium is an important consideration, and liposomes are intolerant to high temperatures, whereas cyclodextrin is water-soluble and has greater temperature stability. The latter preparation may, therefore, be more effective in protecting α-tocopherol in field conditions.

The principal role of vitamin E is as a cellular antioxidant,[33] although, more recently, the incidence of reproductive disorders and mastitis in dairy cows during the early postpartum period has been shown to be related to low vitamin E intake.[34] Dietary supplementation of this vitamin may also improve immune function.[35] The current National Research Council's[36] requirement for vitamin E (15 mg U/kg of dry matter intake) may not be adequate to promote health and peak immune function in dairy cows. Moreover, dietary requirements may vary depending on feed composition and storage. Thus, supplementation with an encapsulated preparation of α-tocopherol may be beneficial because of the prolonged duration of the vitamin in plasma after administration of a single dose of this preparation.

[a]Schleicher & Schuell, GmbH, Dassel, Germany.
[b]Istituto per le Ricerche Applicate, Usmate Velate, Milan, Italy.
[c]Easy Fir, Istituto Mario Negri, Milan, Italy.
[d]Proc GLM, SAS Institute Inc, Cary, NC.

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