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DEGRADATION OF TOCOPHEROL AND TOCOPHERYL ACETATE BY RUMINAL CONTENTS *IN VITRO*

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Story in Brief

Ruminal fluid, from ruminally cannulated beef steers (300kg) fed a concentrate or a high roughage diet, was used to measure disappearance of α -tocopherol and hydrolysis of α -tocopheryl acetate to α -tocopherol *in vitro*. Undiluted ruminal fluid was used in Exp. 1, while buffer was added in Exp. 2. Degradation of α -tocopherol or conversion of α -tocopheryl acetate into α -tocopherol was estimated after 12 and 24 h incubation of each α -tocopherol in ruminal fluid. Following 24 h incubation in ruminal fluid, the α -tocopherol concentration in ruminal fluid was not changed in either Exp. 1 or Exp. 2 by α -tocopherol or tocopheryl acetate. These results suggest that α -tocopherol is not destroyed in the rumen and that α -tocopheryl acetate was not extensively changed into α -tocopherol by ruminal microbes.

Key Words: *In vitro*, Rumen, Vitamin E, Destruction

Introduction

Most studies of bioavailability of vitamin E have monitored responses in the blood or tissue α -tocopherol concentration. Such studies have been reported for ruminants (Njeru et al., 1995; Hidiroglou et al., 1992), swine (Chung et al., 1992) and horses (Siciliano et al., 1997). Based on those studies, d-α -tocopherol has a higher bioavailability than other forms of vitamin E. Because the acetate form is less susceptible to oxidative destruction, α -tocopheryl acetate is used widely in the feed industry as a source of vitamin E. Prior to absorption from the small intestine of nonruminants, α -tocopheryl acetate ester supposedly needs to be hydrolyzed to the free form (Burton and Traber, 1990).

Because the digestive tract of ruminants differs from that of nonruminants, especially in lipid metabolism, vitamin E metabolism has been of interest. Failure to recover dosed vitamin E at the duodenum has led researchers to conclude that vitamin E is extensively destroyed in the rumen (Alderson et al., 1971; Shin and Owens, 1990). However, *in vitro* studies, both with ruminal fluid from fasted sheep (Astrup et al., 1974) and ruminal fluid from a steer fed high-concentrate diet (Leedle et al., 1993), have detected little if any microbial destruction of dl-α -tocopherol after incubation. The purpose of this study was to monitor the disappearance of free α -tocopherol and hydrolysis of α -tocopheryl acetate to α -tocopherol during incubation with

ruminal fluid. Both a high concentrate and a high roughage diet were used in this experiment in order to match previous *in vitro* studies concerning vitamin E degradation (Astrup et al., 1974; Leedle et al., 1993). Therefore, in our study, we used two different diets and two different *in vitro* methods to investigate whether free tocopherol is destroyed in the rumen and whether tocopheryl acetate is converted to free tocopherol in the rumen.

Materials and Methods

Six beef steers (300 kg) were used as a source of ruminal fluid. Each steer had a ruminal and duodenal fistula. The high roughage group steers received a diet of 30% alfalfa hay and 45% cottonseed hulls. The high concentrate-fed steers received a diet containing 75% corn grain, 7% cottonseed hulls, and 5% alfalfa hay (Table 1). The high concentrate group steers were adapted to diet for 7 d. On d 8, ruminal contents were collected and squeezed through four layers of cheesecloth. Ruminal fluid was transferred to the laboratory where it was flushed with CO_2, and held in a water bath (39^{o}C) until it was dispensed in 50 ml aliquots into incubation tubes. No buffer or mineral was added into the ruminal fluid. Tocopherol and tocopheryl acetate stock solutions were made by dissolving them in 95% ethanol and storing them at 4^{o}C in the dark.

Tocopherol or tocopheryl acetate solution (100 or 200 µl at 2,500 ppm) was dispensed into each incubation tube to achieve final concentrations of 5 and 10 ppm of tocopherol in ruminal fluid. Each incubation bottle was set in a water bath (39^{o}C) and incubated for 24 h. Another bottle, stored in the freezer without incubation served as a control. After 24 h, all bottles were frozen and stored at
-20^{o}C until being analyzed for tocopherol.

In Exp. 2, ruminal fluid was obtained from steers fed a high roughage diet. McDougal□s buffer was added to ruminal fluid in a 50:50 ratio and incubation procedures were the same as in Exp. 1.

Alpha-tocopherol, extracted from ruminal fluid with chloroform and methanol (Folch et al., 1957), was analyzed by High Pressure Liquid Chromatography (HPLC) using reverse phase chromatography at ambient temperature. Mean values and standard deviations were calculated. Data were analyzed by PROG GLM (SAS, 1988).

Results and Discussion

Experiment 1. Undiluted Ruminal Fluid Incubations. Initial pH was in the normal range, but pH decreased during the 24-h incubation period (Table 2). During incubation with ruminal fluid from steers fed the high roughage diet, pH dropped from 7.0 to 5.9. During 24-h incubation in strained ruminal fluid without buffers, Leedle et al. (1993) suggested that it was not

necessary to add buffer. Although they implied that use of buffers or added energy may not be required for 24-h incubation, final pH, both in their study and in ours, was lower than typically found in the rumen of cattle fed roughage.

The average initial concentration of α -tocopherol in undiluted ruminal fluid was 0.95 μ g/ml (Figure 1). In ruminal fluid from steers fed the concentrate diet, α -tocopherol concentration increased significantly (P<.05) during 24-h incubation with α -tocopheryl acetate at both 5 and 10 ppm and with free α -tocopherol at 5 ppm, while there was no significant change after incubation with free α -tocopherol at 10 ppm. The α -tocopherol concentration in control fluid was also greater (P<.05) after 24-h incubation even when no tocopherol was provided (1.32 vs 1.03 μ g/ml). With the high roughage diet, concentration of tocopherol increased over time only when acetate was added at 10 ppm though mean concentrations tended to increase slightly during incubation.

Overall, no loss of free acetate was detected during *in vitro* incubation of free acetate with ruminal fluid from cattle fed either roughage or concentrate diets. Increased concentrations of free tocopherol during incubation of tocopherol acetate should serve as an index of ruminal hydrolysis. Again, extent of ruminal hydrolysis of tocopherol acetate to free tocopherol was very minimal (average of under 10% of dosed acetate). Consequently, if the acetate form must be converted to the free acid form prior to absorption as suggested by Burton and Traber (1990), such hydrolysis must be occurring post-ruminally because hydrolysis in the rumen was very limited.

Nevertheless, hydrolysis of the acetate to the free form also could occur in the small intestine.

Experiment 2. Diluted Ruminal Fluid Incubations. During incubation of diluted ruminal fluid from steers fed concentrates with 10 ppm α -tocopheryl acetate, α -tocopherol concentration had increased (P<.05) after 24 h of incubation (Figure 2). This supports the results above indicating that hydrolysis of α -tocopheryl acetate can occur in the rumen even though its extent is limited. No change in α -tocopherol concentration was detected with free α -tocopherol as a substrate supporting the above results that free tocopherol is not destroyed within the rumen. Concentration of α -tocopherol increased in control tubes. Leedle et al. (1993) used only α -tocopheryl acetate in their study to investigate ruminal tocopherol disappearance; therefore, one cannot compare their study directly with ours on hydrolysis of α -tocopheryl acetate. However, they reported that they did not detect any free α -tocopherol concentration after incubation, supporting the idea that ruminal hydrolysis may be limited. Astrup et al. (1974) also

reported no destruction of vitamin E after 24-h *in vitro* incubation with radiolabeled dl-α -tocopherol.

Tocopherol is stable to heat and alkali in the absence of oxygen. It is not affected by acids up to 100°C (Machlin, 1984). However, it is readily oxidized in the presence of oxygen. The ester form of tocopherol is used widely as an antioxidant to prevent the oxidation of free tocopherol. Whether α -tocopherol is destroyed in the digestive tract prior to absorption is not clear (Tucker et al., 1971). Shin and Owens (1990) also measured the ruminal disappearance of α -tocopherol and α -tocopheryl acetate in young steers. When vitamin E was fed with a marker, they reported that disappearance of vitamin E in the rumen, from highest to lowest, ranked α -tocopherol, α -tocopheryl acetate (liquid form), α -tocopheryl acetate (absorbate), and α -tocopheryl acetate (spray dried) at 52, 50, 40 and 36%, respectively. In contrast, Astrup et al. (1974) detected no destruction of vitamin E when incubated with ruminal contents from fasted sheep fed a high forage diet. Leedle et al. (1993), who incubated α -tocopheryl acetate with undiluted strained ruminal fluid, detected no ruminal degradation of α -tocopherol. In their study, to verify the degradation of tocopherol in the rumen, three different extraction procedures for α -tocopherol were used. These included hot ethanol in a Soxhlet apparatus (Cort et al., 1983), methanol/hexane extraction method (Schuep and Steiner, 1988), and the chloroform/methanol extraction (Folch et al., 1957) method. These three different extraction methods resulted in different recoveries. The chloroform/methanol extraction method yielded over 96% recovery. They found that vitamin E disappeared when spiked ruminal contents held at 39°C for a few minutes. However, it seems questionable that vitamin E is unstable at 39°C because vitamin E is quite stable to heat. They suggested that ruminal disappearance of vitamin E *in vitro* might be an artifact of inadequate extraction rather than destruction of vitamin E in the rumen.

Even though our results indicate that α -tocopheryl acetate was partially hydrolyzed after 24-h incubation with ruminal fluid, this increase is not large enough to explain the high bioavailability of α -tocopheryl acetate *in vivo* if hydrolysis is required prior to absorption unless intestinal hydrolysis is extensive. Nevertheless, results from this work indicate that both free tocopherol and tocopherol acetate are stable in the rumen contrary to previous suggestions.

Implications

In contrast to previous suggestions α -tocopherol and α -tocopheryl acetate were not degraded nor destroyed during incubation with ruminal fluid. Some rumen hydrolysis of α -tocopheryl acetate was detected but the degree of hydrolysis was very limited.

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Table 1. Composition of diet in Exp. 1.

Ingredients	Roughage diet, %	Concentrate diet, %
Corn, ground	15	74.3
Soybean meal	4	8
Cottonseed hulls	45	7

Alfalfa hay	30	5
Cane molasses	4.84	4.54
Limestone, 38%	.83	.83
Salt	.3	.3
Rumensin 80	.0175	.0175
Tylan 40	.0115	.0115

Table 2. Ruminal fluid pH during *in vitro* incubation in Exp. 1.

Item	0 h	24 h
High concentrate ruminal fluid		
Control	5.8	4.9
Acetate, 5 ppm	5.9	5.0
Acetate, 10 ppm	5.9	5.0
Free, 5 ppm	6.0	5.1
Free, 10 ppm	5.9	5.0
High roughage ruminal fluid		
Control	6.5	5.9
Acetate, 5 ppm	6.8	5.8
Acetate, 10 ppm	7.0	5.8
Free, 5 ppm	7.0	5.8
Free, 10 ppm	6.8	5.8

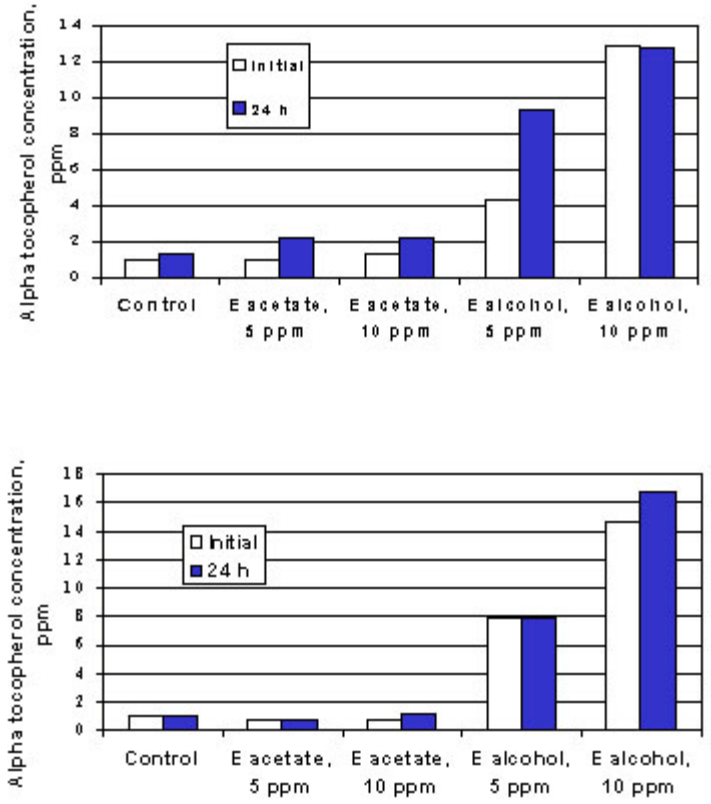

Figure 1. Alpha tocopherol concentration (μ g/ml) in ruminal fluid after 24-h incubation *in vitro*.

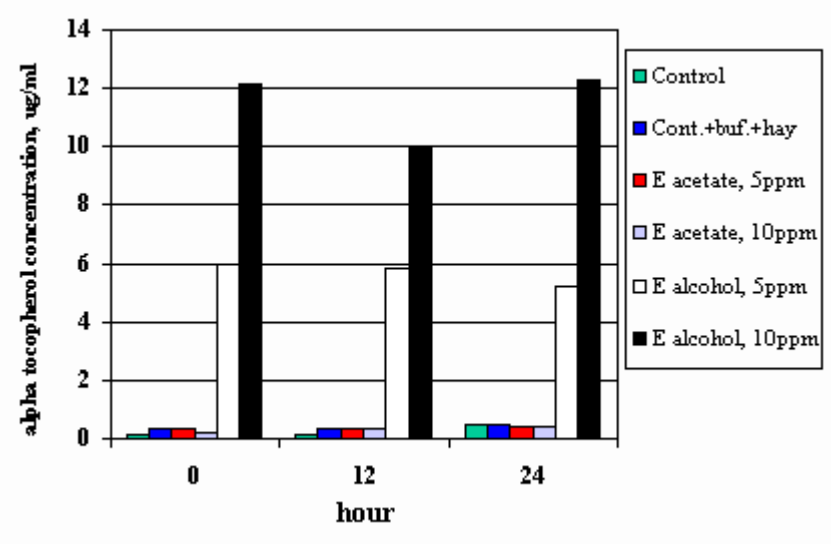

Figure 2. Alpha tocopherol concentration (μ g/ml) during incubation with diluted, buffered ruminal fluid fed a concentrate diet.

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