UTILIZATION OF DIFFERENT SOURCES AND FORMS OF VITAMIN E IN POULTRY

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ABSTRACT

Of vitamin E sources and forms tested in poults and chicks, micellized, natural tocopherol (Micellan®-E, Virbac GmbH) administered in drinking water resulted in the highest blood and tissue *alpha*–tocopherol concentrations. In one study, day-old poults were fed either 12, 46, 81, 115 IU synthetic vitamin E-acetate (all-***rac***-α–tocopheryl acetate) per kg diet or 12 IU synthetic E-acetate per kg diet plus 150 IU micellized natural tocopherol (RRR-α-tocopherol) per kg drinking water from day 3 through day 10. Plasma alpha-tocopherol concentrations at day 10 were 2.32, 3.79, 3.95, 8.22, and 33.66 μg/ml for the five treatments, respectively (P< 0.001), and red blood cells had .25, .58, .82, 1.01, 1.50, and 3.29 μg/ml, respectively (P< 0.001). Tissues measured at day 10 and percent increases in alpha-tocopherol concentrations (μg/g) with micellized natural tocopherol compared to 115 IU synthetic-acetate were: bursa of Fabricius, 320% (P< 0.001); lungs, 485% (P< 0.001); pancreases, 414% (P< 0.001); and livers, 459% (P< 0.001). Adrenal glands were the only tissues not affected by treatment (P< 0.27). Body weights of poults receiving micellized, natural tocopherol at day 10 were 6.8% higher (P< 0.008) and feed efficiency was not different. In a second study, five liquid vitamin E formulations (two commercial and three experimental) were administered via drinking water to chicks from day 3 through day 17 at four different levels (0, 50, 100, or 200 IU per kg drinking water). At 200 IU per liter drinking water, plasma alpha-tocopherol levels at 17 days of age were 134.2, 91.8, 104.9, 95.1 and 104.0 μg/ml for micellized natural tocopherol (Micellan®-E), water-dispersible natural tocopherol, micellized natural tocopheryl acetate, water-dispersible natural tocopheryl acetate and water-dispersible synthetic tocopheryl-acetate (BASF), respectively. At levels of 50 or 100 IU per kg drinking water, the micellized formulations had higher plasma alpha-tocopherol concentrations than water-dispersible natural acetate and water-dispersible synthetic acetate. Adjusted mean plasma concentration for chicks receiving no supplemental vitamin E was 5.96 μg/ml. These and other studies in chicks and poults clearly demonstrate the superiority of micellized, natural tocopherol over feed-grade synthetic vitamin E-acetate and other water-soluble formulations for young poults and chicks. The inclusion of 50-200 IU micellized, natural tocopherol per kg drinking water dramatically increased vitamin E status in young poults and chicks.

Key Words: Vitamin E, Micellized, Tocopherol, Poultry

INTRODUCTION

Vitamin E has been shown to be a critically important nutrient in poultry. The major function of vitamin E is its role as the primary fat-soluble biological antioxidant in tissues. Since vitamin E is fat-soluble, its primary site of antioxidant activity is in the lipid bi-layers of cellular membranes and mitochondria. Classical vitamin E deficiency symptoms are an indication of destruction of cellular membranes (Reference). In the 1970's, research indicated an improved immune system with vitamin E supplemented at levels up to 300 I.U. per kg diet (Tengerdy and Nockels, 1975; Tengerdy and Brown, 1977). Bendich et al, 1986, demonstrated that in order for improved immunocompentency in rodents, plasma tocopherol levels had to be much higher than levels normally fed to prevent deficiency symptoms. Recent research in turkeys has tested the effectiveness of vitamin E levels up to 800 I.U. per kg on immune reactions (Heffels-Redmann et al., 2001). Presently, dietary vitamin E levels up to 250 IU per kg are commonly recommended for improved immunocompentency in young poultry.

Various liquid and powdered sources and forms of supplemental vitamin E (alpha-tocopherol) are commercially available for use in animal diets. Both natural (RRR-*alpha*-tocopherol) and synthetic (all-*rac*-*alpha*-tocopherol) vitamin E is available in both ester and non-ester forms. The acetate-esters are utilized in complete diets due to superior stability compared to non-esterified *alpha*-tocopherol that can be readily oxidized in complete feeds after prolonged storage. Esters are available in either adsorbed or water-soluble powders. Although not as biologically active as natural vitamin E, synthetic vitamin E-acetate powders are typically used in poultry diets, mainly due to cost. Liquid, water-soluble products may contain either tocopherol or tocopheryl-acetate in either micellized or water-dispersible liquids.

As is the case with most other vitamins, synthetic-source vitamin E (all-*rac*) is not chemically identical to natural vitamin E (RRR). One mg RRR-alpha-tocopherol provides 1.49 IU activity and is one isomer, while synthetic alpha-tocopherol (all-*rac*-alpha-tocopherol) is a racemic mixture of eight stereoisomers, one of which is identical to RRR-alpha-tocopherol and the other seven have reduced potencies. One mg all-*rac*-alpha-tocopherol provides 1.1 IU activity (U.S. Pharmacopia).

In young poultry, efficiency of absorption of supplemental vitamin E is low because vitamin E absorption depends upon several factors including adequate pancreatic secretion to de-esterify the vitamin, and bilary secretion and subsequent micelle formation prior to absorption (Gallo-Torres, 1980). Reasons for the low efficiency of absorption may be two-fold. Firstly, the limited ability of young poultry to utilize dietary fat during the first weeks after hatch could negatively impact the absorption of fat-soluble vitamin E, since the two are related (Bjorneboe et al., 1990). Thus, the provision of micellized vitamin E (alpha-tocopherol) should enhance absorption. The micellization of alpha-tocopherol prior to supplementation utilizes an emulsifier that surrounds the fat-soluble vitamin thus making the vitamin more biologically available. Secondly, since dietary supplemental vitamin E is typically provided as the acetate-ester and the ester has to be removed prior to absorption, a lack of esterase enzymes to cleave the acetate-moiety from alpha-tocopherol prior to absorption could possibly reduce efficiency of absorption (Gallo-Torres, 1980). Providing non-esterified, alpha-tocopherol improves efficiency of absorption. Lack of pancreatic carboxyl ester hydrolase occurs in piglets immediately after weaning (Lauridsen et al., 2001). Since unesterified alpha-tocopherol is less stable than the acetate-ester in complete diets, a superior method to supplement vitamin E to poultry is to administer a micellized, unesterified source through the drinking water. A new product has recently been introduced in Germany and other EU countries. Micellan®-E (Virbac) is a clear micellized, liquid-source of natural vitamin E (RRR-alpha-tocopherol). The water-soluble product contains 500 IU vitamin E per ml and is recommended to be administered through drinking water or mixed into feed immediately prior to feeding.

Research in chicks (Wills and Rodick, 1993), poults (Waibel et. al., 1994), piglets (Mahan, 2002, personal communication), calves, horses, and exotic animals (Stuart et al., 1992) has shown micellized natural tocopherol to be an excellent vitamin E supplement that dramatically increases vitamin E status compared to conventional methods. Interestingly, very limited research has been published on comparative utilization of the various formulations, forms and sources of vitamin E commercially available for poultry.

This paper will focus on two studies in young poultry. One study was conducted in young poults to evaluate the efficacy of various levels of dietary synthetic vitamin E-acetate compared to drinking water-administered micellized natural vitamin E. A second study was conducted in chicks to compare the effectiveness of various liquid sources of vitamin E activity on enhancing plasma alpha-tocopherol status.

MATERIALS AND METHODS

Study One. This study is part of a dissertation by Soto-Salanova, Iowa State University, Ames. Sixteen 1-day-old male turkey poults were allocated to each of twenty pens and each of the five treatments was randomly assigned to four pens. The diet was a typical corn-soybean meal based diet formulated to provide all nutrients at adequate levels. The only variables were dietary level of synthetic tocopheryl acetate (IU/kg) and the inclusion of micellized, water-dispersible natural tocopherol (Micellan®-E, Virbac) in drinking water of one treatment. Treatments 1 through 4 were 12, 46, 81, and 115 IU synthetic vitamin E-acetate/kg (Rovimix E-50, Hoffmann-LaRoche, Inc.), respectively. Treatment 5 received 12 IU synthetic vitamin E-acetate/kg diet continuously plus 150 IU micellized, natural tocopherol/kg drinking water from days 3 through 10. The reader is referred to Soto-Salanova, 1995 for specific methods utilized during the study.

Study Two. This study was conducted in 2002 by Maurice and Lightsy, Clemson University, Clemson, SC. Day-old commercial broiler chicks were fed a corn-soy diet with no supplemental vitamin E. Each treatment consisted of five replicates with 8 chicks per replicate. From days 3 to 17, drinking water was supplemented with five different water-soluble vitamin E supplements to provide a target of 0, 50, 100, and 200 IU/kg drinking water. Two of the liquid supplements were commercially available and three were experimental. The supplements were: 1) micellized, natural tocopherol (Micellan®-E, Virbac); water-dispersible natural tocopherol (experimental); micellized natural tocopheryl acetate (experimental); water-dispersible natural tocopheryl acetate (experimental) and water-dispersible synthetic tocopheryl acetate (BASF). Blood samples were collected at the end of the study from five birds at each level for each supplement and subsequently analyzed for alpha-tocopherol. Supplemental vitamin E intake, expressed as µg alpha-tocopherol intake per bird per day constituted the dose and plasma alpha-tocopherol concentration (µg/ml) after supplementation from days 3 through 17 was the response. Due to a difference in vitamin E intake among the treatments, an efficiency of utilization value was determined for each treatment.

RESULTS AND DISCUSSION

Administering micellized natural α–tocopherol (Micellan®-E) in drinking water of young poults and chicks was the most effective method tested to dramatically increase vitamin E status in blood and tissues. Table 1 shows poult plasma and erythrocyte responses to micellized natural tocopherol in drinking water compared to various levels of dietary synthetic vitamin E acetate (Soto-Salanovo, 1995). Water supplementation of micellized natural α- tocopherol was initiated on day 3 and continued for one week through day 10.

Table 1. Plasma and erythrocyte alpha-tocopherol (µg/ml) in poults receiving dietary synthetic vitamin E or micellized natural tocopherol (Micellan-E) in drinking water[a] (Soto-Salanova, 1995)

Treatment	Day 1	Day 5	Day 10
Plasma			
12 IU synthetic vitamin E acetate/kg diet	15.22	7.07	2.32
46 IU synthetic vitamin E acetate/kg diet		4.38	3.79
81 IU synthetic vitamin E acetate/kg diet		5.28	3.95
115 IU synthetic vitamin E acetate/kg diet		8.31	8.22
12 IU synthetic vitamin E acetate/kg diet + 175 IU Micellized natural tocopherol from days 3-10/kg drinking water		13.00 (P<.001)	33.66 (P<.001)
Erythrocytes			
12 IU synthetic vitamin E acetate/kg diet	.44	.49	.25
46 IU synthetic vitamin E acetate/kg diet		.65	.58
81 IU synthetic vitamin E acetate/kg diet		.50	.82
115 IU synthetic vitamin E acetate/kg diet		.58	1.02
12 IU synthetic vitamin E acetate/kg diet + 175 IU Micellized natural tocopherol from days 3-10/kg drinking water		2.06 (P<.001)	3.29 (P<.001)

[a]Day I values represent the mean of twelve sacrificed poults. Day 5 and day 10 values represent the mean of 4 pens (2 poults per pen).

Poults and chicks have high levels of vitamin E at hatch and previous research has shown that plasma tocopherol levels begin to decline around day 3 after hatch and after 10 days of age, poults appear to be better able to utilize ester-forms of supplemental vitamin E (Soto-Salanova, 1995). The day-5 plasma concentrations in poults that received the micellized product reflected consumption of the micellized source for two days, and the 10-day result was after 7 days of water supplementation. After 7 day of supplementation, plasma tocopherol was 309% higher than in poults fed 115 IU per kg diet (P<0.001). Erythrocyte tocopherol concentrations followed a similar pattern with day-10 levels being 223% higher than in poults receiving the highest dietary level (Table 1). Tissue tocopherol levels are presented in Table 2. All tissues sampled on day 10 from poults receiving the micellized product from day 3 through 10 had dramatically higher tocopherol levels than poults receiving the highest dietary level. Bursa of Fabricius, lungs and livers had tocopherol levels up to five-fold higher than did the highest level of dietary supplementation (115 IU/kg).

Table 2. Tissue alpha-tocopherol (µg/g) in poults receiving dietary synthetic vitamin E or micellized natural tocopherol in drinking water[a] (Soto-Salanova, 1995)

Treatment	Day 1	Day 10
Bursa of Fabricius		
12 IU synthetic vitamin E acetate/kg diet	7.09	3.26
46 IU synthetic vitamin E acetate/kg diet		9.04
81 IU synthetic vitamin E acetate/kg diet		10.47
115 IU synthetic vitamin E acetate/kg diet		12.51
12 IU synthetic vitamin E acetate/kg diet + 175 IU Micellized natural tocopherol from days 3-10/kg drinking water		52.54 (P<.001)
Lungs		
12 IU synthetic vitamin E acetate/kg diet	8.85	3.06
46 IU synthetic vitamin E acetate/kg diet		7.14
81 IU synthetic vitamin E acetate/kg diet		9.23
115 IU synthetic vitamin E acetate/kg diet		11.65
12 IU synthetic vitamin E acetate/kg diet + 175 IU Micellized natural tocopherol from days 3-10/kg drinking water		68.14 (P<.001)
Pancreases		
12 IU synthetic vitamin E acetate/kg diet	12.28	3.33
46 IU synthetic vitamin E acetate/kg diet		5.92
81 IU synthetic vitamin E acetate/kg diet		7.23
115 IU synthetic vitamin E acetate/kg diet		7.31
12 IU synthetic vitamin E acetate/kg diet + 175 IU Micellized natural tocopherol from days 3-10/kg drinking water		37.62 (P<.001)
Livers		
12 IU synthetic vitamin E acetate/kg diet	176.6	3.49
46 IU synthetic vitamin E acetate/kg diet		8.66
81 IU synthetic vitamin E acetate/kg diet		13.00
115 IU synthetic vitamin E acetate/kg diet		17.24
12 IU synthetic vitamin E acetate/kg diet + 175 IU Micellized natural tocopherol from days 3-10/kg drinking water		96.30 (P<.001)
Adrenal Glands		
12 IU synthetic vitamin E acetate/kg diet	14.4	428.8
46 IU synthetic vitamin E acetate/kg diet		521.6
81 IU synthetic vitamin E acetate/kg diet		536.9
115 IU synthetic vitamin E acetate/kg diet		525.7
12 IU synthetic vitamin E acetate/kg diet + 175 IU Micellized natural tocopherol from days 3-10/kg drinking water		445.4 (P<.27)

[a]Day I values represent the mean of twelve sacrificed poults. Day 10 values represent the mean of four pens (2 poults per pen).

In the chick study, all liquid products increased plasma concentration of alpha-tocopherol compared to chicks receiving a non-vitamin E supplemented diet that naturally contained 6 IU per kg diet (Table 3). At all levels tested, the synthetic tocopheryl-acetate product supplied approximately 50% more vitamin E activity (I.U.'s) in the drinking water than all other products. Of the five liquid products tested, the micellized formulations were superior to either the water-dispersible natural products or the water-dispersible synthetic vitamin E when supplemented at 50 and 100 IU/kg water. At the inclusion level of 200 IU/kg water, Micellan-E supplementation resulted in the highest average plasma α–tocopherol level and the highest efficiency of utilization (Tables 3 and 4).

Table 3. Plasma alpha-tocopherol (µg/ml) in chicks receiving different water-soluble formulations of vitamin E at different levels[a] (Maurice and Lightsey, 2002)

Formulation	No supplement	50 IU/kg drinking water	100 IU/kg drinking water	200 IU/kg drinking water
Micellized RRR-alpha-tocopherol (Micellan-E)	5.96	44.3	74.8	134.2
Water-dispersible RRR-alpha-tocopherol (Exp.)		39.1	72.8	91.8
Micellized RRR-alpha-tocopheryl acetate (Exp.)		37.7	73.0	104.9
Water-disp. RRR-alpha-tocopheryl acetate (Exp.)		28.6	48.6	95.1
Water-disp. all-*rac*-alpha-tocopheryl acetate[b] (BASF)		42.6	92.4	104.0

[a] Each value represents the mean of 5 chicks per treatment level.
[b]Synthetic acetate provided approximately 50% more vitamin E activity than other formulations.

The dose response to synthetic vitamin E acetate was not linear since there was no significant increase in plasma levels when intake was increased from 100 to 200 IU/kg. The results of this study indicate that micellized products were superior to water-dispersible natural or synthetic products and resulted in the highest efficiency of utilization of supplemental vitamin E. It may have been appropriate to obtain plasma samples on day 10 of the 17-day study to ascertain if micellized tocopherol was superior to micellized tocopheryl-acetate.

Results of these two studies are in agreement with results previously reported in turkeys by Waibel, et.al., 1995, and chicks (Wills and Rodick, 1993) in which the water-supplementation of micellized natural tocopherol dramatically improved vitamin E status when compared to either feed sources or water-dispersible non-micellized esterified products.

Table 4. Efficiency of utilization of different water-soluble vitamin E formulations administered at different levels to chicks from day 3 through 17[a] (Maurice and Lightsey, 2002)

Formulation	50 IU/kg drinking water	100 IU/kg drinking water	200 IU/kg drinking water	Average
(Efficiency of utilization) Increase in plasma concentration above non-supplemented / average daily vitamin E intake (μg/day)				
Micellized RRR-alpha-tocopherol (Micellan-E)	13.68	11.74	10.66	12.02
Water-dispersible RRR-alpha-tocopherol (Exp.)	11.54	11.30	7.22	10.02
Micellized RRR-alpha-tocopheryl acetate (Exp.)	11.64	12.38	9.97	11.33
Water-disp. RRR-alpha-tocopheryl acetate (Exp.)	8.25	8.74	9.13	8.70
Water-disp. all-*rac*-alpha-tocopheryl acetate (BASF)	8.69	10.15	6.19	8.34

[a]Plasma alpha-tocopherol concentration minus plasma alpha-tocopherol in non-supplemented chicks divided by average daily intake (μg) (See table 3 for concentrations utilized).

Summary

Previous research has shown that immune function is enhanced when vitamin E status is elevated. Administering vitamin E as micellized, natural α–tocopherol to young poultry is an excellent method to maintain and/or enhance vitamin E status during the critical period immediately after hatch. In the study with poults, plasma α–tocopherol decreased markedly through 10 days of age in poults receiving synthetic vitamin E acetate irrespective of the concentration of synthetic vitamin E supplied in the diet (12 to 115 IU/kg), while those poults that received micellized natural α–tocopherol increased to 33.66 μg/ml. The recommendation use of **Micellan-E** is to administer 50-200 IU /kg drinking water which corresponds to 0.1 to 0.4 ml Micellan-E per kg drinking water for up to two weeks after hatch.

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Common units utilized to report serum or plasma alpha-tocopherol

mg/dl, µg/ml, µM/L
1 mg/dl = 10 µg/ml = 23.2 µM/L
1 µg/ml = 0.1 mg/dl = 2.32 µM/L
1 µM/L = 0.43 µg/ml = 0.043 mg/dl
Molecular weight of α-tocopherol = 429.6

Conversions of vitamin E sources from International Units (I.U.) to mg[a]

Form	**Natural Source**	**Synthetic Source**
α–tocopherol	1.49 I.U. /mg	1.10 I.U. /mg
α–tocopheryl acetate	1.36 I.U. /mg	1.00 I.U. /mg
α–tocopheryl succinate	1.21 I.U. /mg	0.89 I.U. /mg

[a]Determined utilizing rat fetal resorption test