

Original Research Paper

The Vitamin A and E Forms Influence Differently the Plasma Vitamin Concentrations in Newly Received Calves

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Abstract: Ten Holstein steer calves (105±3 kg) were used to evaluate bioavailability of two Vitamin A or E forms, “natural” (EA1) or “synthetic” (EA2), based on plasma vitamin concentrations during a 3-d period post injection(subcutaneous). Plasma retinyl palmitate and plasma Vitamin A concentration (retinol equivalent of all forms) was greater 24 and 48 h (P<0.01) post injection of retinyl palmitate vs retinyl propionate. Likewise, plasma retinol was greater (P = 0.04) 72 h post injection of retinyl palmitate. Plasma retinyl propionate was undetected for either treatment, indicative that it is not absorbed as a parent compound. Plasma retinyl palmitate, retinol and total Vitamin A concentrations peaked 24 h (P<0.01) post injection, decreasing toward baseline levels by 72 h post injection. Plasma tocopherol concentration was greater 24 h post injection (P<0.01) for calves injected with D- α -tocopherol vs DL- α -tocopherol. Levels were not different among treatments 48 h post injection (P>0.10). Plasma tocopherol concentrations were maximal (P<0.01) 24 h post injection of D- α -tocopherol, promptly decreasing by 72 h post injection (a level comparable to that observed with DL- α -tocopherol injection). We concluded that based on plasma concentrations, bioavailability of parenterally supplemented retinyl palmitate and D- α -tocopherol are greater than that of retinyl propionate and DL- α -tocopherol under acute Vitamin A or E supplementation.

Keywords: Holstein Calves, Retinyl Palmitate and Propionate, Tocopherol

Introduction

During shipping, cattle suffer oxidative stress from physiological stressors, food deprivation and physical exertion resulting in the activation of signaling pathways that lead to generation of Reactive Oxygen Species (ROS) in response to transit and handling (Deters and Hansen, 2020). Elevated ROS production compromises genomic stability and disrupts protein and lipid structures (Koch and Hill, 2017), overwhelming cellular antioxidant defense system (Burton and Jauniaux, 2011). Supplemental antioxidants delay or inhibit oxidation of the oxidizable substrates (Betteridge, 2000) that play an important role for the immune defense and health of stressed cattle (McDowell, 2000; Mattioli *et al.*, 2020). Antioxidants such as Vitamin E and β -carotene are considered chain-breaking antioxidant (Betteridge, 2000; McDowell, 2000; Baj *et al.*, 2019). Accordingly, these two vitamins are routinely supplemented in receiving diets to counteract pro-

oxidant status, promoting both health and growth performance (Alosilla Jr *et al.*, 2007; Abuelo *et al.*, 2015; Mattioli *et al.*, 2020). In vivo and in vitro Vitamin A and E evaluations indicate a greater efficiency of utilization when supplemented parenterally rather than orally (Rode *et al.*, 1990; McDowell, 2000; Snider *et al.*, 2014). Vitamin bioavailability is assessed by the elevation of the plasma or serum vitamin concentration, itself (Horwitt *et al.*, 1984; Baker *et al.*, 1986; Dersjant-Li and Peisker, 2010) and remains the most common biomarker for vitamin status today (Sheftel *et al.*, 2019). A drawback for this approach is that it does not distinguish the dosed vitamin from other sources (Green and Fascetti, 2016). Although differences in vitamin formulation may influence vitamin bioavailability (Snider *et al.*, 2014), there is limited research evaluating sources of Vitamin A or E parenterally supplemented on plasma retinol and

tocopherol bioavailability in newly received calves. Hence, the objective of the present study was to compare bioavailability of combinations of retinyl palmitate and D- α -tocopherol versus retinyl propionate and DL- α -tocopherol following parenteral supplementation in newly received calf-fed Holstein calves.

Materials and Methods

Animals, Diet and Treatments

All animal care, handling and sample techniques followed protocols approved by the University of California, Davis, Animal Use and Care Committee.

Ten Holstein steer calves (105 \pm 3 kg BW) were used in a 3-d trial to assess the bioavailability of two forms of injectable vitamins A and E based on plasma vitamin concentrations. Calves were obtained from a commercial calf ranch (CalfTech, Tulare, CA). The rearing program included vaccination for Bovine Rhinotracheitis virus (IBR), Bovine Viral Diarrhea virus types 1 and 2 (BVD) and parainfluenza-3 virus (PI3; 2 mL s.c., Bovi-Shield Gold 4, Zoetis Animal Health, New York, NY) on d 25 and 50, and *Moraxella bovis* (2 mL s.c., I-Site XP Pinkeye, AgriLabs, St. Joseph, MO) on d 30 and 60 of age. Steers were fed a conventional steam-flaked corn-based growing-finishing diet that did not contain supplemental Vitamin A or E (Table 1). Processing on arrival included branding, vaccination for IBR-PI3 (2 mL, SC, Cattle Master Gold w/lepto), Clostridials/Haemophilus, Pasturella, (5 mL, SC, One Shot Ultra 8, Pfizer) and treatment for internal and external parasites (3 mL Ivermectin, SC). The study was initiated upon calf arrival at the feedlot. Calves were randomly assigned to two treatments and housed in open-lot pens with 5.5 \times 9 m with 27 m² of shade equipped with automatic waterers and fence-line feed bunks. Steers were provided ad libitum access to experimental diets. Fresh feed was provided twice daily (0600 and 1400 h). Treatments and their dosage used in current trail were: Vitamin A and E “natural” form (EA1) 500,000 IU Vitamin A as retinyl palmitate, 50,000 IU vitamin D₃ and 1,500 IU Vitamin E as D- α -tocopherol (5.0 cc Vital E \oplus -A + D, Stuart products, Bedford, TX) or Vitamin A and E “synthetic” form (EA2) 500,000 IU Vitamin A as retinyl propionate; 50,000 IU vitamin D₃ and 1,500 IU Vitamin E as DL- α -tocopherol/cc (5.0 cc VitaJec EA-D RXV Products, Porterville, CA). Vitamin treatments were applied subcutaneously in the neck following label dosage.

Sampling Procedure and Vitamin Analysis

Blood samples were collected via jugular puncture into heparinized tubes before the subcutaneous application of vitamins on d 1 and then subsequently at

24, 48 and 72 h. Blood samples were immediately centrifuged at 3500 rpm for 5 min. Plasma was stored at -20°C until subsequent analysis. Plasma α -tocopherol and retinol analyses were conducted by the Veterinary Diagnostic Laboratory (College of Veterinary Medicine, Iowa State University, Ames). Briefly, 2 mL of ethanol and 4 mL of 95/5 hexane/chloroform was added to 0.5 mL of sample plasma (tocopheryl acetate and retinyl acetate added as internal standards). A 2 mL portion of the upper layer (0.25 mL equivalent) is transferred to a 7 mL vial. The sample is then concentrated to dryness using nitrogen. The sample is reconstituted in 100 μ L of methanol and a 20 μ L portion is injected on an HPLC equipped with a PDA detector (photodiode array) and a C18 column (Perkin Elmer Pecosphere, 3 μ M, 4.6 \times 33 mm (mobile phase: 95/5 where the 95 portion is 90/10 methanol/chloroform and the 5 is water). Flow rate was 1 mL/min.

Statistical Analysis

The trial was analyzed as a completely randomized design using linear mixed model for analysis of repeated measures (SAS Inst. Inc., Cary, NC; Version 9.1). Covariance structure (selected for minimum AIC, AICC and BIC) was unstructured.

Table 1: Composition of experimental diet

Item	Basal diet %
Steam-flaked corn	62.96
Alfalfa hay	3.87
Sudangrass hay	7.74
Yellow grease	2.67
Molasses cane	5.93
DDGS	14.36
Urea	0.58
Limestone	1.45
Magnesium oxide	0.09
Monensin, ppm	30.00
Trace minerals ^a	0.35
Nutrient composition (DM basis) ^b	
NE Mcal/kg	
Maintenance	2.21
Gain	1.54
Crudeprotein, %	13.91
Calcium, %	0.72
Phosphorus, %	0.35
Vitamin A, IU/kg	418.00
Vitamin E, IU/kg	25.00

^aTrace mineral salt contained: CoSO₄, 0.068%; CuSO₄, 1.04%; FeSO₄, 3.57%; ZnO, 1.24%; MnSO₄, 1.07%, KI 0.052%; and NaCl, 92.96%

^bBased on tabular values for individual feed ingredients (National Research Council, 2000) with the exception of Vitamin A which was estimated based on Vitamin A concentration of feed ingredients reported by (Pickworth *et al.*, 2012)

Results and Discussion

The influence of vitamin form on plasma retinol ester and tocopherol concentration are shown in Table 2. Retinyl propionate was not detected in plasma of retinyl palmitate or retinyl propionate treated calves, indicative that it was not absorbed as a parent compound and metabolized into retinol (Bjerke *et al.*, 2021). Consistent with previous studies (Zinn *et al.*, 1996), when retinyl palmitate was not administered, plasma retinyl palmitate concentration was nil (0.02 µg/mL). Increased plasma retinyl palmitate was appreciable (P<0.01) at 24 and 48 h post injection. Comparisons of the 2 forms of Vitamin A on plasma retinyl palmitate or retinol has been limited (Bjerke *et al.*, 2021). Plascencia *et al.* (2018) observed greater plasma retinol concentrations (measured on d 28 and 56 post injection) for calf-fed Holstein steers injected subcutaneously with 500,000 IU retinol as retinyl palmitate vs retinyl propionate. Salinas-Chavira *et al.* (2014) compared dietary supplementation of Vitamin A as palmitate vs propionate esters (vitamin treatments diluted in water before top dressing onto feed delivered to feed bunk). Compared

with non-supplemented steers, vitamin supplementation increased 56-d plasma retinol concentration independently of ester form, concluding that the two forms have similar bioavailability when orally ingested. The majority (>75%) of Vitamin A is stored in the liver as retinyl esters of long chain fatty acids (Majchrzak *et al.*, 2006; Lee *et al.*, 2010; Schreiber *et al.*, 2012; Shirakami *et al.*, 2012; Senoo *et al.*, 2017; Haaker *et al.*, 2020). Consistent with Snider *et al.* (2014), plasma retinyl palmitate concentration peaked 24 h post injection, decreasing to near baseline levels by 72 h post injection. Likewise, at 72 h post injection, plasma retinol concentration was greater (P = 0.04) for retinyl palmitate vs retinyl propionate. Plasma retinol concentration prior to Vitamin A injection averaged 0.13 µg/mL. It is considered that plasma retinol levels < 0.15 µg/mL may indicate that liver stores are in a state of depletion (Raila *et al.*, 2017). Although plasma retinol concentrations for both treatments peaked (P<0.05) 24 h post injection, differences within treatment in plasma retinol concentrations at 48 and 72 h post injections were marginally appreciable.

Table 2: Treatment effects of supplemented Vitamin E and A form on plasma retinol ester and tocopherol concentrations of newly arrived feedlot calves

Item	EA1 ^a	EA2 ^b	SEM	P-value
Replications	5	5		
Plasma retinylpalmitate (µg/mL)				
0 h	0.00***	0.00	0.66	1.00
24 h	5.58*	0.00	0.66	< 0.01
48 h	2.61**	0.00	0.66	< 0.01
72 h	1.08**	0.06	0.66	0.13
Plasma retinylpropionate (µg/mL)				
0 h	0.00	0.00		
24 h	0.00	0.00		
48 h	0.00	0.00		
72 h	0.00	0.00		
Plasma retinol (µg/mL)				
0 h	0.13**	0.13**	0.04	1.00
24 h	0.25*	0.17*	0.04	0.06
48 h	0.18**	0.10**	0.04	0.06
72 h	0.13**	0.08**	0.02	0.04
Plasma Vitamin A (retinol equivalent, all forms; µg/mL) ^c				
0 h	0.13***	0.13*	0.04	1.00
24 h	3.30*	0.17*	0.37	< 0.01
48 h	1.60**	0.10*	0.52	0.02
72 h	0.72**	0.11*	0.35	0.12
Plasma tocopherol (µg/mL)				
0 h	0.53***	0.57**	0.21	0.86
24 h	21.34*	5.72*	2.29	< 0.01
48 h	5.70**	3.73*	1.54	0.23
72 h	4.52**	4.00*	1.40	0.72

EA1: Single subcutaneous injection of 500,000 IU Vitamin A as retinyl palmitate, 50,000 IU vitamin D3 and 1,500 IU Vitamin E as D-α-tocopherol (5.0 cc Vital E®-A+D, Stuart products, Bedford, TX)

EA2: Single subcutaneous injection of 500,000 IU Vitamin A as retinyl propionate; 10,000 IU vitamin D3 and 300 IU Vitamin E as DL-α-tocopherol/cc (5.0 cc VitaJec EA-D RXV Products, Porterville, CA)

Total plasma Vitamin A as retinol equivalent (retinol = retinol + (retinyl palmitate/1.83)

*-***. Means within treatment across sampling time for various plasma measures with different superscripts differ (P<0.05)

This may indicate a state of Vitamin A sufficiency (Vogel *et al.*, 1999), where hepatic stellate cells maintain plasma retinol level under daily Vitamin A intake fluctuations (Senoo *et al.*, 2017). The elevated plasma Vitamin A concentration (retinol equivalent of all forms) for up to 48 h post retinyl palmitate injection may be ascribed to both dosage and route of administrations (Rode *et al.*, 1990; McDowell, 2000; Snider *et al.*, 2014). When orally supplemented, plasma Vitamin A subsides to baseline within 6 to 8 h post feeding (Vogel *et al.*, 1999).

As expected, initial (upon arrival at the feedlot) plasma tocopherol concentration was not different ($P>0.99$) between treatment, averaging 0.54 $\mu\text{g/mL}$ (82 IU/dL). Consistent with previous studies (Zinn *et al.*, 1996), form of Vitamin E supplementation (D- α -tocopherol vs DL- α -tocopherol) did not affect plasma tocopherol concentration.

Consistent with previous studies (Hidiroglou and McDowell, 1987; Hidiroglou *et al.*, 1988a; 1988b), 24-h plasma tocopherol concentration was 3.7-fold greater ($P<0.01$) for calves injected with D- α -tocopherol vs DL- α -tocopherol. This, notwithstanding the expectation that both treatments had the same label equivalent Vitamin E activity (1,500 IU). However, by 48 h post injection treatment differences were less appreciable ($P \geq 0.13$). Whereas injection with DL- α -tocopherol increased plasma tocopherol concentration at 24 h, the plasma concentration for 24, 48 and 72 h were not different. In contrast, at 24 h following parenteral administration of D- α -tocopherol, plasma tocopherol concentration increased 40-fold (21.34 $\mu\text{g/mL}$ or 3185 IU/dL; $P<0.01$), promptly decreasing to 4.52 $\mu\text{g/mL}$ by 72 h post injection (a level comparable to that observed with DL- α -tocopherol injection).

Conclusion

Based on plasma concentrations, bioavailability of parenterally supplemented retinyl palmitate and D- α -tocopherol are greater than that of retinyl propionate and DL- α -tocopherol. Retinyl propionate supplementation had limited or null influence on plasma retinol or total Vitamin A under acute vitamin supplementation.

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Author's Contributions

Lorenzo Buenabad: Analyzed and interpreted the data, wrote the manuscript.

Alberto Barreras: Collaborated with statistical analysis.

Alejandro Plascencia: Analyzed and interpreted the data.

Richard Avery Zinn: Designed and supervised the experiment and laboratory work, proofread the manuscript.

Ethics

No potential conflict of interest was reported by the authors.

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