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Bioavailability of vitamin A sources for cattle¹

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ABSTRACT: An experiment was conducted to evaluate the bioavailability of 5 sources of vitamin A. It was hypothesized that some vitamin A products have protective coatings that are more resistant than others to rumen destruction and that such protection would result in greater tissue concentrations of vitamin A. Fifty-three yearling Angus × Brahman cattle, consisting of 39 steers and 14 heifers, were stratified by BW and sex and randomly assigned to 6 high-concentrate diet groups receiving no vitamin A supplementation (control) or vitamin A supplemented from the following sources: Microvit A (Adisseo, Acworth, GA), Rovamix A (DSM, Parsippany, NJ), Sunvit A, Lutavit A, and Microvit A DLC (Adisseo). The vitamin A treatment groups were fed daily 80,000 IU of retinol/animal in a low-retinol concentrate diet (78.5% oats, 10% cotton-

seed hulls, 8% molasses, and 2% cottonseed meal; DM basis) and a free-choice, poor quality (low carotene) hay for 84 d. Every 28 d, BW was determined and liver biopsies and plasma were collected and analyzed for retinol concentrations. All retinol treatments showed significant increases in liver retinol concentrations compared with control animals ($P < 0.0001$), which steadily decreased over time. At all collection times, Microvit A led to numerically, but not significantly, greater concentrations of retinol in liver than did all other treatments. However, at the end of the experiment, there was no significant difference in liver retinol concentration among Microvit A, Rovamix A, Lutavit A, and Microvit A DLC diets. When liver retinol concentrations at all collection times were considered, Microvit A and Rovamix A appeared to provide the most bioavailable vitamin A.

Key words: bioavailability, cattle, retinol, rumen destruction, vitamin A

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INTRODUCTION

Vitamin A generally is supplemented to ruminant diets to insure maximum health and productivity. Unfortunately, considerable supplemental retinol is destroyed by ruminal microbes. The amount of concentrate in a diet is one factor associated with ruminal destruction. Rode et al. (1990) reported an 80% loss of vitamin A when cattle were fed 70% concentrate diets, but when fed high-forage diets, losses were only 20%. Another study by Weiss et al. (1995) demonstrated that the form of retinol supplementation had no effect, but increased concentrate in the diet resulted in greater ruminal vitamin A loss. Therefore, there is a need for minimizing ruminal destruction to increase the amount of vitamin A that reaches the duodenum. To protect vitamin A from preintestinal destruction, gelatin beadlets have been devel-

oped commercially that contain not only vitamin A but also carbohydrates and antioxidants to stabilize the vitamin A (Hoffmann-La Roche, 1994).

The objective of this study was to compare the bioavailability of 5 forms of supplemental vitamin A fed to beef cattle.

MATERIALS AND METHODS

Cattle Management and Treatments

The University of Florida Animal Care and Use Committee approved all procedures involving animals for this experiment. Fifty-three yearling Angus × Brahman cattle, consisting of 39 steers and 14 heifers, which weighed 341 ± 20 kg, were stratified by sex and BW and assigned randomly to 1 of 7 pens (100 m² each) and 1 of 8 Calan gates (American Calan, Northwood, NH) within each pen at the University of Florida Beef Research Unit, in August 2002.

The high-concentrate dietary treatments included control (no supplemental vitamin A), Microvit A (Adisseo, Acworth, GA), Rovamix A (DSM, Parsippany, NJ), and Sunvit A (Sunvit GmbH, Bardowick, Germany), Lutavit

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Table 1. Concentrate diet composition (DM basis)¹

Item	%
Oats	78.5
Cottonseed meal	2.0
Cottonseed hulls	10.0
Molasses	8.0
Limestone	1.0
Minerals ²	0.5
Vitamin E ³	—
Vitamin A ⁴	—

¹Hay intake per animal ranged from 1.8 to 4.4 kg of DM/d.

²Contained 17.0 to 20.0% Ca as calcium carbonate, 9.0% P as mono-calcium phosphate and dicalcium phosphate, 25% NaCl, 0.25% Cu as copper sulfate, 0.01% Co as cobalt sulfate, 0.01% I as calcium iodate, 0.20% Mn as manganous oxide, 0.004% Se as sodium selenite, and 0.4% Zn as zinc sulfate.

³Provided 50 IU of DL- α -tocopheryl acetate per kilogram of diet.

⁴Various sources provided 80,000 IU per animal daily.

A (BASF, Florham Park, NJ), and Microvit A DLC (Adis-seo) fed daily at 80,000 IU/animal. The analyzed values of vitamin A (IU/g) in the commercial products were Microvit A, 1,063,000; Rovamix A, 513,600; Sunvit A, 542,000; Lutavit A, 545,000; and Microvit A DLC, 1,000,000. The vitamin A premixes were formulated and mixed every 2 wk. Intakes gradually increased; therefore, vitamin A additions changed so that cattle always received 80,000 IU/d. A total of 9 cattle were used per treatment except for 8 in the control group.

Before treatment allotment, the cattle were trained to use the Calan Gates for a 21-d period. For the training period, cattle consumed an average of 3.64 kg/d of feed (Table 1). Preweaning care for these animals included vaccinations for Clostridia, Pasteurella, Leptospirosis, and Vibriosis (Cattle Master 4, Pfizer, New York, NY; and Clostridial 7-Way, Agri Laboratories, St. Joseph, MO). No other dewormer or implant was administered. An insecticide was impregnated into the ear tags (Dominator, Schering-Plough, Madison, NJ).

In each pen, poor quality Bermudagrass hay (low vitamin A content; 0.71 μ g of β -carotene/g) and water were supplied for ad libitum consumption. The control diet is described in Table 1.

Sample Collection

On d 0, 28, 56, and 84, all animals were restrained and weighed, and liver and blood samples were collected. Animals were weighed and sampled before the morning feeding. Liver biopsy samples were obtained at the 11th intercostal space on the right side as described by Chapman et al. (1963). Approximately 2 g (wet weight) of liver was collected and blotted on filter paper to remove blood. Liver samples were frozen at -80°C until analysis. Blood was collected by jugular venipuncture with 20-ga. needles into 10-mL, heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Blood was centrifuged for 25 min at $700 \times g$, and plasma was frozen at -80°C until analysis. Plasma and liver samples were analyzed for retinol concentrations using HPLC. Plasma was pre-

pared for retinol analysis as described by Brocas et al. (1997).

Liver biopsy samples were thawed and minced, and then approximately 200 mg were placed into tubes of at least 10-mL capacity with caps. All reagents were supplied by Fisher Scientific, USA. To each tube, 2.4 mL of ethanol containing 1% (wt/vol) ascorbic acid and 0.6 mL of 30% (wt/vol) KOH were added. The tubes were capped, shaken hard, and mixed by inversion. The capped tubes were incubated in a water bath at 70°C for 30 min. Tubes were removed and vortexed thoroughly. They were cooled in an ice bath and then centrifuged at $4,000 \times g$ for 15 min. The supernatant was aspirated into another tube, capped, and frozen at -80°C . To extract for analysis, a 1-mL sample of the supernatant was pipetted into a 16×125 -mm glass tube, and 1 mL of acetone, 1 mL of 0.9% (wt/vol) NaCl, and 3 mL of petroleum ether were added. The sample was vortexed for 6 min. The upper phase was removed and placed into another tube on ice. The lower phase was reextracted as above, centrifuged, and the upper phase was removed and pooled with the previous upper phase on ice. The samples were reconstituted with plasma, as described by Brocas et al. (1997).

The HPLC system consisted of an ABI Spectroflow 400 pump (ABI Analytical, Ramsey, NJ), a Model L-7200 Hitachi autosampler (Hitachi Ltd., Tokyo, Japan) with a 20 μ L loop, and a Lichrosorb 10- μ m I 60A SI, 4.6 \times 250-mm column (Alltech Chromatography, Deerfield, IL). The mobile phase consisted of 70% (vol/vol) isooctane, 29.5% (vol/vol) tetrahydrofuran, and 0.5% (vol/vol) acetic acid. The UV detector was an ABI Analytical Spectroflow 757 (ABI Analytical) set at a wavelength of 325 nm and a sensitivity of 0.005 absorption units at full scale. The data were collected with an LCI-100 Laboratory Computing Integrator (Perkin-Elmer Corp., Norwalk, CT). The flow rate was 1 mL/min. The retention time of retinol was 7.2 min. The standard was 10 ng of retinol (Sigma Chemical Co., St. Louis, MO). The procedure was validated with standard reference material 968c, fat-soluble vitamins, carotenoids, and cholesterol in human serum (National Institute of Standards & Technology, Gaithersburg, MD).

Statistical Analysis

The experiment was analyzed as a completely random design using the MIXED procedure (SAS Inst. Inc., Cary, NC) for repeated measures. The model included terms for a covariate (value from d 0), treatment, time, and the treatment \times time interaction. Animal within treatment was included as a random effect, and time was included as the repeated variable, with animal within treatment as the subject. The model used the compound symmetry covariance structure. Differences among treatments as well as among treatments on specific days were separated using *F*-protected, pairwise *t*-tests (PDIF option of SAS).

Table 2. Effect of vitamin A source on BW of cattle

Item	Vitamin A source						SEM ¹
	Control	Microvit A	Rovamix A	Sunvit A	Lutavit A	Microvit A DLC	
No. of cattle	8	9	9	9	9	9	
	Pretrial BW, kg						
D 0	355	341	351	352	317	333	21.4 ²
	Covariate-adjusted BW, kg						
D 28	364 ^a	357 ^a	359 ^a	359 ^a	359 ^a	362 ^a	1.6 ³
D 56	367 ^b	365 ^b	369 ^b	363 ^b	367 ^b	366 ^b	
D 84	378 ^c	384 ^c	383 ^c	385 ^c	386 ^c	384 ^c	

^{a-c}Treatments within the same row not bearing a common superscript differ ($P \leq 0.05$).

¹SEM were the largest among treatments.

²SEM for d 0.

³SEM for covariate-adjusted day means.

RESULTS AND DISCUSSION

The initial DMI per animal for the preexperimental period was 3.64 kg/d. Feed intake was increased every 2 wk until cattle were consuming 6.82 kg/d at the 12-wk termination. Average 14-d DMI over the 84-d experiment were 3.64, 4.09, 4.55, 5.45, 6.36, and 6.82 kg/d.

Body weights (Table 2) increased with time ($P < 0.0001$), but there were no effects of treatment ($P = 0.86$) or of treatment \times time ($P = 0.31$) detected. Because there were no differences among treatments, control cattle with minimal dietary vitamin A were able to rely on storage reserves of retinol for body growth.

Retinol concentrations in plasma (Table 3) were affected by treatment ($P = 0.01$) and time ($P = 0.04$). There were no interactions between treatment and time ($P = 0.96$). Plasma retinol concentrations decreased over time, but differences among dietary treatments were more readily detected at later stages of feeding (i.e., d 84). Using d 0 values as the covariate, Microvit A and Rovamix A increased ($P < 0.05$) plasma retinol (d 84 and overall) compared with control and also compared with the Sunvit A treatment groups ($P < 0.05$). On d 84, Lutavit A and Microvit A DLC were intermediate and did

not differ from control; Lutavit A also did not differ from Microvit A or Rovamix A; and Microvit A DLC did not differ from Microvit A. Similar trends at d 28 and 56 were evident.

Retinol concentrations in liver (Table 4) were affected by treatment ($P < 0.01$) and a treatment \times time interaction was detected ($P < 0.0001$); retinol-supplemented cattle had greater ($P < 0.05$) and more sustained concentrations of liver retinol compared with a steady decline for the control group through d 84 (Table 4). Overall Microvit A had the numerically highest concentration of liver retinol, but it did not differ statistically from Rovamix A and Lutavit A. However, averaged over all sampling times, Microvit A led to greater liver retinol ($P < 0.05$) than did Sunvit A ($P < 0.05$) and Microvit A DLC ($P < 0.05$). Control animals clearly had lower retinol concentrations in liver compared with all vitamin A dietary supplements.

According to previous studies (Kelley and Green, 1998; Hammell et al., 2000; McDowell, 2000), plasma retinol concentration is a less reliable indicator of vitamin A status than is liver retinol concentration. Unless there is a severe deficiency, the liver maintains relatively normal

Table 3. Effect of vitamin A sources on plasma retinol concentrations of cattle

Item	Vitamin A source						SEM ¹
	Control	Microvit A	Rovamix A	Sunvit A	Lutavit A	Microvit A DLC	
No. of cattle	8	9	9	9	9	9	
	Pretrial plasma retinol, $\mu\text{g/mL}$						
D 0	0.329	0.365	0.333	0.310	0.297	0.329	0.021 ²
	Covariate-adjusted plasma retinol, $\mu\text{g/mL}$						
D 28	0.331 ^{ab}	0.389 ^a	0.381 ^{ab}	0.321 ^b	0.350 ^{ab}	0.335 ^{ab}	0.010 ³
D 56	0.312 ^{ab}	0.350 ^a	0.365 ^a	0.281 ^b	0.339 ^{ab}	0.345 ^a	
D 84	0.284 ^c	0.357 ^{ab}	0.369 ^a	0.286 ^c	0.324 ^{abc}	0.305 ^{bc}	
Overall mean	0.308 ^b	0.366 ^a	0.372 ^a	0.296 ^b	0.337 ^{ab}	0.328 ^{ab}	0.017 ⁴

^{a-c}Treatments within a row not bearing a common superscript differ ($P \leq 0.05$).

¹SEM were the largest among treatments.

²SEM for d 0.

³SEM for covariate-adjusted day means.

⁴SEM for covariate-adjusted overall means.

Table 4. Effect of vitamin A sources on liver retinol concentrations of cattle

Item	Vitamin A source						SEM ¹
	Control	Microvit A	Rovamix A	Sunvit A	Lutavit A	Microvit A DLC	
No. of cattle	8	9	9	9	9	9	
	Pretrial liver retinol, µg/g of wet liver						
D 0	158	160	146	131	158	151	27 ²
	Covariate-adjusted liver retinol, µg/g of wet liver						
D 28	121 ^c	183 ^a	153 ^{ab}	141 ^{bc}	161 ^{ab}	158 ^{ab}	5 ³
D 56	90 ^c	178 ^a	163 ^{ab}	153 ^{ab}	151 ^{ab}	135 ^b	
D 84	70 ^c	187 ^a	183 ^a	143 ^b	168 ^{ab}	156 ^{ab}	
Overall mean	94 ^c	183 ^a	166 ^{ab}	145 ^b	160 ^{ab}	150 ^b	10 ⁴

^{a-c}Treatments within a row not bearing a common superscript differ ($P \leq 0.05$).

¹SEM were the largest among treatments.

²SEM for d 0.

³SEM for covariate-adjusted day means.

⁴SEM for covariate-adjusted overall means.

plasma retinol concentrations. Plasma retinol concentrations are maintained until a severe deficiency occurs (Kelley and Green, 1998; Hammell et al., 2000). Our study demonstrated large differences due to vitamin A supplementation between treatments and control in liver retinol concentrations, but only subtle differences in plasma retinol. The liver is the site for greatest storage of retinol and is the best indicator of vitamin A status (Olson, 1991; McDowell, 2000).

Increasing the amount of vitamin A reaching the duodenum increases the availability for absorption and storage as illustrated by elevated liver retinol concentrations (Table 4). Vitamin A availability is limited in ruminants due to losses by ruminal destruction. Ruminal destruction is especially high when ruminants are fed high-concentrate diets. Rode et al. (1990) reported that in vitro ruminal microbial degradation of vitamin A was 80% when the diet contained 70% concentrate, whereas diets high in forage only resulted in a 20% destruction of vitamin A in vitro. Weiss et al. (1995) demonstrated that diets consisting of 50% forage led to greater vitamin A destruction (72%) than diets that contained 80% forage, which only experienced a 20% loss. Similarly, Warner et al. (1970) showed this trend but on a smaller scale.

It was hypothesized that some vitamin A supplements with protective coatings are more resistant to rumen destruction or have improved duodenal availability and that these would result in greater liver concentrations of retinol. If these coatings were resistant to intestinal digestion, then supplemented vitamin A could pass the duodenum, the site of vitamin A absorption, and therefore be excreted. Certain products, like Microvit A and Rovamix A, appear to have better resistance to ruminal destruction or improved duodenal availability than other products tested in this experiment.

In conclusion, vitamin A destruction occurs in the rumen with retinol losses up to 80%. Technology that reduces ruminal degradation can ultimately increase the amount of vitamin A being absorbed by the animal. When a vitamin A product is supplied that is more efficient at elevating liver retinol concentrations, less needs to be fed. For the sources of vitamin A being studied, Microvit A and Rovamix A appear to be more available to cattle.

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