# PEER REVIEWED

# Effects of injectable fat-soluble vitamins and selenium on serum status in young beef calves

Carl R. Dahlen<sup>1</sup>, PhD; Rob L. Stuart<sup>2</sup>, PhD; Bryan W. Neville<sup>3</sup>, PhD

<sup>1</sup>Department of Animal Sciences, North Dakota State University, Fargo, ND 58108 <sup>2</sup>Stuart Products, Inc., Bedford, TX 76022

<sup>3</sup>Central Grasslands Research Extension Center, North Dakota State University, Streeter, ND 58483 Corresponding author: Carl R. Dahlen, Tel: 701-231-5588; Fax: 701-231-7590; Carl.Dahlen@ndsu.edu

#### Abstract

Crossbred beef calves (age 3 to 20 days) were used to determine effects of injectable vitamins on serum concentrations of selenium, vitamin A (retinol), and vitamin E (alpha-tocopherol). Spring-born calves were assigned to 1 of 4 treatments: 1) no treatment (**Control**, n = 10); 2) 4 mL of a product containing selenium and vitamin E(SE, n = 10); 3) 5 mL of a product containing vitamin E, vitamin A, and vitamin D (EAD, n = 10); or 4) 4 mL SE and 5 mL EAD (**Both**, n = 10). Blood samples were taken from calves immediately prior to (day 0) and 48 hours after treatment, and from their dams on day 0. Concentrations of selenium, vitamin A, and vitamin E in the dams were 112.8 ng/mL, 0.21 µg/mL, and 2.84 µg/mL, respectively. Calves administered SE (70.3 ng/mL) or Both (72.9 ng/mL) had greater (P < 0.05) concentrations of selenium on day 2 compared with Control (57.2 ng/mL) or EAD (56.0 ng/mL) calves. Calves administered EAD  $(0.21 \,\mu g/mL)$  or Both  $(0.22 \,\mu g/mL)$  had greater (P < 0.05)concentrations of vitamin A compared with SE (0.17 µg/ mL) or Control calves (0.15 µg/mL). Calves administered EAD (13.8 µg/mL) or Both (15.0 µg/mL) had greater (P < 0.05) concentrations of vitamin E compared with SE (6.2 µg/mL) or Control calves (3.4 µg/mL). Administration of injectable EAD increased serum concentration of vitamin A and vitamin E in young beef calves, whereas SE increased concentrations of selenium. Neither duration nor clinical significance of increased concentrations of vitamins and selenium were investigated.

Key words: beef calves, selenium, vitamin A, vitamin E

#### Résumé

Des veaux de boucherie de race croisée (entre 3 et 20 jours d'âge) ont été utilisés afin de déterminer l'effet de vitamines injectables sur la concentration sérique du sélénium, de la vitamine A (rétinol) et de la vitamine E (alpha-tocophérol). Des veaux nés au printemps ont été alloués à l'un des quatre traitements suivants : 1) pas

de traitement (Témoin, n = 10); 2) 4 mL d'une solution contenant du sélénium et de la vitamine E (SE, n = 10); 3) 5 mL d'une solution contenant de la vitamine E, de la vitamine A et de la vitamine D (EAD, n = 10); 4) 4 mL du traitement SE et 5 mL du traitement EAD (Conjoint, n = 10). Des échantillons de sang ont été prélevés des veaux juste avant (Jour 0) et 48 heures après le traitement et de leur mère au jour 0. Chez les mères, la concentration sérique du sélénium était de 112.8 ng/mL, de 0.21 µg/mL pour la vitamine A et de 2.84 µg/mL pour la vitamine E. La concentration sérique de sélénium était significativement plus élevée (P< 0.05) au jour 2 chez les veaux du traitement SE (70.3 ng/mL) ou du traitement Conjoint (72.9 ng/mL) que chez les veaux du traitement Témoin (57.2 ng/mL) ou du traitement EAD (56.0 ng/mL). La concentration sérique de vitamine A était significativement plus élevée chez les veaux du traitement EAD (0.21 µg/mL) ou du traitement Conjoint (0.22 µg/mL) que chez les veaux du traitement SE (0.17 ug/mL) ou du traitement Témoin (0.15 µg/mL). La concentration sérique de vitamine E était significativement plus élevée chez les veaux du traitement EAD (13.8 µg/mL) ou du traitement Conjoint (15.0 µg/mL) que chez les veaux du traitement SE (6.2 µg/mL) ou du traitement Témoin (3.4 µg/mL). L'administration d'un produit injectable contenant les trois vitamines a augmenté la concentration sérique de vitamine A et de vitamine E chez les jeunes veaux de boucherie alors que l'injection de sélénium a augmenté la concentration de sélénium. Ni la durée ni l'impact clinique d'une concentration plus élevée de vitamines et de sélénium n'ont été étudiés.

#### Introduction

Fat-soluble vitamin and selenium status of beef females is important to their overall health and the health of their offspring. Some disorders associated with selenium and vitamin E deficiency in cattle include white muscle disease, retained placenta, mastitis, metritis, abortion, stillbirths, weak-calf syndrome, infertility, and impaired immune function.<sup>8</sup> Taken together, disorders associated with fat-soluble vitamin deficiencies can result in an overall loss of profit.  $^{\rm 4}$ 

Blood concentrations of vitamins and selenium in cows are influenced dramatically by diet and indirectly by timing of the calving season. Vitamin content of forages can be decreased with increasing plant maturity, dry environmental conditions, and storage time. For example, cows grazing on irrigated pastures had greater serum concentrations of vitamin E compared with cows grazing dry foothill pastures.<sup>7</sup> In addition, serum vitamin E status was lower for cows fed stored hay compared with cows fed corn silage.<sup>6</sup>

The indirect impact that timing of calving season can have on cow vitamin status is related to the specific management system and diet being consumed around the time of calving. Pregnant heifers and cows that calve in early spring are maintained on stored roughages during winter months prior to calving. Concentrations of vitamins are greater in cows grazing lush forage compared with cows consuming stored forage.8 Therefore, cows calving late in the winter and consuming stored forages can benefit from receiving supplemental vitamin E.1 In addition, supplementing cows with vitamin A resulted in a reduction in calf death loss.<sup>9</sup> Many commercial supplements may provide vitamin A, but not enough vitamin E to meet the pregnant cow's needs. Furthermore, unlike vitamin A that is stored in the liver, there are no true vitamin E stores, thereby resulting in vitamin E deficiencies occurring more quickly than vitamin A deficiencies.

Since very little vitamin A or vitamin E are transferred through the placenta, calves rely on colostral transfer of vitamins to attain sufficient status.<sup>10,13</sup> In addition, calves not consuming colostrum until 24 hours after birth still had decreased plasma concentrations of vitamin E and vitamin A at 7 days of age compared with calves consuming colostrum within the first hours of life.<sup>2</sup>

In cases where dams were receiving inadequate vitamin supplies through feed resulting in low colostrum vitamin content, or when intake of colostrum by calves was delayed, supplementing winter or early springborn calves may be essential for adequate fat-soluble vitamin status and subsequent disease prevention. Several products are available to increase vitamin and selenium status in livestock species, but the impact of administering combinations of these products to young beef calves is unknown. The objective of this study was to determine the effects of injectable selenium and vitamin products on serum selenium and vitamin concentrations of young beef calves.

#### **Materials and Methods**

All animals in this study were housed at the NDSU Central Grasslands Research Extension Center

in Streeter, ND, and managed according to procedures approved by the North Dakota State University Animal Care and Use Committee.

Forty crossbred beef calves (14 heifers and 26 bulls) born in late March and early April, ranging from 3 to 20 days of age (mean 13.6 days) with a mean body weight of 99.4  $\pm$  2.9 lb (45.1 kg  $\pm$  1.3 kg), were used in the study. To ensure similar calf age and calf sex in each treatment, calves were placed into groups consisting of 4 similar age and sex calves. Within each group, random numbers were drawn to place calves into 1 of 4 treatments:1) no treatment (**Control**, n = 10); 2) 4 mL SC of a product containing selenium and vitamin E<sup>a</sup> (**SE**, n = 10); 3) 5 mL SC of a product containing vitamin E, vitamin A, and vitamin D<sup>b</sup> (**EAD**, n = 10); or 4) 4 mL of a product containing vitamin E, vitamin A, and vitamin D<sup>b</sup> (**Both**, n = 10).

Whole blood samples were collected from calves via jugular venipuncture into vacuum tubes<sup>c</sup> immediately prior to treatment (day 0) and 48 hours later (day 2). Calf body weight and dam blood samples were also collected on day 0. Blood was immediately placed on ice in a dark cooler for 3 hours after collection, centrifuged at 1,500 × g for 15 min, and serum was placed in sample vials and stored at -4° F (-20° C) until analysis. All serum samples were submitted to the Iowa State University Veterinary Diagnostic Laboratory for analysis to determine concentrations of selenium, vitamin A (retinol), and vitamin E (alpha-tocopherol).

Calves were all born to heifers that were maintained in a lot and offered a diet consisting of 47% corn silage, 37% alfalfa/grass hay, 11% barley, and 5% liquid supplement, on a dry-matter basis (100% of National Research Council requirements for energy and protein), and was delivered once daily. The liquid supplement did not include added selenium or vitamin E, but did contain 40,000 IU vitamin A per lb.

Prior to statistical analysis, data for response variables were evaluated to ensure equal variance and normal distribution. Data for concentrations of selenium, vitamin A, and vitamin E 48 hours after treatment administration were analyzed with calf as the experimental unit with the GLM procedure of SAS<sup>d</sup>. The model included treatment, sex, and the baseline concentrations (day 0) of each respective response variable as a covariate.

#### Results

Concentrations of selenium, vitamin A, and vitamin E in the dam serum were  $112.8 \pm 2.09 \text{ ng/mL}$  (range of 92 to 152 ng/mL),  $0.21 \pm 0.01 \mu \text{g/mL}$  (range of 0.1 to 0.44  $\mu \text{g/mL}$ ), and  $2.84 \pm 0.11 \mu \text{g/mL}$  (range of 1.3 to 4.4  $\mu \text{g/mL}$ ), respectively. In addition, concentrations of

selenium, vitamin A, and vitamin E were not different (P < 0.10) among calf treatment groups prior to administration of treatments  $(72.5 \pm 1.44 \text{ ng/mL}, 0.11 \pm 0.01 \text{ µg/mL}, \text{ and } 1.02 \pm 0.06 \text{ µg/mL}$  for selenium, vitamin A, and vitamin E, respectively).

On day 2 after treatment, calves administered SE or Both products had greater (P < 0.05) serum concentrations of selenium compared with calves in the Control or EAD treatments (Table 1). Calves administered EAD or Both products had greater (P < 0.05) serum concentrations of vitamin A on day 2 compared with calves in the SE or Control treatments. Calves administered EAD or Both products had greater (P < 0.05) serum concentrations of vitamin E compared with calves in the SE or Control treatments. Calves administered EAD or Both products had greater (P < 0.05) serum concentrations of vitamin E compared with calves in the SE or Control treatments.

#### Discussion

The current experiment was designed to test the impact of 2 injectable products and their combination, delivered per label instructions, on the concentrations of selenium, vitamin A, and vitamin E measured 48 hours after treatment in crossbred beef calves. Results highlight the fact that products differ in their abilities to increase concentrations of fat-soluble vitamins. This difference is likely due to different vitamin A, vitamin E, and selenium concentrations of the products. In addition, results of other research efforts<sup>3,5</sup> indicate that the bioavailability of vitamin E preparations used in the 2 products tested may be different.

Only the SE product elevated concentrations of selenium in the serum. This was expected, as the EAD product did not contain a selenium source. Conversely, only the EAD product elevated concentrations of vitamin A and vitamin E in the serum. Again, this was expected, as the SE product did not contain a source of vitamin A. Both products did contain a source of vitamin E, but the label dose offered with the EAD product was much greater (total of 1,500 IU per calf) compared with the label dose of the SE product (total of 272 IU per calf). The greater vitamin E content in the EAD product led to a 4.8-fold increase in the concentration of vitamin E compared to Control calves, whereas the SE product did not significantly elevate vitamin E. In a study evaluating forms of vitamin E administration in cows, cows receiving products containing 7500 IU of vitamin E had greater concentrations of plasma vitamin E after IM administration, compared with cows receiving 4500 IU vitamin E.<sup>3</sup>

The form of vitamin E offered in each respective product also differed. The EAD product provided vitamin E as d-alpha-tocopherol, whereas the SE product offered vitamin E as d-alpha tocopheryl acetate. The reason the SE product has the vitamin E ester is because d-alpha-tocopherol is not compatible with sodium selenite. While we were unable to find papers reviewing the bioavailability of different injectable vitamin forms in baby calves, several authors have evaluated different forms of orally administered and injectable vitamin E in older cattle. Crossbred beef cows receiving d-alphatocopherol in the feed had greater plasma concentrations of vitamin E compared with cows fed a preparation of d-alpha tocopheryl acetate.<sup>5</sup> In addition, non-lactating dairy cows that received 4,500 IU of a preparation of d-alpha-tocopherol administered IM had greater concentrations of plasma vitamin E for up to 4 weeks after

nves 40 nours alter treatment.					
Item	Control	SE*	$\mathbf{EAD}^{\dagger}$	$\mathbf{Both}^{\ddagger}$	Std. Error
Selenium, ng/mL	57.2×	70.3 <sup>y</sup>	56.0×	72.9 <sup>y</sup>	2.4
Vitamin A, µg/mL	0.15×	0.17 <sup>x</sup>	0.21 <sup>y</sup>	0.22 <sup>y</sup>	0.02
Vitamin E, µg/mL	3.4×	6.2×	$13.8^{y}$	15.0 <sup>y</sup>	1.8

**Table 1.** Serum concentrations of selenium, vitamin A (retinol), and vitamin E (alpha-tocopherol) in young beef calves 48 hours after treatment.

\*BO-SE®, Merck Animal Health, Inc., Summit, NJ, contains 1 mg/mL selenium and 68 IU/mL Vitamin E as d-alpha tocopheryl acetate

<sup>†</sup>VITAL-E<sup>®</sup> A+D, Stuart Products, Inc., Bedford, TX, contains 300 IU/mL vitamin E as d-alpha-tocopherol, 100,000 IU/mL vitamin A as retinyl-palmitate, and 10,000 IU/mL vitamin D<sub>3</sub>

<sup>‡</sup>Calves received both the SE and EAD preparations.

<sup>xy</sup>Means within row lacking common superscript differ (P < 0.05).

administration, compared with cows given 4500 IU of a d-alpha tocopheryl acetate preparation IM.<sup>3</sup> Perhaps bioavailability of vitamin E was different among the products evaluated in the current study. The SE treatment administered did not raise serum concentrations of vitamin E above the control, and administering the combination of SE and EAD also failed to elevate concentrations of vitamin E above calves given only the EAD product.

Calves used in the current study were born from first-calf heifers consuming stored feed around the time of calving. Though no overwhelming consensus exists regarding the absolute threshold values for vitamin deficiency, the ranges of vitamin A (0.04 to 0.2 µg/mL) and vitamin E (0.5 to 2.1 µg/mL) observed in the current study indicate that at least a portion of the calves were deficient prior to the time of treatment administration. A population of calves slightly older than calves in the current report had greater baseline concentrations of vitamin E (values of 1.8, 2.62, and 4.19 µg/mL for different treatments).7 In addition, calves with vitamin E concentrations similar to the baseline measures reported in the current study were reported to experience fatal diarrhea as a result of vitamin E deficiency.<sup>11</sup> Vitamin A deficiencies were present in 95% of calves that died within 3 days of birth in a Saskatchewan study, whereas 80% were deficient in vitamin E and 33% were deficient in selenium.<sup>14</sup> Though it is unknown whether the calf vitamin deficiencies were the result of inadequate colostrum vitamins,<sup>10,13</sup> delayed colostrum intake,<sup>2</sup> or a combination of the 2 factors in the current study, administration of the EAD treatment increased vitamin A and vitamin E concentrations relative to untreated calves at 48 hours post-treatment.

The current report did not attempt to evaluate a therapeutic impact of the preparations given, nor did it evaluate the length of time vitamin and selenium status would be elevated after a single administration in young calves. Supplementing vitamin E can have an immunostimulatory effect leading to increased titer levels after vaccination,<sup>12</sup> and practitioners recommended parenteral administration of vitamin E and selenium products in cases where vitamin E deficiency was identified.<sup>11</sup> However, determining the true therapeutic response to the treatments tested would be a difficult endeavor. Given the binomial nature of disease incidence and cure rates, almost 400 sick calves per treatment would be needed to determine whether there was a 5% difference in recovery rate of ill calves after administration of the treatments tested in the current study. Finding this many sick calves would be a challenge, and not taking other steps to improve the overall vitamin and selenium status of cow herds identified for this type of study would be putting more animals at a risk of illness, which would not be acceptable from an animal welfare standpoint.

Our results show that products do differ in their respective ability to increase serum concentrations of vitamin A, vitamin E and selenium, but whether this difference in vitamin status results in better response to treatment is unknown. However, the increase in serum vitamin A and E and selenium status observed in this study indicated that intervention with injectable products is useful in cases where blood vitamin and selenium concentrations need to be elevated. Further research is needed to determine the duration of increased vitamin and selenium levels in serum of young beef calves following injection.

## Conclusion

Administration of a SE product increased serum concentrations of selenium, and administration of EAD increased serum concentrations of vitamin E and vitamin A. In cases where calves are symptomatic of selenium or vitamin deficiencies, or calves are identified that have inadequate or delayed colostrum intake, injectable products are a viable option to rapidly increase blood vitamin and selenium supply. However, specific products differ in their abilities to elevate specific nutrients in the blood of young beef calves, and the duration of elevated serum concentrations are unknown.

## Endnotes

<sup>a</sup>BO-SE<sup>®</sup>, Merck, Animal Health, Inc., Summit, NJ <sup>b</sup>VITAL-E<sup>®</sup> A+D, Stuart Products, Inc., Bedford, TX <sup>c</sup>Red-top Vacutainer tubes, Becton, Dickinson and Company, Franklin Lakes, NJ <sup>d</sup>SAS Version 9.3, SAS Institute, Carv. NC

# <sup>d</sup>SAS Version 9.3, SAS Institute, Cary, NC

# Acknowledgements

The authors thank staff and students from the NDSU Central Grasslands Research Extension Center in Streeter, ND, and the North Dakota State University Department of Animal Sciences in Fargo, ND, for their assistance in sample collection and processing, and the personnel at the Iowa State University Veterinary Diagnostic Laboratory for evaluating vitamin A, vitamin E, and selenium concentrations in serum samples. This project was partially funded by Stuart Products, Inc., the makers of VITAL-E® A+D, and Dr. Stuart is employed by Stuart Products, Inc.

# References

1. Bass RT, Swecker WS, Eversole DE. Effects of oral vitamin E supplementation during late gestation in beef cattle that calved in late winter and late summer. Am J Vet Res 2001;62:921-927.

2. Blum JW, Hadorn U, Sallmann HP, Schuep W. Delaying colostrum intake by one day impairs plasma lipid, essential fatty acid, carotene, retinol, and  $\alpha$ -tocopherol status in neonatal calves. *J Nutr* 1997;127:2024-2029.

3. Charmley E, Hidiroglou N, Ochoa L, McDowell LR, Hidiroglou M. Plasma and hepatic  $\alpha$ -tocopherol in cattle following oral or intramuscular supplementation. *J Dairy Sci* 1992;75:804-810.

4. Frye TM, Williams SN, Graham TW. Vitamin deficiencies in cattle. Vet Clin North Am Food Anim Pract 1991;7:217-275.

5. Hidiroglou N, Laflamme LF, McDowell LR. Blood plasma and tissue concentrations of vitamin E in beef cattle as influenced by supplementation of various tocopherol compounds. *J Anim Sci* 1988;66:3227-3234.
6. Lynch GP. Changes of tocopherols in blood serum of cows fed hay or silage. *J Dairy Sci* 1983;66:1461-1465.

7. Maas J, Hoar BR, Myers DM, Tindall J, Puschner B. Vitamin E and selenium concentrations in month-old beef calves. *J Vet Diagn Invest* 2008;20:86-89.

8. McDowell LR. Vitamins in animal and human nutrition. 2<sup>nd</sup> ed. Ames: Iowa State University Press, 2000.

9. Meacham TN, Bovard KP, Priode BM, Fontenot JP. Effects of supplemental vitamin A on the performance of beef cows and their calves. J Anim Sci 1970;31:428-433.

 Quigley JD, Drewry JJ. Symposium: Practical considerations of transition cow and calf management. J Dairy Sci 1998;81:2779-2790.
Radostits OM, Booker CW, Shepherd G. Fatal non-responsive diarrhea in beef calves 6 to 8 weeks of age. Proceedings. 24<sup>th</sup> Annu Conf Am Assoc Boy Pract 1991;101-109.

12. Reddy PG, Morrill JL, Minocha HC, Stevenson JS. Vitamin E is immunostimulatory in calves. *J Dairy Sci* 1987;70:993-999.

13. Van Saun RJ, Herdt TH, Stowe HD. Maternal and fetal vitamin E concentrations and selenium-vitamin E interrelationships in dairy cattle. *J Nutr* 1989;119:1156-1164.

14. Waldner C. Weak calf syndrome. *Report to the Alberta beef prod.* 2007. Project No. 0007-034.