

Effects of d- α -Tocopherol and Dietary Energy on Growth and Health of Pre-Ruminant Dairy Calves

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Lucas Krueger, Graduate Research Assistant; Donald Beitz, Distinguished Professor of Animal Science; Ken Onda, Visiting Scientist from Japan; Mohammed Osman, Post-doctoral Fellow; Matt O'Neil, Graduate Research Assistant; Samantha Lei, Laboratory Manager; Robert Stuart, Stuart Products, Inc.; Howard Tyler, Professor of Animal Science; Brian Nonnecke, National Animal Disease Center

Summary and Implications

Newborn Holstein bull calves were fed milk to support low or moderate growth and were supplemented with a complement of vitamins A, D, and E. The objective of the study was to determine the effects of dietary energy and vitamin supplementation on inflammation at the whole-body level. Calves were assigned randomly to one of four treatment groups (low growth, not vitamin supplemented; low growth, vitamin supplemented; moderate growth, not vitamin supplemented; moderate growth, vitamin supplemented) for five weeks. Vitamin supplementation tended to improve average daily gain in moderate-growth calves and significantly increased concentrations of retinol, 25-(OH)-vitamin D, and α -tocopherol in plasma in supplemented groups. Moderate growth calves exhibited lower concentrations of α -tocopherol in plasma and higher concentrations of serum haptoglobin, which is a protein associated with chronic inflammation. All calves exhibited elevated concentrations of the more acute indicator of inflammation, serum amyloid A, during weeks 1-3. These results indicate potential roles for vitamins A, D, and E in moderation of pro-inflammatory responses early in life.

Introduction

Neonatal dairy calves throughout the United States are commonly fed pasteurized whole milk as the primary dietary component during their first several weeks of life. Whole milk fails to meet the recommendations for dietary inclusion of vitamins D and E for neonatal calves put forth by the National Research Council (2001). Vitamins A, D, and E are all thought to affect innate and cell-mediated immune responses by aiding cell proliferation and differentiation as well as oxidative burst function. All of these functions may vary according to energy status of the animal. As the neonatal calf does not produce measurable endogenous antibody until several weeks of age, these innate and cell mediated immune responses are critical for health. The objective of this study was to characterize the

effects of both energy and vitamin supplementation on the growth and health of Holstein bull calves.

Materials and Methods

Experimental Design

Calves entered the study over a period of 14 weeks and were assigned randomly to one of four dietary treatments in a 2-by-2 factorial randomized complete block, split-plot design. Calves were fed 2.85 L and 1.99 L, respectively, of pasteurized whole milk twice daily for five weeks. Diets were formulated for moderate growth (MG) of 0.5 kg/d or low growth (LG) of 0.25 kg/d according to NRC (2001) recommendations. Vitamins A and E were quantified in milk, and vitamin D was assumed to be constant in milk at 307 IU/kg dry matter. Vitamins A, D, and E in dietary milk are summarized in Table 1. Calves were not offered additional hay or grain, and water was offered ad libitum. Calves in these two dietary energy groups were either supplemented (MG-S and LG-S) with vitamins E, A, and D or not supplemented (MG-C and LG-C) as a control. Vitamin-supplemented calves were injected intramuscularly on d 0 with 1500 I.U. vitamin E, 500,000 I.U. vitamin A, and 50,000 I.U. vitamin D₃ (Vital E-A+D $\text{\textcircled{R}}$, Stuart Products, Inc., Bedford, TX 76022) and daily administered 500 I.U. of d- α -tocopherol (Emcelle Tocopherol $\text{\textcircled{R}}$, 500 I.U./mL, - Stuart Products, Inc., Bedford, TX 76022) via milk. Control calves were not supplemented during the study period.

Table 1. Vitamin E, A, and D₃ content in dietary milk.

Wk.	Vitamin E		Vitamin A		Vitamin D ₃	
	Dietary Milk ¹	NRC Rec ²	Dietary Milk ¹	NRC Rec ²	Dietary Milk ^{1,3}	NRC Rec ²
0	1.8	26.5	<	5,500	38.4	582
4	1.5		<	3,300		
8	1.1		<	3,300		
11	1.1		<	3,300		
14	1.7		<	3,300		

¹Values are reported as IU/L.

²Values are reported as IU/d.

³IU/L according to NRC characterization of whole milk

Sample Collection and Analysis

Calves were weighed and blood samples were collected via jugular venipuncture at birth and again each week for 5 weeks. Plasma was sent to Iowa State Veterinary Diagnostic Laboratory for quantification of retinol and α -tocopherol. Vitamin D in plasma was quantified via equilibrium radioimmunoassay procedure after extraction with acetonitrile. Haptoglobin was quantified in serum by using bovine-specific ELISA (Immunology Consultants Laboratory, Inc., Newberg, OR). Serum amyloid A was quantified by using multispecies serum amyloid A ELISA kit (Tridelta Development Ltd. Maynooth, Co. Kildare, Ireland).

Statistical Analysis

Data were analyzed by using mixed procedures of SAS (Statistical Analysis Software, Version 9.3, SAS Institute, Inc., Cary, NC). Calf served as the experimental unit for all analyses. Units were blocked within each treatment group by order of birth. The final model included main effects of energy, vitamin, time, and all possible interactions of main effects. The energy-by-vitamin-by-block interaction was included as a random effect. Least squares means were compared via the *pdiff* statement in SAS, and *P*-values were adjusted by the Tukey-Kramer method. Post-hoc analyses were triggered when main effects exhibited a strong trend ($P < 0.1$). Means were considered different when $P < 0.05$. Results are reported as least squares means \pm SEM. ADG of MG-C and MG-S calves were analyzed via an ad-hoc two-sample, one-tailed Student's *t*-test, by using SAS. Results are reported as means \pm SEM.

Results and Discussion

Body weight gain of calves is shown by treatment group during the progression of the five-week feeding period (Figure 1). Moderate growth calves gained significantly more weight than did LG calves. Vitamin supplementation tended to increase average daily gain between the two MG groups.

Vitamin supplementation significantly increased concentrations of vitamins A (Figure 2), D (Figure 3), and E (Figure 4) in plasma of both supplemented groups by week 1. Retinol and α -tocopherol were lower in MG calves than in LG calves at week 2, indicating greater utilization of these compounds by tissues. Calves not supplemented with

vitamins were deficient in vitamins D and E. Vitamin D concentrations in plasma of greater than 20 ng/mL are required for proper mineral regulation, and concentrations greater than 30 ng/mL may aid T lymphocyte proliferation during immune responses. Concentration of α -tocopherol in plasma above 3000 ng/mL is required in the adult cow to improve resistance to intramammary infections; calves in this study did not achieve this status when fed unsupplemented whole milk. Calves that were supplemented with vitamins in this study accumulated α -tocopherol, indicating that this compound was supplemented in excess.

Acute phase proteins measured in jugular blood to assess inflammation at the whole-body level are shown in Figure 5. Haptoglobin was highest for the MG-control group at week 2. The elevation of haptoglobin coincided with increased incidence of scours, but the cause of scours was not determined. Serum amyloid A, an indicator of acute inflammation, was elevated in all groups at week 1. Inflammation indicated by elevated serum amyloid A is likely the outcome the neonate's initial series of encounters with environmental microbes in all exposed tissues including the intestine. Calves in all groups exhibited significantly lower serum amyloid by week 4. Neither vitamin supplementation nor dietary intake significantly lowered serum amyloid A during early life. Collectively, these results indicate that vitamins D and E are deficient in whole milk and that calves not supplemented with these vitamins may be more susceptible to disruption of homeostasis in exterior tissues including the intestine. This condition may be exacerbated as dietary energy increases. Therefore, it is advisable to supplement whole-milk diets with vitamins D and E in concentrations at least equal with those recommended by NRC.

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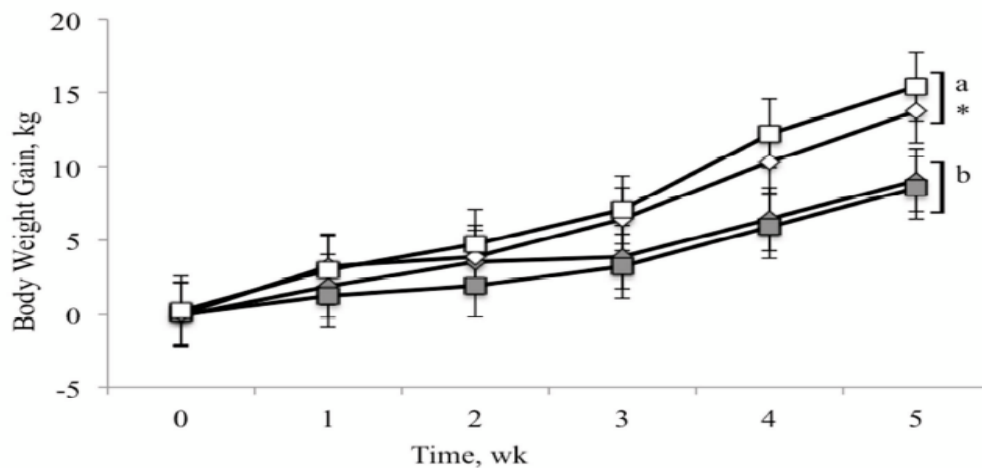


Figure 1. Body weight gain of calves. ◆ LG-C, ■ LG-S, ◇ MG-C, □ MGS. *ADG of MG-S and MG-C calves are different ($P = 0.066$). a,b Brackets with different superscripts denote pooled means that are different ($P < 0.0001$).

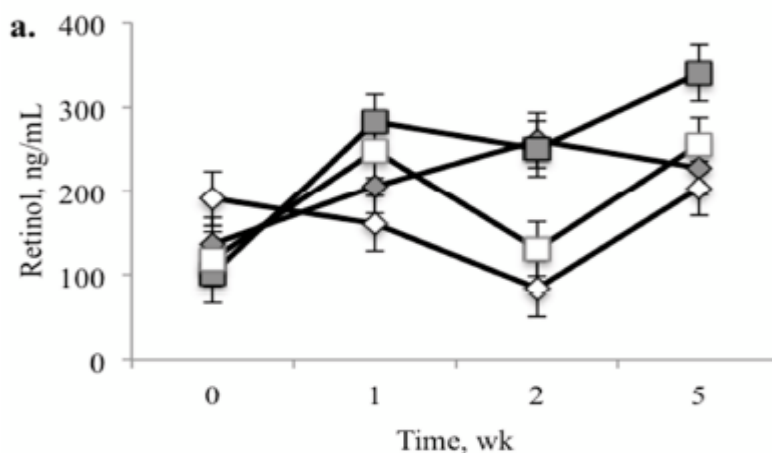


Figure 2. Plasma retinol of calves. ◆ LG-C, ■ LG-S, ◇ MG-C, □ MGS.

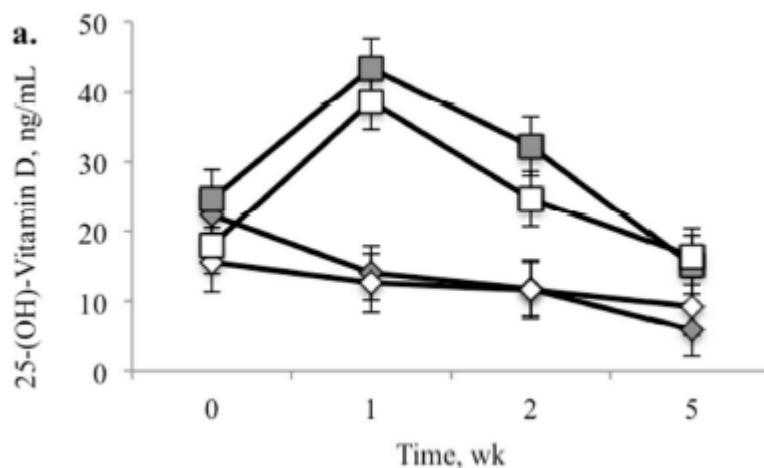


Figure 3. Plasma 25-(OH)-vitamin D in calves. ◆ LG-C, ■ LG-S, ◇ MG-C, □ MGS.

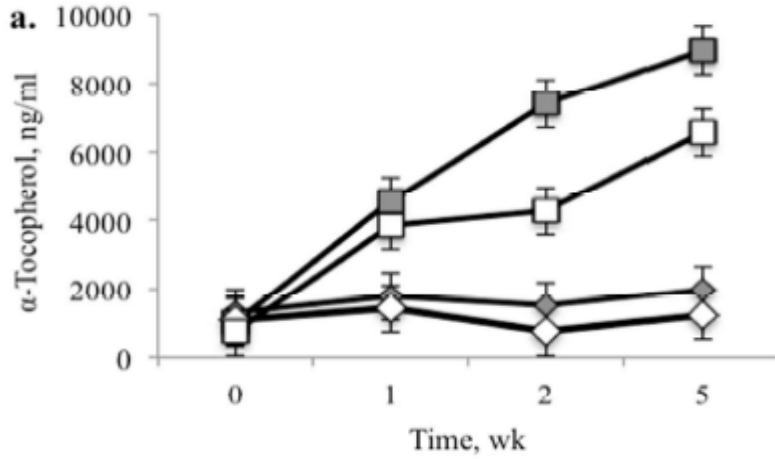


Figure 4. Plasma α -tocopherol in calves. \blacklozenge LG-C, \blacksquare LG-S, \blacklozenge MG-C, \square MGS.

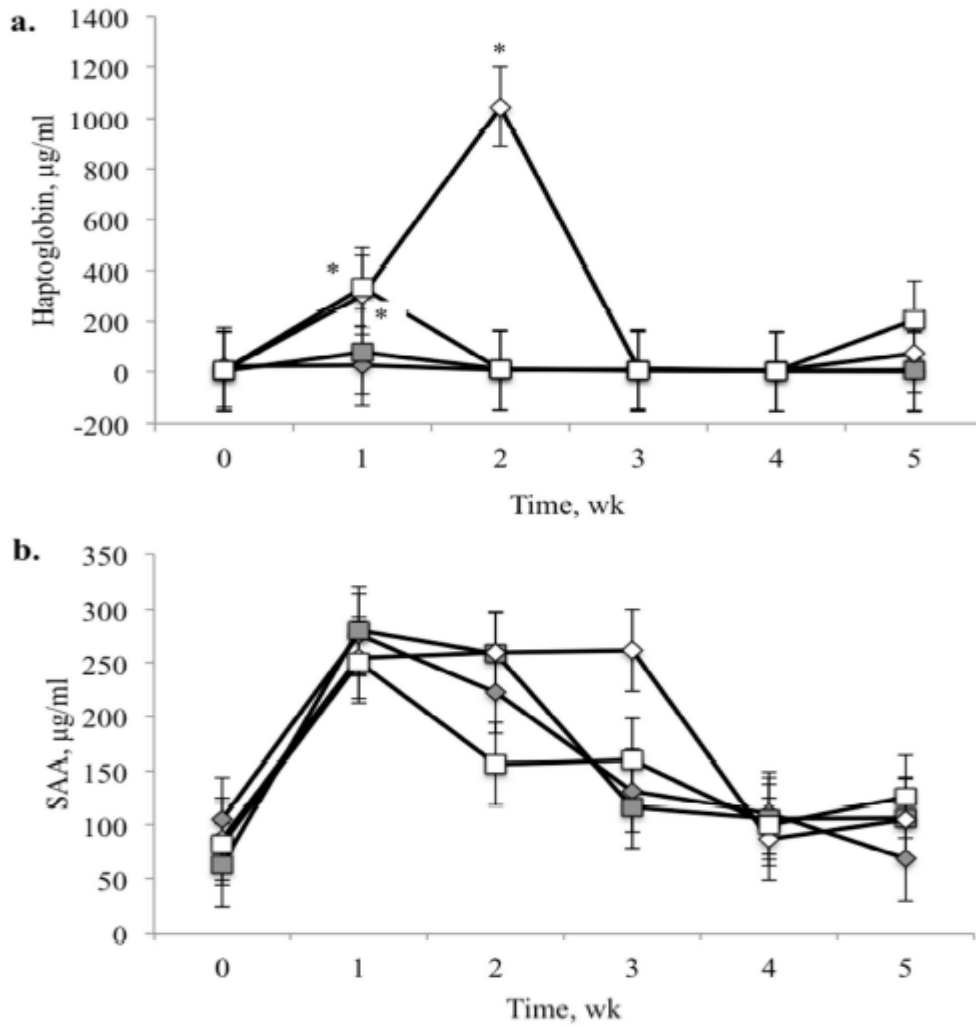


Figure 5. (a) Serum haptoglobin, and (b) serum amyloid A in calves. \blacklozenge LG-C, \blacksquare LG-S, \blacklozenge MG-C, \square MGS.