LEUKOCYTE FUNCTION AND HEALTH STATUS OF CALVES SUPPLEMENTED WITH VITAMINS A AND E

S. D. Eicher-Pruiett, J. L. Morrill, F. Blecha[1], *C. G. Chitko-McKown*[1], *and N. V. Anderson*[2]

Summary

Forty-four Holstein calves were fed milk replacers with varied concentrations of vitamins A and E from 3 to 45 d of age to determine their effects on concentrations of plasma vitamin A (retinol and retinyl palmitate) and vitamin E (α-tocopherol), lymphocyte and neutrophil functions, and health of calves. Plasma α-tocopherol was unaffected by increased vitamin A supplementation. Fecal scores, and eye and nose membrane responses were improved with increased vitamin A and lower vitamin E concentration, whereas the same treatment tended to reduce neutrophil cytotoxic and bactericidal activity by 6 wk of age. Increased supplemental vitamin E tended to enhance neutrophil functions. However, age appeared to have an effect on response to both vitamins.

(Key Words: Calves, Leukocytes, Vitamins, Health.)

Introduction

Previous research has shown improved immune function of lymphocytes with increased vitamin E supplementation to young calves. However, research with other species indicated that absorption of α-tocopherol diminished with increased dietary vitamin A, leading to the hypothesis that increased dietary vitamin A may interfere with absorption of dietary vitamin E in the calf. Therefore, vitamin A may limit availability of vitamin E to enhance immune functions. Many milk replacers contain more than 10 times the NRC requirement of vitamin A and amounts less than or equal to NRC recommendations of vitamin E. This experiment was conducted to determine if 1) increased vitamin A interferes with plasma α-tocopherol concentrations and 2) various concentrations of vitamins A and E in the diet affect lymphocyte and neutrophil functions and other health traits. All concentrations of vitamins that were used reflect concentrations present in milk replacers on the market.

Procedures

Forty-four Holstein calves, blocked by sex and age, were fed colostrum and then transition milk for 3 d. They were then fed experimental milk replacer at 10% of body weight, adjusted weekly. Vitamin A concentrations provided in milk replacers were low (LA; 3,200 IU/lb) or high (HA; 39,900 IU/lb) and vitamin E concentrations were low (LE; 5.1 IU/lb) or high (HE; 25.9 IU/lb). Concentrations of vitamin A and vitamin E reflect those amounts contained in milk replacers. The four experimental milk replacers were designated LA-LE, HA-LE, LA-HE, and HA-HE. Twice daily fecal scores and discharges of eyes and nose were recorded. Calves were weighed weekly. At 0, 3, and 6 wk, blood was sampled for determination of plasma retinol, retinyl palmitate, and α-tocopherol. Blood samples were collected at 3 and 6 wk to determine lymphocyte proliferation and neutrophil cytotoxicity and bactericidal and chemotactic functions (measures of immune health of calves). Concanavalin A was used as the mitogen for lymphocyte proliferation. The cytotoxicity assay was an

[1]Department of Anatomy and Physiology.
[2]Department of Surgery and Medicine.

antibody-dependent cellular-cytotoxicity (ADCC) assay using chicken red blood cells as the target cells. The neutrophil bactericidal assay targeted *Staphylococcus aureus*. Chemotaxis was measured under agarose with zymosan-activated serum as the chemoattractant for directed:random migration.

Results and Discussion

Plasma Vitamin Concentrations

Concentrations of plasma α-tocopherol were not affected adversely by increased supplementation of vitamin A at 6 wk (Table 1) but reflected the supplementation of vitamin E. However, α-tocopherol concentrations tended to increase overall with high vitamin A supplementation and were higher ($P<.05$) at 3 wk. Plasma retinol and retinyl palmitate did not consistently reflect the increased supplementation of vitamin A. Some of the inconsistencies may have been due to a retinol ester that is formed or because of tissue stores (neither measured in our analysis).

Growth and Health

Gain in body weight was similar between treatments for the total 6-wk period (72, 71, 64, and 66 lb for LA-LE, HA-LE, LA-HE, and HA-HE, respectively). The mean fecal score (1=solid, 4=fluid) for the 6-wk period of the HA-LE calves was lower ($P<.10$) than the scores of both LA treatment groups. The HA-LE group tended to have the lowest fecal score at 2 to 5 wk (Figure 1). The increase for the LA-HE group at 2 wk may explain the decrease in gain of that group that occurred then. The eye discharges increased, beginning at 2 wk for all treatments and remained high through 5 wk (Figure 2). The discharges observed in this study were clear, probably in response to fly irritation. Therefore, an increased discharge was considered a healthy response of the eye membrane. The HA-LE treatment tended to have the greatest occurrence of eye discharges. Total nasal discharges across weeks were greater for the LA-HE treatment ($P<.10$; data not shown). These discharges were thick mucous that occurred in few calves and for short periods of time and were considered a sign of infection.

Leukocyte Function

No differences in lymphocyte blastogenesis occurred among treatments at 3 or 6 wk (Table 2). Neutrophil phagocytosis and bactericidal activity tended to be lowest at 6 wk for calves on HA-LE treatment. Significant differences ($P<.05$) in bactericidal activity occurred between HA-HE and LA-HE treatments at 3 wk. The chemotaxis index indicated a greater response to a chemoattractant at 6 wk for LA-HE-supplemented calves.

Conclusion

Increased supplementation of vitamin A tended to improve responses that rely on a healthy mucous membrane. Simultaneously, the immune functions that utilize vitamin E tended to be improved by increased vitamin E and were inhibited when lower vitamin E and higher vitamin A concentrations were fed. The response of neutrophils to the chemoattractant, although enhanced by HE supplementation, was inhibited when HA was fed simultaneously, indicating possible interference of vitamin A with vitamin E utilization when both are fed at high concentrations. An age effect on vitamin E was seen both in plasma concentrations and leukocyte responses.

Table 1. Plasma Retinol, Retinyl Palmitate, and α-Tocopherol Concentrations in Calves Fed Experimental Milk Replacers

	Vitamin supplementation				
	LA-LE	HA-LE	LA-HE	HA-HE	SE
Vitamin & wk	---------- (µg/dl) ----------				
α-Tococpherol					
3 wk	266[c]	255[c]	298[b]	354[a]	7.6
6 wk	285[b]	297[b]	439[a]	452[a]	8.4
Retinol					
3 wk	101[b]	95[c]	102[b]	109[a]	2.2
6 wk	72[b]	191[a]	89[b]	77[b]	11.7
Retinyl Palmitate					
3 wk	51[b]	55[b]	52[b]	63[a]	3.1
6 wk	50[c]	66[b]	72[ab]	84[a]	5.5

[a,b,c]Means within a row without a common superscript letter differ (P<.05).

Table 2. Cellular Functions Weeks 3 and 6 of Calves Fed Experimental Milk Replacers

Measurement	Vitamin supplement				
and Wk	LA-LE	HA-LE	LA-HE	HA-HE	SE
Lymphocyte Blastogenesis (CPM)					
3 wk	193244	179062	179798	167536	18715
6 wk	191916	170630	169899	195659	17076
ADCC (%Lysis)					
3 wk	40.1	42.4	35.1	34.5	4.1
6 wk	42.4	37.5	44.8	45.4	6.1
S. aureus (% Kill)					
3 wk	27.1[ab]	20.5[b]	19.7[bd]	31.7[ac]	6.3
6 wk	24.0	18.8	25.6	26.9	5.6
Chemotaxis Index[1]					
3 wk	3.8	2.5	3.8	3.2	.1
6 wk	4.2[ab]	5.1[ab]	7.9[a]	4.0[b]	.3

[a,b,c,d]Means within row with different superscripts differ ([ab]P<.10); [cd]P<.05).

[1]For description of test see test.

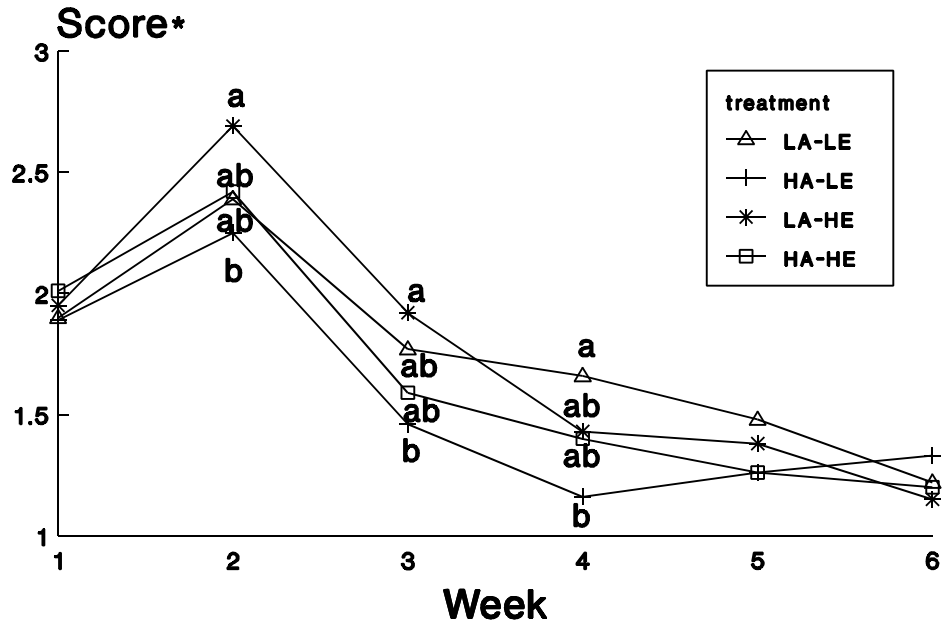

Figure 1. **Weekly Fecal Scores of Calves Fed Experimental Milk Replacers. Means Within a Week with Different Superscripts Differ (P<.10). 1 = Solid to 4 = Fluid.**

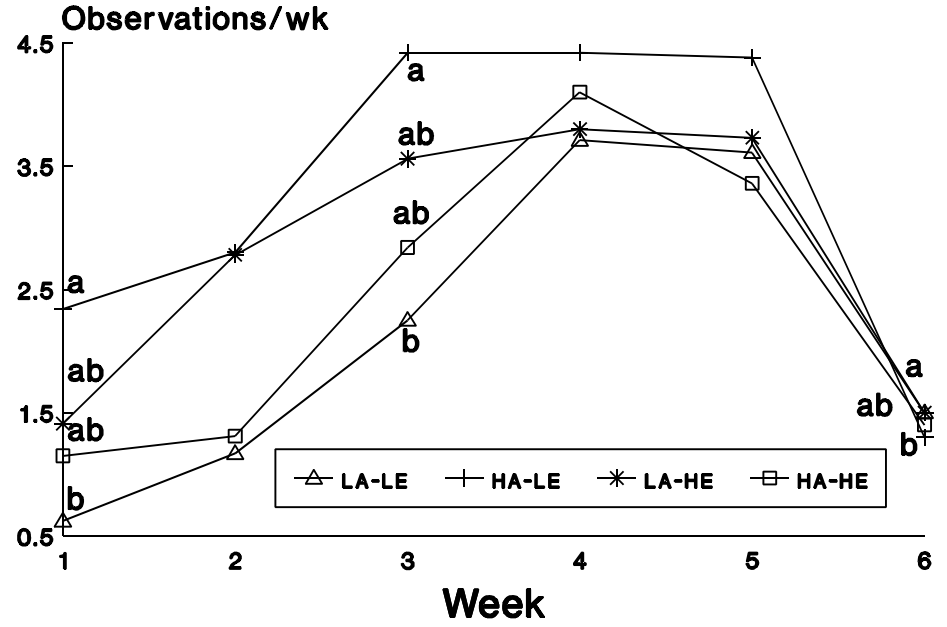

Figure 2. **Weekly Eye Discharges of Calves Fed Experimental Milk Replacers. Means Within a Week with Different Superscripts Differ (P<.10).**