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

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## 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

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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.

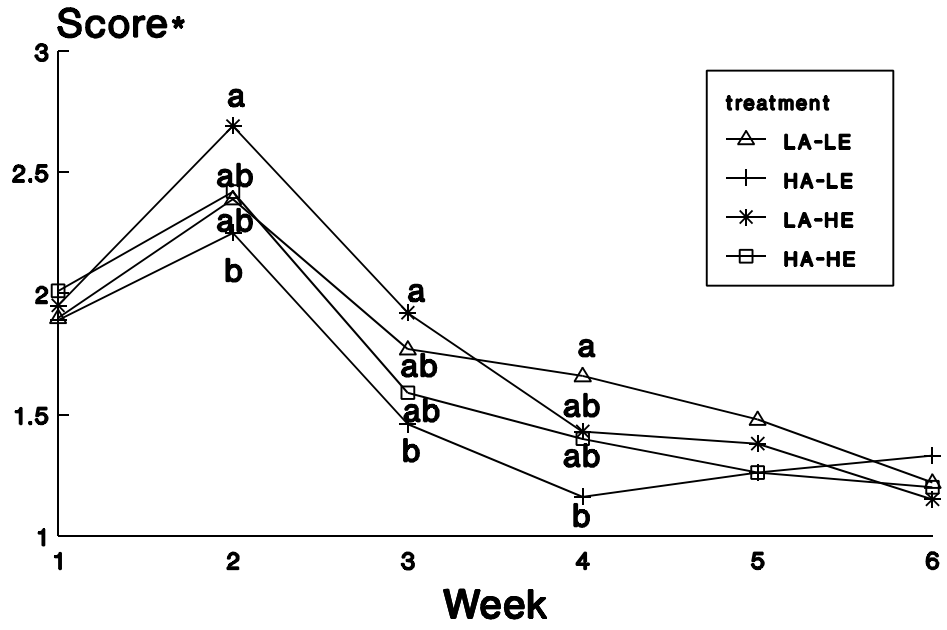


**Figure 1.** **Weekly Fecal Scores of Calves Fed Experimental Milk Replacers. Means Within a Week with Different Superscripts Differ (P<.10). 1 = Solid to 4 = Fluid.**

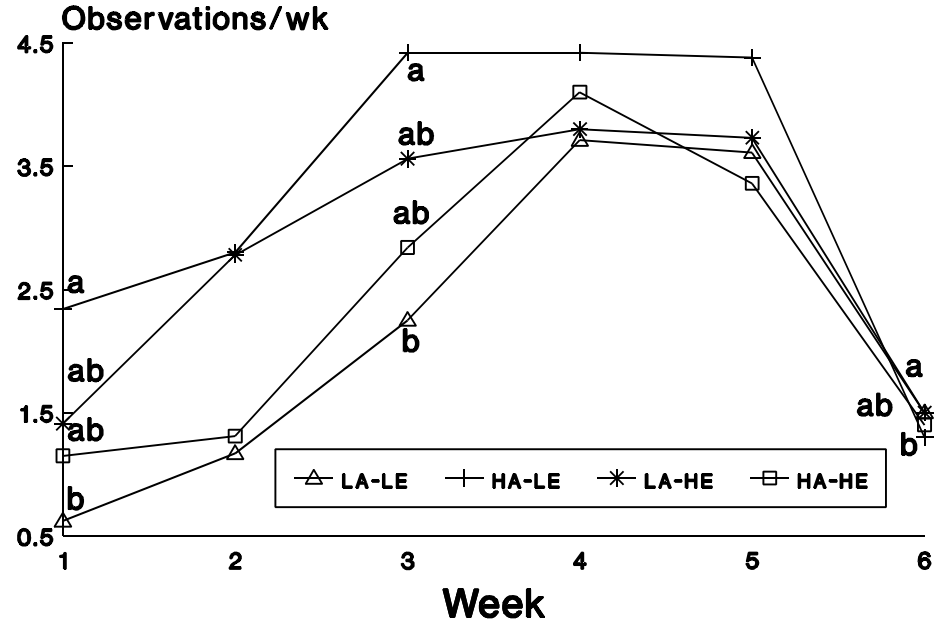


**Figure 2.** **Weekly Eye Discharges of Calves Fed Experimental Milk Replacers. Means Within a Week with Different Superscripts Differ (P<.10).**