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## Neutrophil and lymphocyteresponse to vitamins C and E supplementation in young calves

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# **NEUTROPHIL AND LYMPHOCYTE RESPONSE TO VITAMINS C AND E SUPPLEMENTATION IN YOUNG CALVES**

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## **Summary**

Calves were bottle-fed milk replacers at 10% of weekly adjusted body weight for 8 wk. Treatments were 1) no supplements (control), 2) .16 oz vitamin C, or 3) .16 oz vitamin C plus 125 IU/lb vitamin E. Lymphocytes and neutrophils isolated from day 14 and day 28 blood samples were assayed for neutrophil-mediated *S. aureus* phagocytosis and antibody-dependent cellular cytotoxicity, and for mitogen induced lymphocyte proliferation. Eye and nasal discharges of calves supplemented with vitamin C and vitamins C plus E were less than those of control calves for wk 1 to 8. Lymphocyte proliferation with the mitogens showed a trend for higher responses at wk 2 in vitamin C plus E supplemented calves. Neutrophils of calves supplemented with vitamin C showed decreased phagocytosis and lysis functions compared to those of control calves at wk 2 and 4. Neutrophil function of calves supplemented with vitamins C plus E was near or slightly higher than that of controls at wk 2 and 4, suggesting that the addition of vitamin E negated the adverse effects that vitamin C alone had on neutrophil functions.

## **Introduction**

Ascorbic acid (vitamin C) is produced by the liver of many animals, including cattle. Ascorbic acid synthesis begins in calves between the second and third wk of life and reaches adult concentrations of vitamin C around 3 mo of age. Milk, which has a relatively low ascorbic acid content, is often exposed to air and light before being consumed by the calf. Both are destructive agents of vitamin C. Therefore, vitamin C deficiency is a potential problem with the young milk-fed calf. This would be especially true for dairy calves, whose only source of vitamin C for the first few wk of life is bucket- or bottle-fed milk or milk replacer.

Vitamin C deficiency has been linked to decreased immune response, and elevated ascorbic acid concentrations have been linked to increased immune response in catfish, poultry, cattle, and swine. Conversely, others have found that vitamin C supplementation had no beneficial effect on the immune responses measured. The value of vitamin E to the immune system of the young calf has been established. It is primarily responsible for protection of the cell membrane. In other species, vitamins C and E have been shown to work cooperatively to protect the cell membranes against peroxidation.

The purpose of the present study was to determine the effects of supplements of vitamin C alone and vitamins C and E together on the function of neutrophils and lymphocytes of the young calf's

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immune system, as well as their effect on the growth and general health of the calf during the first 8 wk of life.

### **Procedures**

Thirty Holstein bull calves were removed from their dams at 24 h following birth. They were given transition milk for 2 more days, then assigned to each of three treatment groups. The treatments consisted of milk replacers 1) without supplemental vitamins (control), 2) with .16 oz vitamin C per kg, or 3) with .16 oz vitamin C plus 125 IU vitamin E per lb. Milk replacer was reconstituted to 13.5% dry matter and was fed at 10% of body weight, adjusted weekly, which was divided into two equal daily feedings. Calves were housed in individual hutches with straw bedding. Water was available ad libitum.

Body weights were measured and milk replacer allocations were adjusted weekly. Twice daily fecal and general appearance scores were recorded, and eye or nasal discharge or signs of enteric or respiratory illness were noted. Blood samples were collected in heparinized tubes on experimental day 1, 14, 28, and 56 for vitamin C and E determinations. Day 14 and day 28 samples were used to assay lymphocyte proliferation and neutrophil function. A lymphocyte transformation assay (LTA) and neutrophil-mediated antibody-dependent cellular-cytotoxicity (ADCC) and *S. aureus* phagocytosis assays were used to determine lymphocyte and neutrophil cellular function.

### **Results and Discussion**

Vitamin C was stable in the milk replacers after a decrease that occurred prior to sampling. Plasma vitamin C concentrations of the control group dropped throughout the study until wk 8, when concentrations for all treatments were approximately equal (Figure 1). The vitamin C-only group maintained a high concentration until wk 8. The plasma vitamin C content of vitamin C plus E-supplemented calves decreased greatly at wk 2, but later recovered to levels near those of the vitamin C-only group. Plasma vitamin E concentrations reflected the supplementation with that vitamin.

No significant differences occurred between treatments in gain or feed efficiency until wk 6 (Table 1). The vitamin C plus E supplemented group had greater gains over the entire 8 wk period than the vitamin C supplemented group and was more efficient in feed conversion (lb feed/lb gain) than both the control and vitamin C supplemented group.

Table 2 shows weekly mean fecal scores and discharge observations for all groups. The control group tended to have the lowest fecal scores (more solid feces) throughout the 8 wk, and the vitamin C supplemented group tended to have the highest scores. The control and vitamin C supplemented groups were different ( $P < .10$ ) at wk 2, 6, and 8, and at wk 8 the vitamin C group was also significantly higher than the vitamin C plus E supplemented group. Mean eye and nose discharges tended to be higher for the control group for all 8 wk. At wk 3, the vitamin C plus E supplemented calves had significantly less discharges than the control group, and the vitamin C only supplemented group was significantly lower than the control calves at wk 7.

**Table 1. Total and Daily Gain by Week and Feed Efficiency**

| Wk     | Gain, lb/d              |                    |                   | Feed efficiency, lb feed/lb gain |                   |                   |
|--------|-------------------------|--------------------|-------------------|----------------------------------|-------------------|-------------------|
|        | Vitamin supplementation |                    |                   | Vitamin supplementation          |                   |                   |
|        | None                    | C                  | C + E             | None                             | C                 | C + E             |
| 1      | 1.19                    | 1.08               | 1.54              | 1.3                              | 2.5               | 1.0               |
| 2      | .75                     | .81                | .99               | 1.5                              | 1.5               | 1.6               |
| 3      | .77                     | .90                | .31               | 2.2                              | 2.0               | 1.9               |
| 4      | .90                     | .92                | 1.03              | 1.7                              | 1.8               | 1.8               |
| 5      | .99                     | 1.12               | .99               | 2.2                              | 1.9               | 1.9               |
| 6      | 1.34 <sup>ab</sup>      | .81 <sup>a</sup>   | 1.76 <sup>b</sup> | 1.8 <sup>b</sup>                 | 3.6 <sup>a</sup>  | 1.2 <sup>b</sup>  |
| 7      | 1.50 <sup>a</sup>       | 1.39 <sup>ab</sup> | .92 <sup>b</sup>  | 1.8 <sup>b</sup>                 | 1.7 <sup>b</sup>  | 4.6 <sup>a</sup>  |
| 8      | 1.32 <sup>ab</sup>      | 1.19 <sup>a</sup>  | 1.91 <sup>b</sup> | 1.9                              | 1.5               | 1.6               |
| Totals | 61.8 <sup>ab</sup>      | 57.6 <sup>a</sup>  | 64.5 <sup>b</sup> | 1.65 <sup>a</sup>                | 1.61 <sup>a</sup> | 1.41 <sup>b</sup> |

<sup>ab</sup>Differing superscripts within the same week denote means with statistically significant differences (P<.10).

Because of variability in the lymphocyte functions of the 2- and 4-wk-old calf, no significant differences between the treatment groups could be noted in the Con-A and PHA mitogen-induced proliferation means. Week 2 lymphocytes of vitamin C plus vitamin E supplemented calves showed trends toward greater proliferation. By wk 4, the lymphocytes of vitamin C supplemented calves had proliferative responses equal to or greater than those of the C plus E supplemented calves. The lack of differences in lymphocyte proliferation between treatments may have been partly due to very little environmental stress experienced by these calves. Environmental stress has been shown to increase the use of vitamin C and antibody production in young calves supplemented with vitamin C. Increased antibody production would be preceded by increased lymphocyte proliferation.

The neutrophil mediated ADCC at wk 2 (Figure 2) showed significant differences between the two vitamin supplemented groups, and at wk 4 between the vitamin C supplemented group and the control as well. The *S. aureus* phagocytosis assays showed the vitamin C group to be significantly different from the control at wk 2, but no significant differences were detected at wk 4.

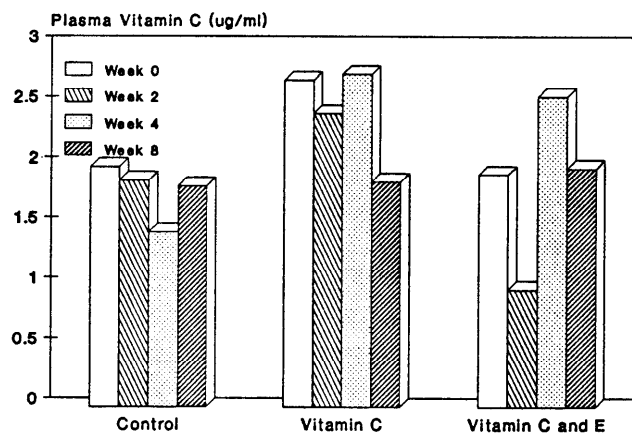
**Table 2. Weekly Eye and Nasal Discharge and Weekly Fecal Scores**

| Wk     | Eye and nasal discharge |                   |                  |      | Fecal score             |                  |                   |     |
|--------|-------------------------|-------------------|------------------|------|-------------------------|------------------|-------------------|-----|
|        | Vitamin supplementation |                   |                  |      | Vitamin supplementation |                  |                   |     |
|        | None                    | C                 | C + E            | SE   | None                    | C                | C + E             | SE  |
| 1      | 1.5                     | .2                | .4               | .26  | 2.0                     | 2.2              | 2.1               | .13 |
| 2      | 2.5                     | 1.6               | 1.3              | .47  | 2.0                     | 2.6              | 2.2               | .15 |
| 3      | 3.4 <sup>a</sup>        | 1.9 <sup>ab</sup> | 1.5 <sup>b</sup> | .85  | 2.3                     | 2.3              | 2.1               | .16 |
| 4      | 3.1                     | 2.8               | 1.6              | .92  | 1.8                     | 1.9              | 1.9               | .10 |
| 5      | 1.7                     | .9                | 1.6              | .64  | 1.6                     | 1.8              | 1.8               | .12 |
| 6      | 1.9                     | .7                | .8               | .56  | 1.5 <sup>b</sup>        | 1.9 <sup>a</sup> | 1.7 <sup>ab</sup> | .12 |
| 7      | 2.0 <sup>a</sup>        | .5 <sup>b</sup>   | .7 <sup>ab</sup> | .59  | 1.6                     | 1.6              | 1.6               | .12 |
| 8      | 1.9                     | 1.9               | .6               | .54  | 1.5 <sup>b</sup>        | 1.8 <sup>a</sup> | 1.6 <sup>b</sup>  | .12 |
| Totals | 17.0                    | 10.5              | 9.0              | 3.61 | --                      | --               | --                | --  |

<sup>ab</sup>Differing superscripts within the same week denote means with statistically significant differences ( $P < .10$ ).

### Conclusion

Supplementation with vitamin C alone and vitamins C and E together did not have a beneficial effect on any of the immune responses measured here. However, the lower incidence of mucous discharge from eyes and noses suggests a beneficial effect that was not reflected by the cellular functions we measured. The use of vitamins C and E together appeared to negate the adverse effects of vitamin C alone on neutrophil functions at both 2 and 4 wk and on lymphocyte proliferation at wk 2.



**Figure 1.** Plasma vitamin C concentrations at 0, 2, 4, and 8 wk of treatment.

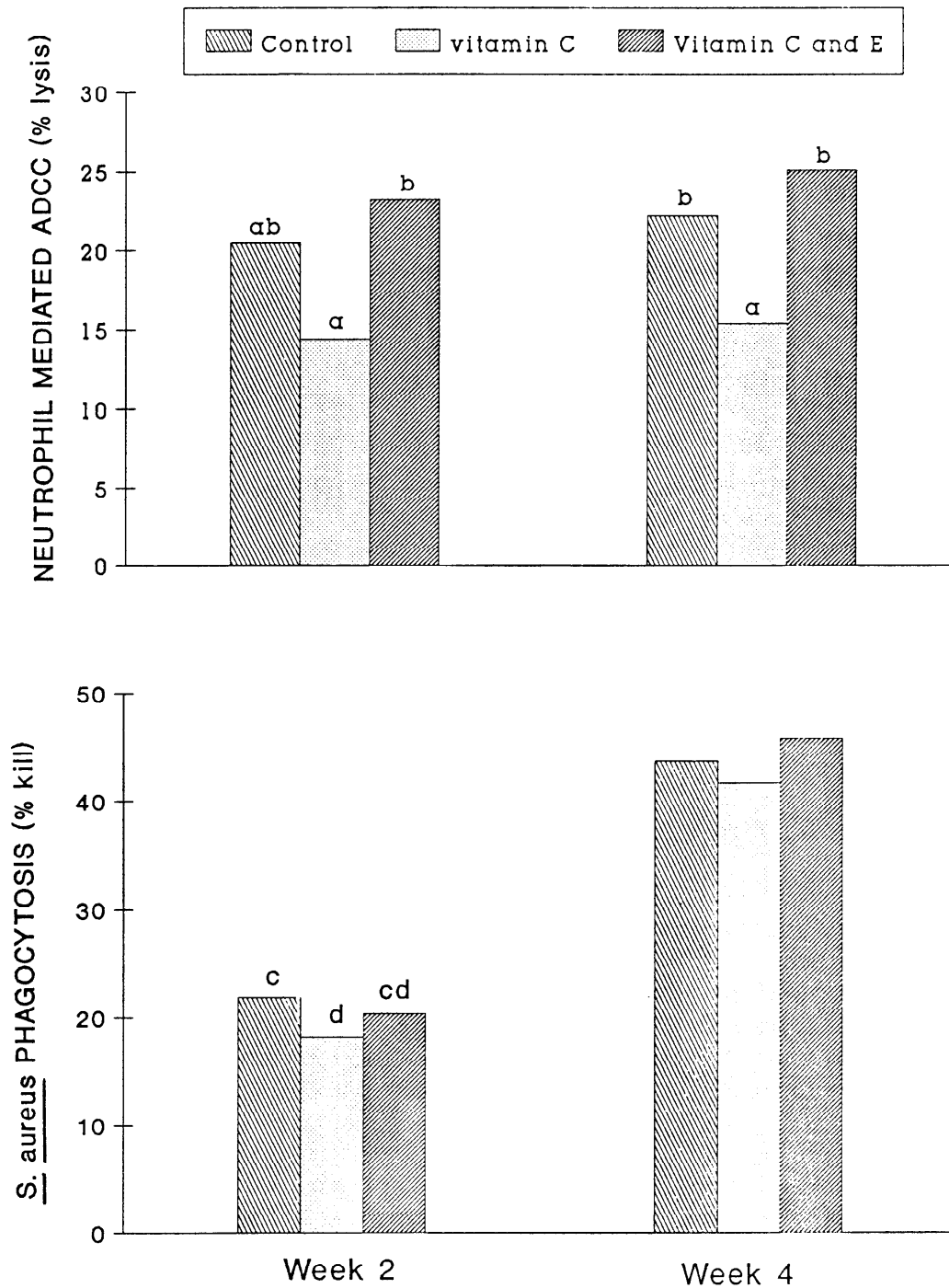


Figure 2. Neutrophil-mediated antibody-dependent cellular cytotoxicity % specific-lysis and *S. aureus* phagocytosis % kill. <sup>ab</sup>Differing superscripts within the same wk denote means with significant differences ( $P < .10$ ). <sup>cd</sup>Differing superscripts within the same wk denote means with significant differences ( $P < .05$ ).