The physiological and production effects of increased dietary intake of vitamins E and C in feedlot cattle challenged with bovine herpesvirus 1

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ABSTRACT: The physiological and production effects of feeding additional vitamin E and ruminally protected vitamin C were examined in cattle challenged with bovine herpesvirus 1. Forty-eight individually penned 6-mo-old Angus and Angus crossbred heifer calves with a mean BW of 151 kg were allocated randomly to four diets in a 2 x 2 factorial arrangement of treatments. Pelleted diets provided either 15 or 185 IU/kg of DM of vitamin E, with or without 3.7 g of ruminally protected vitamin C/kg of DM. Blood samples were taken at start of the experiment and at wk 4, 5, and 6. At the start of wk 5, half of each of the dietary groups was challenged with BHV 1. Feeding additional vitamin E was associated with greater (P < 0.001) mean plasma α-tocopherol. In contrast, feeding ruminally protected vitamin C was not associated with greater (P = 0.59) mean plasma ascorbate concentration; however, feeding ruminally protected vitamin C was associated with lower (P = 0.03) mean blood total superoxide dismutase (Cu/Zn SOD and Mn SOD) concentration.

Key Words: Alpha-Tocopherol, Antioxidants, Ascorbate, Cattle, Infectious Bovine Rhinotracheitis

Introduction

Numerous studies have illustrated a positive effect of antioxidants on cattle health. Most have examined the effects of vitamin E. McDowell et al. (1994) reported that feedlot cattle supplemented with 450 IU of vitamin E-animal−1·d−1 after the stress of long-distance transport had fewer sick pen days per animal and decreased morbidity. Hill et al. (1990) reported a lower incidence of liver abscesses in cattle supplemented with 1,000 IU of vitamin E-animal−1·d−1. Secrist et al. (1997) pooled the results of five studies in which supplemental vitamin E was assessed at intakes of 450 to 1,600 IU-animal−1·d−1. Morbidity tended (P = 0.14) to be less with vitamin E supplementation (48 vs. 55%). Carter et al. (2002) fed 2,000 IU of vitamin E-animal−1·d−1 to cattle newly arrived at the feedlot, and although they did not detect an effect on morbidity, mortality, or weight gain, they reported that 28-d vitamin E supplementation decreased treatment costs for respiratory disease.

There has been limited investigation of the potential benefits of vitamin C supplementation to cattle health (Roth and Kaeberle, 1985; MacLeod et al., 1996). Cummins and Brunner (1989) reported a trend (P < 0.10) toward a lower incidence of scouring in calves supplemented with vitamin C at 1.75 g/d of the free acid. The authors are unaware of any experiment that specifically addresses the potential of vitamin C to improve the production of feedlot cattle; however, studies have found improved production of feedlot cattle in response...
to vitamin E supplementation (Hutcheson and Cole, 1985; Hill et al., 1990; Secrist et al., 1997). The present study was designed to investigate the effects of vitamins E and C on physiological measurements and production in feedlot cattle, the effects of bovine herpesvirus 1 (BHV 1) infection, and the potential of the antioxidant vitamins E and C to affect physiological and performance outcomes in feedlot cattle challenged with BHV 1.

Materials and Methods

Animals and Management

Fifty-two ruminating heifer calves that were seronegative for BHV 1 and had an average BW of 151 kg (range = 110 to 193 kg) were obtained from the New South Wales (NSW) Department of Agriculture Trangie Research Station, Australia, and transported by truck to Coota Park Research Facility, Cowra, NSW, Australia. The calves were Angus, Angus × Hereford, and Angus × Shorthorn, and were from one calf crop, with a maximum difference in age of 3 mo. The calves were weighed, vaccinated against clostridial diseases (Ultravac, CSL Ltd., Parkville, Australia; Clostridium perfringens type D, Clostridium tetani, Clostridium novyi type B, Clostridium septicum [as ultrafiltered toxoids], Clostridium chauvoei [as formal culture], and ear-tagged. Numbered ear tags were allocated randomly to divide the calves into four dietary groups of 12 animals. Each calf was placed in an individual pen, with pen number corresponding to ear tag number. An extra calf was allocated to each of the four dietary treatment groups, with these cattle being individually penned adjacent to the research facility. These additional calves were treated the same in all respects as the 48 animals initially allotted to the experiment, and were replacements to be used if required. One calf died after 22 d on feed and was replaced with the calf on the same diet. All data of the former calf were replaced with those of the latter. This experiment had Animal Care and Ethics Committee approval.

Experimental Design and Treatments

Diets. Two levels each of vitamins E and C were provided for the duration of the experiment, and BHV 1 challenge of half the calves occurred at wk 4. The experiment was a split-plot design, with a 2 × 2 factorial arrangement of whole-plot treatments. Pens were arranged in six blocks of eight pens each. Each block of eight pens was divided into two sets of four pens using electric wires and tarpaulins. Calves in four pens were challenged, and calves in the remaining four pens were not challenged with BHV 1. Within each of these blocks, all vitamin combinations were allocated randomly to pens. All calves were fed a basal diet providing the minimum requirements of vitamin E, vitamin A, and trace minerals (NRC, 1996), including Se (0.05 mg/kg; CSIRO, 1990). The diets included 1) basal diet alone; 2) 185 IU of added vitamin E/kg of dietary DM; 3) 3.7 g of added ruminally protected vitamin C/kg of dietary DM (MacLeod et al., 1996); and 4) the addition of both vitamins E and C (Table 2). Four weeks after the start of the experiment, half the calves from each dietary treatment were challenged with a field strain of BHV 1. The BHV 1 used was an NSW field isolate (Z154, Elizabeth Macarthur Agricultural Institute, Camden, Australia) that had undergone limited (less than five) passages in cell culture. Challenged calves had 1 mL of BHV 1 culture fluid with a virus titer of 6.9 log10/mL delivered into each nostril using a 2.5-mL syringe. Calves challenged with BHV 1 were separated from the nonchallenged calves by a double physical barrier, using two sealed tarpaulins. Transmission of BHV 1 to unchallenged calves via fomites was avoided by hand washing with chlorhexidine when moving in the feed alley between challenged and unchallenged groups and by the use of separate feed-handling facilities. The effectiveness of serological conversion in the challenged calves and the containment of a serological response to those calves were checked by serological testing at the conclusion of the experiment.

Allocation to Treatments. Calves were allocated randomly to the four pelleted diets at the start of the experiment on the basis of ear tag numbers. The different diets were coded with a separate letter and color. The research facility staff and researchers were unaware of the letter and color code applied to each diet for the duration of the experiment.

Diet Formulation. The ingredient composition of the basal diet is shown in Table 1. The mean analytical results are compared with the calculated nutrient composition in Table 2. The vitamin-mineral premixes (Hoffmann-LaRoche, Wagga Wagga, Australia) were mixed into the feeds before pelleting by Young Stock Feeds, Young, Australia. A sample of the base mix without vitamin-mineral premix was analyzed for the concentrations of vitamin A, vitamin E, Co, Cu, I, Fe, Mn,
Se, and Zn by Hoffmann-La-Roche (Dee Why, Australia). The vitamin-mineral premix was added to meet the formulated concentrations, with the exception of vitamin A, for which the final diet contained 2,109 IU/kg of diet DM. The vitamin-mineral premix carrier contained 40% ground rice hulls and limestone. For the purposes of calculation, it was considered that vitamin C (Rovimix Stay C, Hoffmann-LaRoche) was 100% ascorbyl-2-polyphosphate.

Diet Analyses. Immediately after manufacture, samples of the diets were analyzed by three different feed analysis laboratories to compare with formulation (Table 2). Residual feed remaining in the individual feed bins was sampled for every animal during the fifth week on feed. Pooled samples for each diet were analyzed to check that there were no substantial differences between feed eaten and the residual feed, which was weighed and disposed of daily.

Feed Delivery and Measurement. The calves were fed daily at 0730 at the rate of 2.7% of individual BW of dietary DM. Records were kept of all feed delivered and residuals to calculate DMI, ADG, and G:F. The calves were weighed every 2 wk, and the weight of feed delivered to each animal was calculated on the basis of the most recent weight of each calf (with no withholding period from feed and water). Feed was delivered each morning at approximately 0830 to the individual feed bins in dedicated color-coded containers with each feed corresponding to the color coding of the feeder bins. Body weight, feed amounts, and feed residuals were measured using portable electronic scales (model KM2, Ruddweigh, Guyra, NSW, Australia) that were calibrated before each feeding. Except for samples taken during wk 5, residuals from each calf were discarded at the completion of each feeding.

Measurements

Blood samples were obtained from the calves by jugular venipuncture. Sampling was commenced at 0800 at initiation of the experiment; after 4 wk of feeding immediately before BHV 1 challenge; after inoculation with BHV 1 during wk 5; and after 6 wk of feeding. Plasma and serum samples were obtained using evacuated tubes (Interleuvenlaan 40, Terumo Corp., Leuven, Belgium). Plasma samples were inverted five times immediately after collection, and both plasma and serum samples were placed in ice baths. The tubes were centrifuged at 1,056 × g for 10 min and samples harvested within 1 to 2 h of collection. Concentrations of plasma β-carotene, retinol, ascorbate, α-tocopherol, blood glutathione peroxidase, total superoxide dismutase (SOD; Cu/Zn SOD and Mn SOD), and serum ceruloplasmin were measured. Differential leukocyte counts were used to monitor cellular responses to immune challenge. Production was assessed by comparing ADG and G:F. The calves were weighed on successive days at the start of the experiment, and then at 2-wk intervals with duplicate weighing 1-d apart at the end of the experiment. The duplicate weighing at the start and finish were designed to decrease variation in results due to gut fill.

Analytical Methods

Measurement of Retinol, α-Tocopherol, β-Carotene, and Ascorbate Concentrations. For analysis of retinol,
α-tocopherol, and β-carotene, a 1-mL sample of plasma was placed in a capped plastic container and frozen immediately at −8°C. For analysis of ascorbate, a 0.5-mL plasma sample was placed in a capped plastic tube and stabilized with 4.5 mL of 5% (wt/vol) metaphosphoric acid, and then the tube was immediately frozen at −8°C. Retinol, α-tocopherol, and β-carotene concentrations were determined simultaneously by HPLC according to the method of Hess et al. (1991), except that the retinol absorbance of 1,826 units was read at 325 nm. The plasma ascorbate concentration was determined by fluorometric assay using a centrifugal analyzer with fluorescence attachment, according to the method of Vuilleumier and Keck (1989).

Measurement of Glutathione Peroxidase and Ceruloplasmin Concentrations. Blood glutathione peroxidase concentrations and serum ceruloplasmin concentrations were measured with an Abbott Spectrum Autoanalyzer, Series 2 (Abbott Laboratories, Abbott Park, IL). Glutathione peroxidase concentration was determined by the Elizabeth Macarthur Agricultural Institute Glutathione Peroxidase Method (No. GSHPx.0001), using the technique of Paynter et al. (1987). Serum ceruloplasmin was determined by the Elizabeth Macarthur Agricultural Institute Copper Method (No. Copper.0001) and expressed as copper with a range of 3 to 15 μmol/L.

Measurement of Total Superoxide Dismutase Concentration. Total SOD concentrations were determined for whole blood samples in lithium heparin 10-mL Vacutainer tubes. Total SOD was measured by calculating the degree of inhibition of generation of superoxide radical by a xanthine/xanthine oxidase generating system using the compound para-iodonitrotetrazolium violet as the detection system, as superoxide converts para-iodonitrotetrazolium violet to a red formazan dye (Randox, Catalog No. SD125, Bay Scientific, Dandenong, Australia).

Measurement of Malondialdehyde Concentration. The method for measuring malondialdehyde concentration was based on the reaction of malondialdehyde with thiobarbituric acid to form a pink chromogen (thiobarbituric acid – malondialdehyde) adduct. This adduct was separated by reverse-phase HPLC on a C18 column, with the adduct detected either by absorbance (532 nm) or fluorescence (EX: 532, EM: 553) according to the procedure of Halliwell and Chirico (1993).

Measurement of Blood Responses. Samples for differential leukocyte count and measurement of packed cell volume, total plasma protein (TPP), fibrinogen, plasma protein:fibrinogen ratio, and platelets, were collected in 10-mL EDTA evacuated tubes (Interleuvenlaan 40). Blood smears were made from these samples within 1 h of collection. Packed cell volume and white blood cell count (WCC) were measured with a Cobas Mira Vet analyzer (Roche Diagnostics, F. Hoffmann-LaRoche Ltd., Basel, Switzerland). Total plasma protein, fibrinogen, plasma protein:fibrinogen ratio, and white cell differential counts were determined according to the methods of Jain (1986).

Statistical Methods

Pre-experiment measurements (d 1) were used as covariates in the repeated-measures analyses to account for the initial variation. Animals on the four dietary treatments were in six blocks, with half of each of the blocks challenged with BHV 1 inoculation after 4 wk. The model for analyses where there was no time factor was:

\[ Y_{ijkl} = \mu + \rho_i + \tau_j + \alpha_k + \gamma_l + (\tau\alpha)_{jk} + (\tau\gamma)_{jl} + (\alpha\gamma)_{kl} + (\tau\alpha\gamma)_{jkl} + \varepsilon_{ijkl} \]

where \( \rho_i \) is the term for the ith block, \( \tau_j \) is the term for the jth level of virus challenge, \( \alpha_k \) is the term for the kth level of vitamin C, \( \gamma_l \) is the term for the lth level of vitamin E, and the terms in brackets are the interaction terms. The model for analyses where there was a time factor was:

\[ Y_{ijkl} = \mu + \rho_i + \tau_j + \alpha_k + \gamma_l + (\tau\alpha)_{jk} + (\tau\gamma)_{jl} + (\alpha\gamma)_{kl} + (\tau\alpha\gamma)_{jkl} + \delta_{ijkl} + \lambda_m + (\lambda\tau)_{jm} + (\lambda\alpha)_{hm} + (\lambda\gamma)_{lm} + (\gamma\tau\alpha)_{jkl} + (\tau\alpha\gamma)_{jklm} + \varepsilon_{ijklm} \]

where \( \lambda_m \) is the term for the mth level of time (weeks of the experiment), and \( \delta_{ijkl} \) is the error for comparisons not involving time. The main effects and interactions are as described in the first model. Analyses for a randomized complete block design with repeated measures were done for the measurements repeated over wk 4, 5, and 6. Malondialdehyde was measured during the wk 5 sampling only. Due to heterogeneous variances, log transformations were made on the data for ceruloplasmin, fibrinogen, plasma protein:fibrinogen, and β-carotene. All data were analyzed using GLM in Genstat Version 3.2 (Numerical Algorithms Group Ltd., Wilkinson House, Oxford, U.K.).

Results

Effects of Increased Dietary Vitamin E. The intakes and performance of the calves are shown in Table 3. Feeding additional vitamin E had no effects on feedlot performance, including DMI (\( P = 0.21 \)), ADG (\( P = 0.40 \)), and G:F (\( P = 0.997 \)). Mean plasma α-tocopherol concentration in calves fed additional vitamin E was greater (4.12 vs. 1.54 \( \pm 0.192 \) mg/L; \( P < 0.001 \)) than the mean plasma α-tocopherol concentration for calves fed the lower dietary concentration of vitamin E, both for the entire feeding period and at each sampling. Calves fed additional vitamin E had greater (167.2 vs. 139.3 ± 9.63 \( \mu \)g/L; \( P = 0.05 \)) mean plasma β-carotene concentrations than calves fed a lower dietary concentration of vitamin E. In contrast, a higher dietary intake of vitamin E had
Table 3. Feed and calculated vitamin intakes, average daily gain, and gain:feed for the duration of the experiment

<table>
<thead>
<tr>
<th>Vitamin supplementation per kg of dietary DM</th>
<th>Vitamin E</th>
<th>Vitamin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itema</td>
<td>15 IU</td>
<td>183.5 IU</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>4.73</td>
<td>4.48</td>
</tr>
<tr>
<td>Vitamin intake, animal/d</td>
<td>71 IU</td>
<td>822 IU</td>
</tr>
<tr>
<td>Mean BW, kg</td>
<td>184.0</td>
<td>176.7</td>
</tr>
<tr>
<td>Vitamin intake, kg BW/d</td>
<td>0.39 IU</td>
<td>4.65 IU</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>1.15</td>
<td>1.09</td>
</tr>
<tr>
<td>G:F</td>
<td>0.24</td>
<td>0.24</td>
</tr>
</tbody>
</table>

aData are means of 12 calves.

no effect \( (P = 0.98) \) on retinol concentration (data not shown). Calves fed additional vitamin E had a greater \((2.77 \text{ vs. } 2.36 \pm 0.14 \mu\text{mol/L}; P = 0.05)\) mean plasma malondialdehyde concentration at wk 5.

**Effects of Ruminally Protected Vitamin C.** There were no significant effects of feeding ruminally protected vitamin C on DMI \((P = 0.33)\), ADG \((P = 0.34)\), or G:F \((P = 0.76; \text{Table 3})\). Feeding ruminally protected vitamin C was not associated with a greater \((1.91 \text{ vs. } 1.79 \text{ mg/L}; \text{SEM} = 0.16; P = 0.59)\) plasma ascorbate concentration but was associated with lower \((3,111 \text{ vs. } 3,367 \pm 77.9 \text{ U/g Hb}; P = 0.03)\) mean blood total SOD concentration.

**Interactions Between Increasing Dietary Vitamin E and Ruminally Protected Vitamin C.** The interactions between vitamins E and C with respect to G:F, ceruloplasmin concentration, and lymphocyte count are shown in Table 4. Calves fed a combination of high vitamin C and high vitamin E had a higher \((P = 0.05)\) mean G:F than those fed high concentrations of each of the vitamins alone. The mean G:F of calves fed additional vitamin E and protected vitamin C did not differ from that of the controls. Calves fed ruminally protected vitamin C alone had a lower \((P = 0.01)\) mean serum ceruloplasmin concentration than calves fed no ruminally protected vitamin C and lower vitamin E and calves fed high dietary concentrations of both vitamins. There was a trend toward higher \((P = 0.07)\) lymphocyte counts in calves fed 822 IU of vitamin E/d and/or 16.6 g of vitamin C/d. The highest counts occurred with calves fed a diet high in vitamin E or vitamin C alone.

**Effects of Intranasal Delivery of Bovine Herpesvirus 1.** Regardless of diet, virus challenge increased \((14.62 \text{ vs. } 14.22 \mu\text{mol/L}; P = 0.048; \text{SE} = 0.15)\) serum ceruloplasmin concentration for the entire feeding period. There was also a time \times virus challenge interaction on mean serum ceruloplasmin concentration (Figure 1). Calves that were not challenged with BHV 1 had a lower \((P = 0.04)\) mean serum ceruloplasmin concentration at the 6-wk sampling.

Inoculation of calves with BHV 1 was associated with lower WCC \((9.05 \text{ vs. } 10.44 \times 10^9/\text{L}; P = 0.007; \text{SEM} = 0.35)\) and lymphocyte count \((4.61 \text{ vs. } 5.76 \times 10^9/\text{L}; P < 0.001; \text{SEM} = 0.18)\) at wk 5, but did not have an effect \((P = 0.46)\) on the plasma concentration of malondialdehyde. Dry matter intake, ADG, and G:F were examined for the last 2 wk of the experiment, the period subsequent to virus challenge of half the calves, and a period of greater growth rates. Virus-challenged calves had lower \((P = 0.03)\) DMI from 48 to 96 h after virus challenge \((4.97 \text{ vs. } 5.52 \pm 0.17 \text{ kg/d})\) and over the last 2 wk \((70.59 \text{ vs. } 77.15 \text{ kg/animal}; P = 0.04, \text{SEM} = 2.22)\) of the feeding period.

**Effects of Ruminally Protected Vitamin C and Dietary Vitamin E on Calves Challenged With Bovine Herpesvi-**

Table 4. Effects of supplemental vitamins E and C on gain:feed, ceruloplasmin concentration, and lymphocyte count^a^

<table>
<thead>
<tr>
<th>Vitamin supplementation</th>
<th>Low E</th>
<th>High E</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No C</td>
<td>+ C</td>
<td></td>
</tr>
<tr>
<td>G:F</td>
<td>0.26</td>
<td>0.24</td>
<td>0.23</td>
</tr>
<tr>
<td>Ceruloplasmin concentration, (\mu\text{mol/L})</td>
<td>14.78</td>
<td>13.98</td>
<td>14.25</td>
</tr>
<tr>
<td>Lymphocyte count, (\times 10^9/\text{L})</td>
<td>5.386</td>
<td>5.852</td>
<td>5.692</td>
</tr>
</tbody>
</table>

^a^Low E = 71 IU of vitamin E \(^{-1}\)animal \(^{-1}\)d \(^{-1}\); High E = 822 IU of vitamin E \(^{-1}\)animal \(^{-1}\)d \(^{-1}\); No C = no ruminally protected vitamin C; and + C = 16.6 g of ascorbyl-2-polyphosphate \(^{-1}\)animal \(^{-1}\)d \(^{-1}\).

^b^Data are means of 12 calves.

^c^Vitamin E \(\times\) ruminally protected vitamin C interaction, \(P = 0.05\).

^d^Vitamin E \(\times\) ruminally protected vitamin C interaction, \(P = 0.01\).

^e^Vitamin E \(\times\) ruminally protected vitamin C interaction, \(P = 0.07\).
Figure 1. Mean serum ceruloplasmin concentrations ± SEM in cattle challenged with bovine herpesvirus 1 nasal inoculation (BHV 1) immediately after the 4-wk sampling compared with cattle not inoculated (BHV 1 −); time × virus challenge interaction; $P = 0.04$; SEM = 0.27.) Data are means of 24 calves.

**rhus 1.** The interaction between feeding ruminally protected vitamin C, increasing dietary vitamin E, and challenge of calves with BHV 1 on mean plasma retinol concentration approached significance for the wk 5 sampling ($P = 0.06$). There was a trend toward an increase in mean plasma retinol concentration in response to virus challenge in calves fed increased vitamin E and no ruminally protected vitamin C (Table 5). There were no effects of virus challenge or supplemental vitamin E and/or C on glutathione peroxidase ($P = 0.35$), serum ceruloplasmin ($P = 0.95$), or the oxidation reaction product malondialdehyde ($P = 0.16$).

**Effects of Dietary Vitamin E on Calves Challenged with Bovine Herpesvirus 1.** There was an interaction ($P = 0.03$) between increased dietary vitamin E and virus challenge with respect to serum ceruloplasmin concentration (Table 6). Higher dietary vitamin E was associated with lower serum ceruloplasmin concentrations in calves challenged with BHV 1 than in those not challenged. The interaction between increasing dietary vitamin E and virus challenge approached significance with respect to TPP ($P = 0.06$; Table 6). A higher intake of vitamin E was associated with a greater TPP concentration in response to challenge with BHV 1. Conversely, TPP values were similar regardless of dietary vitamin E intake in cattle not challenged with BHV 1.

There were no interactions between increasing dietary vitamin E and virus challenge with respect to the plasma concentration of malondialdehyde ($P = 0.76$) or DMI ($P = 0.71$) measured after virus challenge. Furthermore, there were no significant interactions between increasing dietary vitamin E and virus challenge in terms of feedlot performance for the period subsequent to virus challenge ($P = 0.10$ to 0.49).

**Effects of Ruminally Protected Vitamin C on Calves Challenged with Bovine Herpesvirus 1.** There were no significant interactions between the feeding of ruminally protected vitamin C and virus challenge with respect to the blood responses measured at wk 5 after virus challenge ($P = 0.15$ to 0.99). There was no interaction between the feeding of ruminally protected vitamin C and challenge with BHV 1 with respect to mean plasma concentration of malondialdehyde ($P = 0.87$) or DMI ($P = 0.66$) after virus challenge. There were no significant interactions between feeding protected vitamin C and virus challenge in terms of feedlot performance for the period subsequent to virus challenge ($P = 0.54$ to 0.90).

**Discussion**

**Amount of Vitamin E Fed.** The 822 IU·animal$^{-1}$·d$^{-1}$ of vitamin E fed in this experiment is comparable to that fed in several other experiments that found a positive response in immune function, health, or production (Lee et al., 1985; Reddy et al., 1986, 1987a,b; Hill et al., 1990; Pehrson et al., 1991; Hogan et al., 1992; Brzezniak-Slebodzinska et al., 1994). Reddy et al. (1987a,b) estimated that vitamin E intake was 2.4 to 3.4 IU·kg BW$^{-1}$·d$^{-1}$, and Lee et al. (1985) fed 1.8 IU·kg BW$^{-1}$·d$^{-1}$. Other studies have not reported vitamin E intake as a rate based on BW and covered a large range of cattle from calves to cows. The following assumptions were used to calculate vitamin E intakes as rates based on BW for these studies: Holstein calf average BW = 60 kg; Holstein cow average BW = 550 kg; average days on feed for U.S. feedlot cattle = 156 d; ADG for U.S. feedlot cattle = 156 d; ADG for U.S. feedlot cattle = 156 d.
cattle = 1.50 kg/d. Based on these assumptions, the experiments cited found positive responses to vitamin E inclusion rates from 0.58 to 16.7 IU kg\(^{-1}\) BW\(^{-1}\) d\(^{-1}\). Most studies used 3 to 5 IU kg\(^{-1}\) BW\(^{-1}\) d\(^{-1}\) of vitamin E. The 4.65 IU kg\(^{-1}\) BW\(^{-1}\) d\(^{-1}\) of vitamin E fed in this experiment was within the range used in other experiments. The difficulty in comparing experiments illustrates the importance of reporting rates of antioxidant administration on a BW basis rather than amount per animal.

The Effect of Feeding Additional Vitamin E on Blood Concentrations of Antioxidants. In keeping with other studies that found significant responses with similar dose rates of vitamin E, there was a significant increase in plasma \(\alpha\)-tocopherol in calves fed additional vitamin E in the present experiment. Greater plasma concentrations of \(\alpha\)-tocopherol may be warranted with cattle with greater exposure to stress, and vitamin E adequacy should be assessed in relation to selenium, due to the sparing effect exerted by glutathione peroxidase (CSIRO, 1990). There were no significant health problems in either the high vitamin E or low vitamin E groups, suggesting little benefit from increased supplementation when cattle were not stressed. These considerations illustrate the difficulty of arriving at recommendations for dietary and blood antioxidant concentrations regardless of prevailing conditions and emphasize the importance of assessing animal performance and immunocompetence in determining recommended intakes. Antioxidant supply and turnover may be more important to maintaining animal health than plasma concentrations.

Although the high vitamin E diet was associated with greater plasma \(\beta\)-carotene concentration, it had no effect on plasma retinol concentration. \(\beta\)-Carotene is an extremely efficient quencher of singlet oxygen (MacLind and Bendich, 1987; Di Mascio et al., 1991; Chew, 1995) and acts as a chain-breaking antioxidant in membranes (Chew, 1995). Because \(\beta\)-carotene shares this function with \(\alpha\)-tocopherol, feeding a diet high in vitamin E and the resultant increase in plasma concentration of \(\alpha\)-tocopherol may be related to greater concentrations of \(\beta\)-carotene through a sparing effect. Chew (1995) also cited a report by Palozza and Krinsky (1992) that the two antioxidants acted synergistically in rat liver microsomal membranes to inhibit lipid peroxidation. The increased plasma \(\beta\)-carotene associated with increased vitamin E intake contrasts with the findings of Yang et al. (2002), in which an increased intake of \(\alpha\)-tocopherol in pasture-fed cattle was associated with a decrease in plasma \(\beta\)-carotene concentration, but in grain-fed cattle, no effect was observed. Those authors attributed the decrease in plasma \(\beta\)-carotene concentration in the pasture-fed cattle to competition with vitamin E for sites in micelles for intestinal absorption and transport. In the present study, competition for transport sites between fat soluble vitamins would have been very unlikely, for the diet had 3.7\% ruminaly degradable fat. Relative movements in plasma concentrations of \(\alpha\)-tocopherol and \(\beta\)-carotene are therefore more likely to reflect interactions at the cellular level rather than differences in absorption.

The Effect of Ruminally Protected Vitamin C on Blood Concentrations of Antioxidants. Despite a mean intake in the vitamin C fed calves of 0.091 g kg\(^{-1}\) BW\(^{-1}\) d\(^{-1}\), there was no significant increase \((P = 0.59)\) in plasma ascorbate concentration (1.91 vs. 1.79 mg/L; SEM = 0.16). Dubeski and Owens (1993) reported much lower plasma ascorbate concentrations of 0.054 to 0.212 mg/L, 0.022 to 0.076 mg/L, and 0.034 to 0.083 mg/L in suckling calves, transport-stressed calves, and feedlot steers, respectively. The plasma ascorbate concentrations reported in this experiment, however, are comparable to those measured by MacLeod et al. (1996) in Holstein heifers fed 20 g of ascorbyl-2-polyphosphate\(^{-1}\) d\(^{-1}\) and in controls (4.56 ± 0.58 vs. 3.58 ± 0.64 mg/L; \(P < 0.05\)). Assuming a heifer BW of 380 kg in the MacLeod et al. (1996) study, the rate of ascorbyl-2-polyphosphate intake would have been approximately 0.05 g kg\(^{-1}\) BW\(^{-1}\) d\(^{-1}\). Some of the difference in values between this experiment and that of Dubeski and Owens (1993) may be attributed to differences in methods used to fix the plasma sample immediately after collection and the measurement procedure. A substantially greater volume of lower concentration of metaphosphoric acid was used to fix the plasma sample in the present experiment compared with the procedure of Dubeski and Owens (1993).

Table 6. Effects of supplemental vitamin E on serum ceruloplasmin concentrations and total plasma protein (TPP) in cattle challenged with bovine herpesvirus 1 (BHV 1)\(^a\)

<table>
<thead>
<tr>
<th>Measurement(^b)</th>
<th>No BHV 1</th>
<th>+ BHV 1</th>
<th>No BHV 1</th>
<th>+ BHV 1</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceruloplasmin, (\mu)mol/L(^c)</td>
<td>14.73</td>
<td>14.97</td>
<td>14.98</td>
<td>14.34</td>
<td>0.19</td>
</tr>
<tr>
<td>TPP, g/L(^d)</td>
<td>74.71</td>
<td>73.21</td>
<td>73.79</td>
<td>76.62</td>
<td>1.14</td>
</tr>
</tbody>
</table>

\(^a\)Low E = 71 IU of vitamin E animal\(^{-1}\) d\(^{-1}\); High E = 822 IU of vitamin E animal\(^{-1}\) d\(^{-1}\).

\(^b\)Data are means of 12 calves.

\(^c\)Vitamin E × BHV 1 inoculation interaction, \(P = 0.03\).

\(^d\)Vitamin E × BHV 1 inoculation interaction, \(P = 0.06\).
ski and Owens (1993). In addition, plasma ascorbate was determined in this experiment with a fluorometric assay using a centrifugal analyzer with fluorescence attachment, compared with a standard colorimetric method used by Dubeski and Owens (1993).

Although feeding 0.091 g of ascorbyl-2-polyphosphate·animal−1·d−1 did not increase plasma ascorbate concentrations, it was associated with a significant effect on total SOD concentrations. Perhaps the starch in the wheat-based pelleted diet provided a relatively high supply of intestinal starch, and therefore glucose, to the portal circulation, which might have lessened differences in ascorbate concentrations between calves supplemented or not supplemented with vitamin C. Differences could be more marked under conditions of stress that would lower blood glucose concentration or on diets lower in starch. Further research is warranted with diets with more effective fiber. Although the majority of blood copper SOD occurs in erythrocytes (Paynter and Allen, 1981), whole blood total SOD was measured in this experiment, which prevents an assessment of the relative concentrations of SOD in erythrocytes and plasma. The diet higher in vitamin C was associated with lower mean blood total SOD concentrations. Superoxide dismutase activity is highly correlated with Cu intake in ruminants (Andrewartha and Caple, 1980). The reasons for the apparent modification of this relationship between Cu intake and blood SOD in this experiment are unclear.

The absence of an effect of dietary vitamin C on plasma α-tocopherol concentration contrasts with those from previous studies that demonstrated a role for ascorbate in regeneration of α-tocopherol in vitro (Wilson, 1987; Di Mascio, 1991; Forsyth and Guilford, 1995). Regeneration of vitamin E is mediated by reduction of its oxidized form, the tocopheryl radical, through oxidation of vitamin C. The net result of this reaction is a decrease in vitamin C and maintenance of α-tocopherol concentrations (Niki, 1988). When an adequate plasma α-tocopherol concentration is achieved, the ratio between plasma α-tocopherol concentration and plasma ascorbate concentration may provide some indication of oxidative stress.

Interactions Between Increasing Dietary Vitamin E and Ruminally Protected Vitamin C on Leukocyte and Antioxidant Concentrations. Ceruloplasmin acts in the extracellular fluid to maintain Fe in the ferric form (Chan and Decker, 1994; Forsyth and Guilford, 1995) and acts as a superoxide radical scavenger (Chow, 1988). The role of ceruloplasmin is more critical in the presence of high concentrations of plasma ascorbate because ascorbate can provoke the formation of free radicals in the presence of transition metals (Machlin and Bendich, 1987; Niki, 1988). In this experiment, a diet high in vitamin E alone, or in combination with protected vitamin C, did not have a significant effect on plasma ceruloplasmin concentration. Conversely, the relationship between high vitamin C and low ceruloplasmin might indicate increased use of ceruloplasmin in the presence of high ascorbate concentrations.

The role of vitamin E in B-lymphocyte stimulation is well documented (Tanaka et al., 1979; Peplowski, 1981; Reddy et al., 1987b). Vitamin E promotes B-cell proliferation, with the effect being most marked in the primary immune response (Tizard, 1996). In some cases, the associated increased antibody production may lead to increased disease resistance. The role of vitamin C in bovine immunocompetence is less clearly defined. Blair and Cummins (1984) found that vitamin C supplementation increased the concentration of blood immunoglobulins in calves. Furthermore, Cummins and Brunner (1989) and Seifi et al. (1996) reported that calves supplemented with vitamin C had a lower incidence of scouring. An increase in antibody concentration is assumed to reflect B-lymphocyte proliferation. The potential of supplemental vitamin C to stimulate lymphocyte production in calves before weaning presumably also applies to adult ruminants. Results of this experiment indicates that lymphocyte responses in cattle may be increased by feeding ruminally protected vitamin C. Considering the trend toward a positive effect on lymphocyte concentrations by both vitamins E and vitamin C, it is unclear why greater lymphocyte concentrations were not measured when the vitamins were fed together in higher concentrations.

Effects of Vitamins E and C on Plasma Malondialdehyde Concentrations. Malondialdehyde is one of many decomposition products of lipid peroxides formed in the tissues. It is the most extensively investigated of these decomposition products because of its reactivity with a range of biological macromolecules and its association with the pathophysiology of a number of disease states (Draper et al., 1988). Nonetheless, difficulties with the malondialdehyde assay resulting in large CV can make interpretation of the significance of malondialdehyde concentrations difficult. In the present experiment, the interassay CV of the malondialdehyde assay was relatively low at 9%. Increased concentrations of α-tocopherol could be expected to decrease the concentration of malondialdehyde due to a decrease in membrane lipid peroxidation; however, malondialdehyde also is a normal intermediate formed during the synthesis of prostaglandins, leukotrienes, and thromboxanes from arachidonic acid (Chow, 1988). This alternative pathway of malondialdehyde formation has frequently been neglected in previous studies and has added to the difficulties of interpreting changes in malondialdehyde concentration.

Feedlot Performance. The results with G:F indicate that positive production responses are more likely when increased concentrations of vitamins E and C are fed in combination rather than individually. The lack of a difference between the G:F of calves fed the combination of additional vitamin E and protected vitamin C and controls might be explained by the high mean G:F achieved in the experiment (Table 3). More marked effects might be expected during periods of greater
growth rate at heavier BW, where differences in performance might be greater. It also is possible that positive effects of dietary treatments with vitamins E and C may be most evident during periods of stress, such as challenge of cattle with naturally occurring infectious diseases. There seems to be no previous research on the effect of vitamin C alone on the feedlot performance of cattle.

Physiological Effects of Challenge with Bovine Herpesvirus 1. Viral challenge of calves was associated with significantly greater concentrations of serum ceruloplasmin, and a significant time interaction showed greater ceruloplasmin concentrations in virus-challenged calves at the final sampling at 6 wk. Greater serum ceruloplasmin and Cu concentrations were reported by Doyle et al. (1999) in feedlot cattle diagnosed with bacterial and/or viral diseases. Ceruloplasmin is an acute-phase reactive protein that scavenges Fe²⁺ and free radicals, and it has histaminase and ferroxidase activity (Gruys et al., 1998). It is critical under conditions of increased oxidative stress, such as that encountered in response to viral respiratory infection and associated phagocytosis and intracellular cytolysis of virus infected cells, that the potential for transition metal catalysis of oxidative reactions be controlled. Ceruloplasmin performs this function in its role as an acute-phase reactive protein and has a minor role as a scavenger of free radicals.

Although WCC for both groups were within the normal range, challenge of cattle with BHV 1 was associated with a significant decrease in WCC, which was due specifically to a significant decrease in the number of circulating lymphocytes. An extensive review of bovine respiratory disease (BRD; Yates, 1982) concluded that when abnormalities of blood cell measurements were found in previous experiments, leukopenia was reported most frequently. Although the review noted experiments where leukocytosis was found, those experiments used small numbers of animals. Furthermore, Bielefeldt Ohmann and Babiuk (1985) found a decrease in WCC in cattle inoculated with BHV 1 within 24 h of infection, with significant leukopenia on d 3.

Cell counts from the peripheral circulation do not necessarily reflect total numbers in the animal’s body and may reflect a redistribution of the cells of interest to lesions and regions of immunocompromise. This may be the case with challenge or natural infection of cattle with BHV 1. Wyler et al. (1989) noted that the characteristic lesions of infectious bovine rhinotracheitis involve infiltration of the submucosa with lymphocytes, macrophages, and plasma cells, which would redistribute lymphocytes from the peripheral circulation to the tissues of the airways affected by the pathogen. In addition, Wyler et al. (1989) and Liggitt (1985) noted that BHV 1 has an immunosuppressive effect that increases susceptibility to secondary bacterial infections resulting in severe pneumonia. Liggitt (1985) noted this immunosuppressive effect was associated specifically with suppression of peripheral blood lymphocyte responses.

Considering the capacity of respiratory disease to generate oxy-radicals (Mills, 1995; Babiuk et al., 1996), and presumably, therefore, to generate oxidation reaction products, it is unclear why exposure of these cattle to BHV 1 did not result in a significant increase in plasma malondialdehyde concentration. Lack of response may be associated with the absence of moderate to severe clinical respiratory disease in the experimental calves, which is in keeping with the observations of Yates (1982) that experimental production of respiratory disease is not possible without massive doses of causative agents and/or extreme manipulation of stressors. Another possible contributing factor is the imprecision of malondialdehyde as a measurement of oxidative challenge. Development of more comprehensive and accurate measurements of oxidative challenge would further our understanding of the role of antioxidants in the management of diseases such as BRD.

Dry matter intake was significantly lower by virus-challenged calves. The occurrence of inappetence or anorexia in cattle suffering from BRD is widely recognized by feedlot veterinarians and managers, and is noted in the literature (Yates, 1982; Wikse, 1985). Yates (1982) more specifically noted inappetence as an early clinical sign of infection of cattle with BHV 1 causing infectious bovine rhinotracheitis. Interferons are thought to be primarily responsible for the inappetence or anorexia that is a feature early in the course of viral respiratory disease (Tizard, 1996).

Effects of Vitamins E and C in Calves Challenged with Bovine Herpesvirus 1. There was a trend toward greater (P = 0.06) plasma retinol concentration in cattle challenged with BHV 1 and fed a high vitamin E diet compared with the other dietary combinations. A sparing effect of vitamin E on retinol and/or its precursors is of potential importance to immunocompetence. This is relevant to the prevention of BRD, considering the well documented role of vitamin A in maintaining the competency of epithelial surfaces (West et al., 1991) and regulation by T cells of immunoglobulin production (Tizard, 1996). The trend toward greater (P = 0.06) TPP in cattle fed a high vitamin E diet and challenged with BHV 1 also may be relevant to the prevention of BRD. As there was no significant interaction between BHV 1 challenge and dietary vitamin E concentration on fibrinogen concentration or the inflammation index plasma protein:fibrinogen, the TPP response seems to have been due to nonfibrinogen proteins. Logically, major contributors to this protein increase in the face of a pathogenic challenge could have been immunoglobulins and antibodies. With calves exposed to virus challenge, increased dietary vitamin E was associated with significantly lower serum ceruloplasmin concentrations. A greater concentration of membrane-bound α-tocopherol might utilize more ascorbate in regeneration of its active form and in turn lead to the consumption of more
ceruloplasmin as an alternative quencher of free radicals in the aqueous phase.

Although there were no significant feedlot performance responses from feeding increased dietary vitamin E and/or feeding ruminally protected vitamin C with calves challenged with BHV 1, significant interactions may have occurred had virus challenge caused more significant effects on performance, other than decreased intake.

**Implications**

These data showed interactions between vitamins E and C, and other antioxidants, and indicate that vitamin C may exert physiological effects without an increase in the plasma concentration of ascorbate. This finding indicates that utilization of antioxidants rather than simple point measurements should be considered in our assessment of their effects, and it also emphasizes the importance of a multifactorial approach to evaluation of the role of antioxidants. Previous conclusions with regard to the effect of bovine herpesvirus 1 exposure on lymphocytes may need to be reexamined. Epidemiological study of the relationship between tissue concentrations of α-tocopherol, ascorbate, β-carotene, retinol, and ceruloplasmin in commercial feedlot cattle exposed to naturally occurring bovine respiratory disease, and the outcomes of health and growth rate, may help to clarify the potential role of antioxidants.

**Literature Cited**


Vitamins E and C in stressed calves


