Effect of dietary lipoic acid on metabolic hormones and acute-phase proteins during challenge with infectious bovine rhinotracheitis virus in cattle

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Objective—To determine the effect of dietary supplemental lipoic acid (LA) on serum concentrations of metabolic hormones and acute-phase proteins of steers challenged with infectious bovine rhinotracheitis virus (IBRV).

Animals—32 steers.

Procedures—Steers were randomly assigned to 4 treatments: negative control (NC; no LA, no IBRV challenge), control (CON; no LA, IBRV challenge), 16 mg of LA/kg of body weight (BW)/d plus IBRV challenge (LA16), and 32 mg of LA/kg of BW/d plus IBRV challenge (LA32). Following a 21-day adaptation period, CON, LA16, and LA32 steers received IBRV (2 mL/nasal trill [day 0]); NC steers received saline (0.9% NaCl) solution. Blood samples, nasal swab specimens, BW, and rectal temperatures were obtained 0, 1, 3, 5, 7, 14, and 21 days after challenge. Serum was analyzed for concentrations of haptoglobin, amyloid-A, leptin, and anti-IBRV antibodies.

Results—Steers fed LA32 began gaining BW by day 7, whereas BW of CON and LA16 steers declined. Serum haptoglobin concentration of LA32 steers was lower than that of CON and LA16 steers on day 7. Serum neutralization titers for 30 of 32 steers were negative for anti-IBRV antibodies before challenge; however, all steers (including NCs) had antibodies on day 21.

Conclusions and Clinical Relevance—Results suggested that LA supplementation augmented certain aspects of the immune response; LA32 steers had a more rapid recovery from IBRV viral challenge than did others. (Am J Vet Res 2006;67:1192–1198)

Bovine respiratory disease is responsible for >75% of feedlot morbidity and 50% of feedlot deaths, making it the most economically important disease that affects US growing and finishing cattle.1 Gardner et al2 reported that cattle with BRD gained a mean of 0.04 kg/d less than healthy cattle, resulting in carcasses that were 13.5 kg lighter. Carcasses of BRD-affected cattle were worth $46.91 less than carcasses of healthy cattle. Carcasses of BRD-affected cattle were 13.5 kg lighter. Carcasses of BRD-affected cattle were 13.5 kg lighter.

Presented as an abstract at the 2004 Annual Meeting of the American Society of Animal Science, St Louis. Address correspondence to Dr. Berg.
be used as replacements in the event that enrolled steers were judged unfit to remain in the trial. The acclimation period started on day –21. Groups consisted of NC, CON, LA16, and LA32 steers.

Supplemental amounts of LA were chosen on the basis of recent publications in human medicine. Preliminary research has revealed that dry, powdered LA administered directly into the rumen of steers caused a detectable spike in serum LA concentration 30 minutes later. The highest serum concentration was observed 1 hour after administration. Ruminal administration in steers with the same amount of LA in paraffin wax was also followed by increased serum LA concentration; however, the peak in serum LA concentration occurred later and was of lower magnitude, compared with unprotected LA.

Lipoic acid was incorporated into a 90% corn flour extruded pellet (10% LA) to ensure consumption and eliminate loss; CON and NC steers received a 100% corn flour pellet. Lipoic acid pellets and control pellets were created at the University of Missouri Extrusion Laboratory. In a previous research study, the same pellets were fed to steers for 125 days and there was no refusal by any steer to consume the pellets. Steers were allowed a 21-day acclimation period to adjust to diet, LA administration, and environment. During the adaptation period, all 8 replacement steers were randomly incorporated into various groups to replace steers that either became ill or had difficulty adjusting to being individualized. All steers that were removed from the trial because of illness were treated on the basis of the protocol established by the West Texas A&M research feedlot.

After the 21-day adaptation period (day 0), CON, LA16, and LA32 steers received a 4-mL dose of IBRV (2 mL/nostril). Negative control steers received a 4-mL placebo dose of saline (0.9% NaCl) solution (2 mL/nostril). Viral and placebo doses were administered by use of a glass and metal atomizer attached to a compressor-nebulizer set to 30 to 35 psi. The atomizer was connected to the nebulizer via a bleach-type cutoff valve attachment and was positioned on a rolling cart adjacent to a hydraulic chute. Individual steers were restrained securely in the hydraulic chute, and nasal swab specimens were collected to determine prior exposure to IBRV. After collection of nasal swab specimens, the appropriate dose of IBRV or saline solution was placed into the atomizer, which was connected to the nebulizer with the cutoff valve open. The atomizer was inserted into the nasal cavity, and the cutoff valve was closed, resulting in emission of a consistent mist that allowed a dose to be administered to each animal in < 30 seconds. The atomizer was sterilized between administration of viral and placebo doses by flushing with isopropyl alcohol. Prior to viral inoculation on day 0 and subsequently on days 1, 3, 5, 7, 14, and 21, rectal temperature and BW were recorded for each steer. Nasal swab specimens and 2 jugular blood samples (single venipuncture) were also collected from each steer at the same time. Blood samples were allowed to clot for 30 minutes at 21°C and were then refrigerated at 4°C. After 24 hours, chilled blood samples were centrifuged at 3,000×g for 30 minutes (4°C) and serum was separated. Serum was transferred into 15-mL centrifuge tubes for storage and frozen (~70°C) for later analysis.

During the viral challenge at 8AM every morning, the amount of feed remaining in the feed bunk from the prior day's feed allotment was recorded and used to determine the amount of feed delivered that day (this was termed the bunk reading). Bunks were managed so that approximately 100 g of feed remained immediately prior to delivery of the next meal. Steers were fed a common basal diet, and LA was top-dressed after feed delivery (Table 1). As steers became ill, feed delivery was reduced to ensure consumption of the daily allotment of LA-containing pellets. Clinical illness scores were also assigned to each steer at 8AM hours. Scores were based on a 5-point scale (Appendix). The individual who evaluated the bunks and assigned clinical illness scores was unaware of group assignments. Sixteen of the 32 pens were equipped with water meters for daily measurement of water consumption (g steers/group). Waterers were cleaned and meters validated prior to the trial. Water meter readings were recorded daily for each pen at 8AM hours, and water tanks were cleaned weekly, accounting for water lost in the cleaning process.

Serum leptin concentrations were determined via procedures reported by Delavaud et al. A serum amyloid-A assay and haptoglobin assay were used to analyze serum acute-phase proteins. To eliminate errors, each specific assay was conducted on the same day.

Serum neutralization titers were determined by use of serial 3-fold dilutions of serum in cell-culture media and were expressed as the reciprocal of the dilution that fully neutralized infectivity at the 50% end point. The final dilution of serum used in SN assays was 1:512. Approximately 200 tissue culture infectious dose was added to the diluted serum, and this mixture was incubated for 1 hour at 37°C before addition of the cells. Bovine turbinate cells were used for SN assay, and the SN virus strain used was IBRV (Cooper strain). The SN titers were considered positive at dilutions > 1:4.

Virus isolation was performed by inoculation of MDBK cells. Two wells of a 24-well plate (surface area, 2.5 cm2) contained 2 mL of cell culture media containing confluent monolayers of MDBK cells. A 0.2-mL inoculum of frozen cell culture infectious dose was inoculated onto each cell monolayer in triplicate. Following inoculation, tissues culture media were added to each well, and plates were incubated in a CO2 incubator at 37°C. After 2 hours, plates were examined for CPE.

Table 1—Composition (DMI basis) of basal diet of steers challenged-inoculated with IBRV

<table>
<thead>
<tr>
<th>Component</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steam flaked corn</td>
<td>59.30</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>30.00</td>
</tr>
<tr>
<td>Yellow grease</td>
<td>3.00</td>
</tr>
<tr>
<td>Cane molasses</td>
<td>2.70</td>
</tr>
<tr>
<td>Starter supplement</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Nutrient content:
- **Crude protein (%), assayed** 15.00
- **Net energy maintenance (Mcal/kg)** 1.87
- **Net energy gain (Mcal/kg)** 1.26
- **Acid digestible fiber (%)** 13.70

Formulated to provide the following dietary concentrations (DMI basis): 1% K, 0.8% Ca, 0.25% Mg, 0.75% urea, 0.1 mg of Co/kg, 10 mg of Cu/kg, 25 mg of Fe/kg, 0.5 mg of I/kg, 60 mg of Mn/kg, 0.3 mg of Se/kg, 60 mg of Zn/kg, 3.000 U of vitamin A/kg, 15 U of vitamin E/kg, 22 mg of monensin/kg, and 9 mg of tylosin/kg. Cottonseed meal was used as the carrier.
2.0 cm²/well) were inoculated with 0.1 mL of a nasal swab sample and incubated at 37°C in a humidified 5% CO₂ incubator for 2 to 4 hours. Well plates were treated with MDBC cell medium and returned to the incubator. Media were monitored for cytopathic effects for 7 days. To determine the viral titer of individual samples, serial 10-fold dilutions were performed in growth media prior to inoculation of MDBC cells. Fifty-percent end point titers (tissue culture infectious dose50 • mL–1) were calculated.

Statistical analysis—Data were analyzed as a complete random design in time by use of a mixed model. The model accounted for group, day, group X day, and animal within group. Group X day was used as the error term to test whole plot effects. When results of F tests were significant (P < 0.05), group means were compared by use of the method of least significant difference.

Results
There was no group X time interaction (P = 0.40) for clinical illness scores. For the first 3 days after the IBRV challenge, clinical signs of illness for the CON, LA16, and LA32 groups were low (clinical illness score < 1.3; Table 2). Mean score for all 4 groups was 1.00 on day 0. On days 6 and 7, mean scores of CON, LA16, and LA32 steers exceeded 1.5, whereas mean score of NC steers was < 1.4 (P = 0.56). By day 14, scores of all groups returned to a value near 1 (no signs of clinical illness); this condition was maintained to the end of the trial (21 days). These scores indicated that the IBRV challenge technique was successful in causing clinical signs of BRD; moreover, the dose of IBRV resulted in higher mean clinical illness scores at certain time points, compared with the NC group. Clinical signs of BRD developed relatively early, compared with the typical onset of BRD in a commercial feedlot after exposure, and persisted for < 10 days. The greatest increase in clinical illness scores occurred during a 5-day period (days 5 to 9).

Serum neutralization titers for 30 of 32 steers were negative at the end of the 21-day adaptation period (day 0); 1 NC steer and 1 LA16 steer had positive results. All steers had positive results 21 days after viral challenge (NC group included). Twenty-one days after IBRV challenge, geometric mean titers were similar (P = 0.71) for CON, LA16, LA32, and NC steers (24.29, 25.13, 25.13, and 24.50, respectively).

A significant group X time interaction was not observed for rectal temperature. On days 0 and 14, relationship

\[ \text{BW} \text{ (kg)} \]

\[ \text{ΔBW} \text{ (kg)} \]

\[ \text{SE} \]

\[ \text{P-value} \]

\[ \text{ΔΔBW} \text{ (kg)} \]

\[ \text{NA} \]

\[ \text{Within a column, values with different superscripts are significantly (P ≤ 0.05) different.} \]

\[ \text{See Table 2 for remainder of key.} \]
Mean rectal temperatures among groups were similar (Table 2). Mean rectal temperatures of NC steers were significantly lower than those of LA16 and LA32 steers on day 3; mean rectal temperature of CON steers was similar to other groups. By day 5, CON, LA16, and LA32 steers had significantly higher mean rectal temperatures than did NC steers. Mean rectal temperature of LA16 steers was significantly higher than those of LA32 and NC steers on day 7, whereas rectal temperature of CON steers remained similar to the other groups. Rectal temperatures were increased to >39.5°C only on day 5 for all groups challenged with IBRV. This, when considered with clinical illness scores, indicated that clinical signs of BRD in this trial were relatively mild in terms of duration and degree, compared with IBRV infection in commercial feedlots.

There was a group × time interaction (P = 0.02) for BW. No difference among groups in initial BW (day –21; Table 3) was detected. By the day of the IBRV challenge (day 0), LA32 and NC steers had gained significantly more weight than had LA16 steers (54, 54, and 44 kg, respectively). Body weight of CON steers was not significantly different from other groups. One and 3 days after viral challenge, all groups had a similar BW loss, compared with IBRV infection in commercial feedlots. Five days after challenge, CON, LA16, and LA32 steers had lower BW and greater BW loss, compared with NC steers. By day 7, BW and change in BW of LA32 steers were similar to those of NC steers; however, CON and LA16 steers had lower BW and greater BW loss than did LA32 and NC steers. On days 14 and 21, BW of LA16 steers was lower than that of LA32 and NC steers. Body weight of CON, LA32, and NC steers returned to baseline (day 0) by day 14; this was maintained through the end of the trial. Conversely, BW of LA16 steers did not return to baseline, and a net BW loss during the trial was observed.

A group × time interaction (P < 0.001) was observed for DMI. Dry-matter intake (percentage of BW) was similar among groups prior to IBRV challenge (day 0), and there was no readily discernable pattern in DMI on days after viral challenge.
1 and 3 (Table 4). Five and 7 days after challenge, DMI was lower for CON, LA16, and LA32 steers, compared with NC steers. Additionally, DMI of LA32 steers was higher than that of CON and LA16 steers on day 7, which corresponded with the return of BW gain. Dry-matter intake of LA16 steers remained lower than that of NC steers on day 14; however, DMI of CON and LA32 steers was similar to that of the LA16 and NC groups. On day 14, DMI was similar among all groups. As with BW, DMI of LA32 steers returned to baseline sooner, compared with that of CON and LA16 steers.

Water intake (percentage of BW) was similar among groups on days –3, 0, and 5 (Table 4), and no group × time interaction (P = 0.59) was detected. One day after IBRV challenge, water intake of LA16, LA32, and NC steers was similar; however, water intake of CON steers was lower than that of the other groups. Moreover, LA32 steers drank more water than did the LA16 steers, although this difference was not significant. This continued on day 3; water intake by LA32 steers was greater than that of CON, LA16, and NC steers, although this difference was not significant. Water intake by CON steers was lower than that of LA32 steers by day 7; water intake by LA16 and NC steers was similar to that of CON and LA32 steers. On day 14, NC steers had lower water intake than did LA32 steers. Water intake on day 21 was not different among the LA16, LA32, and CON steers.

A group × time interaction was evident for serum haptoglobin and amyloid-A concentrations (P < 0.001). Serum haptoglobin concentration was similar among groups on days 0, 1, 3, 14, and 21 (Table 5). Similarly, there were no group differences in serum amyloid-A on days 0, 1, 3, 14, or 21. Coincident with a general increase in rectal temperature and clinical illness scores (days 5 and 7), group differences were detected in serum acute-phase protein concentrations. On day 5, haptoglobin concentrations of CON steers were highest and NC steers the lowest; mean values of LA16 and LA32 steers were intermediate and similar to both CON and NC. Serum haptoglobin concentrations of LA32 steers was higher than that of NC steers but lower than those of CON and LA16 steers on day 7. Also on day 7, serum amyloid-A concentrations in CON, LA16, and LA32 steers were higher than that in NC steers.

Although there were occasional minor differences in serum leptin concentrations among groups (days 0, 1, 7, 14, and 21), no group × time interaction (P = 0.39) was observed; there were no discernable patterns in these differences, nor did there appear to be any relationship to DMI.

Discussion

Bovine respiratory disease has a multifactorial etiology and develops because of a complex interaction between environmental conditions, host health, and pathogen factors. There are at least 9 viral agents associated with BRD: IBRV, parainfluenza 3 virus, bovine viral diarrhea virus, bovine respiratory syncytial virus, bovine adenovirus, bovine rhinovirus, bovine reovirus, bovine enterovirus, and bovine coronavirus. Of these viral pathogens, only IBRV, parainfluenza 3 virus, bovine viral diarrhea virus, and bovine respiratory syncytial virus are capable of causing acute respiratory disease without interaction with other pathogens. The incubation period for IBRV is typically 2 to 6 days, and recovery generally occurs 4 to 5 days after the onset of signs of illness. The short duration of high clinical illness scores and rectal temperatures in the present study was typical of IBRV infection, but was not typical of the duration of illness seen in cattle with multifactorial BRD infections. Outbreaks of BRD generally occur 10 to 14 days after stress and can commonly last for 2 to 3 weeks. The short duration of clinical signs in the trial reported here can be attributed to allowing the 21-day adaptation period (decreased stress) and inoculation against *M. haemolytica* and *P. multocida*, thus avoiding the multifactorial BRD infection that is typically seen in feedlot cattle. As for the infection of the NC steers, although the steers were individually penned, pen designation was random among the 4 groups; NC steers were penned (individually) but within the same line of pens as the steers that received the viral challenge. Infectious bovine rhinotracheitis virus is a highly contagious virus; thus, the NC steers most likely contracted the virus from nose-to-nose contact with challenge steers.

The transient reduction in BW for all groups was an anticipated result of the viral challenge and provided evidence of its success. We interpreted these results to suggest that supplementation of LA at 32 mg/kg of BW moderated the effects of BRD on weight change by enabling a return of weight gain more rapidly after viral challenge. It was unclear why there appeared to be a dose response associated with LA supplementation and changes in BW. To our knowledge, this was the first trial to investigate supplementation of LA to beef cattle; thus, there are no previous reports of an optimal dose or dose-response curve.

Effects of the IBRV challenge on water intake seemed to be influenced by the amount of LA supplementation when group means were ranked according to DMI and water intake. After viral challenge, NC steers had consistently high DMI and low water intake, compared with other groups. This was unexpected given the direct relationship between DMI and water intake. Water intake and DMI by CON steers were consistently low, relative to other groups. This difference was not significant. Water intake by LA16 and NC steers was similar to that of CON and LA32 steers. On day 14, NC steers had lower water intake than did LA32 steers. Water intake on day 21 was not different among the LA16, LA32, and CON steers.

As with the other indices of IBRV infection measured in this trial, acute-phase protein profiles were interpreted as evidence of a relatively short and mild bout with BRD. This conclusion was also supported by the pattern in DMI in IBRV-challenged groups, in that DMI began to return to prechallenge values after day 7. Presently, there are no data available on the effects of antioxidants on the acute-phase response in cattle. There are, however, data available on the effects of antioxidants on the adaptive immune function of cattle. Nemec et al and Rivera et al both reported that vitamin E supplementation resulted in increased concen-
trations of serum antibodies in virus-challenged steers. Droke and Loerch\(^1\) reported that supplementation of vitamin E to cattle increased serum IgG titers against *M haemolytica* in cattle. Reddy et al\(^2\) reported that calves that received supplemental vitamin E had significantly higher lymphocyte stimulation and increased immunoglobulin M concentration compared with control calves. Although the factor that caused the improvement in immune response associated with LA supplementation in the study reported here was unknown, there is recent research in human medicine that suggests a method of action.\(^3\) Supplementation of LA to HIV-infected patients for 14 days resulted in increased plasma ascorbate concentration, total plasma glutathione concentration, total plasma thiol groups, and total count of T-helper lymphocytes. T-helper-to-suppressor cell ratios were increased, whereas malondialdehyde and 4-hydroxynonenal (lipid peroxidation products) groups were decreased in concentration. These results indicated that LA supplementation changed the redox state of the HIV-infected patients and improved immunologic variables.\(^4\) Additional research indicates that LA can inhibit the replication of the HIV in cultures cells.\(^5\) This downregulation of viral replication may be caused by inhibition of nuclear factor-κB activity imposed by the antioxidant properties of dihydrolipoic acid.\(^6\) The consensus is that supplementation of antioxidants can augment the immune function of growing cattle.

One sign of BRD in feedlot cattle is loss of appetite. The hormone leptin has been implicated in the control of food intake and body composition.\(^7\) Leptin is synthesized and secreted by adipocytes in proportion to the total adipocyte depot and regulates feed intake.\(^8\) As fat stores increase, the amount of circulating leptin increases, causing a hormonal cascade that results in a decreased need for feed. The inverse occurs as fat stores decrease.\(^9\) Leptin has cytokininlike activity in response to inflammation, which may provide a link between immunosuppression and malnutrition because it reduces starvation-induced suppression of cellular immune responses.\(^10\) Leptin has been reported to be secreted in an episodic pattern,\(^11\) and determining this pattern during a viral challenge may be more important than at a single time point. Results of the present study suggested that either fat deposition was not reduced by the viral challenge or the mild nature of the challenge did not induce changes in leptin secretion, production, or both. We speculate that short-term disturbances in appetite may not be reflected in serum leptin concentration when it is measured in blood collected at a single point in time. The viral-induced anorexia that was observed during the viral challenge may have been regulated by other satiety signals such as neuropeptide-Y, urocortin, or ghrelin.\(^12\)\(^13\)

Supplementation of LA at 32 mg/kg appeared to augment certain aspects of immune function of steers challenged with IBRV. Steers in the LA32 group recovered from IBRV challenge more quickly in terms of BW gain and DMI, compared with CON and LA16 steers. The lack of a positive response in LA16 steers suggested that there was a dose-dependent response to LA supplementation. Presently, there are no data available to suggest a recommended amount of supplemental LA for beef cattle. Lack of a discernable pattern in serum leptin concentration, relative to the change in DMI of the infected steers, led us to conclude that leptin concentration may be a poor indicator of short-term disturbances in appetite. The IBRV challenge appeared to reduce DMI to a greater degree than it reduced water intake; however, supplementation with LA at 32 mg/kg appeared to moderate this reduction in DMI.

Various indices of the severity and duration of clinical signs of BRD recorded during this trial (eg, clinical illness score, rectal temperature, and serum acute-phase protein concentrations) were interpreted to suggest that the IBRV challenge technique used in this study was successful in causing clinical signs of BRD. However, most clinical signs of BRD persisted for <10 days, and none appeared to be severe. Further research is needed to evaluate and confirm the role of LA supplementation to feedlot cattle with clinical signs of BRD of greater magnitude. Additional research is needed to determine of the exact role of LA in stimulating the immune response.

### Appendix

Clinical illness scores in cattle challenge-inoculated with IBRV.

<table>
<thead>
<tr>
<th>Clinical illness score</th>
<th>Category</th>
<th>Clinical appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>No abnormal clinical signs.</td>
</tr>
<tr>
<td>2</td>
<td>Slightly ill</td>
<td>Mildly abnormal respiration; dyspnea may be combined with signs of depression, gauntness, and nasal or ocular discharge.</td>
</tr>
<tr>
<td>3</td>
<td>Moderately ill</td>
<td>Moderately abnormal respiration; noticeable dyspnea, gauntness, signs of depression, and nasal or ocular discharge.</td>
</tr>
<tr>
<td>4</td>
<td>Severely ill</td>
<td>Severe abnormal respiration; pronounced dyspnea, gauntness, signs of depression, and nasal or ocular discharge.</td>
</tr>
<tr>
<td>5</td>
<td>Moribund</td>
<td>Recumbent and at point of death; mouth breathing.</td>
</tr>
</tbody>
</table>
References


