Effects of dietary energy and starch concentrations for newly received feedlot calves: II. Acute-phase protein response

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ABSTRACT: Two hundred forty five market-stressed bull and steer calves (205 ± 14 kg) were received in January and September 2001 to determine the response of acute-phase proteins to dietary energy and starch concentrations and to determine whether acute-phase proteins could be used as a diagnostic or prognostic tool for calves affected by bovine respiratory disease. On arrival, calves were randomly assigned to one of two dietary energy levels (0.85 or 1.07 Mcal of NEg/kg of DM) and one of two dietary starch levels (34 or 48% of dietary ME from starch; n = 5 pens/treatment). All calves were weighed, and plasma and serum samples were collected from a subset of animals (n = 6 calves/pen; 30 calves/treatment) on d 0, 7, 14, 28, and 42 of the receiving period. This subset of calves (n = 120) was used for all subsequent analyses. Concentrations of fibrinogen (Fb), haptoglobin (Hp), and serum amyloid-A (SaA) were determined. In addition, samples were collected from the subset of calves when they received medical treatment and 7 d following treatment to measure serum concentrations of Hp and SaA. Serum concentrations of Fb, Hp, and SaA did not differ among dietary treatments, but decreased (P < 0.03) as day of the receiving period increased. Fibrinogen (P < 0.001) and the ratio of Fb:total blood protein were greater (P < 0.003) in calves treated multiple times than in calves never treated or treated once for bovine respiratory disease. In addition, on d 0 and 7, Hp concentration increased (antimicrobial treatment × day interaction, P < 0.03) as the number of antimicrobial treatments increased, and was greater on d 14 and 28 in calves treated multiple times than in calves never treated or treated once. Haptoglobin concentration was greater (P < 0.05) in calves on medical treatment days compared with recovery days (7 d after medical treatment). Although diet seemed to have little effect on acute-phase protein response, these results suggest that haptoglobin may be useful as a diagnostic tool to make management decisions regarding treatment protocols for calves with bovine respiratory disease.

Key Words: Acute-phase Proteins, Bovine Respiratory Disease, Energy, Receiving Calves, Starch


Introduction

Although producers continue to use a number of vaccines and antimicrobials to control the major pathogenic contributors to bovine respiratory disease (BRD), it continues to be the most economically significant disease condition in feedlots (Loneragan et al., 2001). Diagnosis of BRD in cattle is subjective and is often conducted by personnel with limited training. A diagnostic tool to identify infection would be useful to producers and veterinarians by providing a more objective diagnosis of sick animals. The measurement of acute-phase proteins has potential as a diagnostic and prognostic tool in cattle.

Numerous reports on the use of acute-phase protein responses to predict severity or chronicity of cattle diseases have been published (Wittum et al., 1996; Horadagoda et al., 1999; Carter et al., 2002). Alsemgeest et al. (1994) indicated that serum haptoglobin (Hp) and serum amyloid-A (SaA) concentrations, as well as Hp:SaA ratios, were elevated in cattle with inflammatory disease, and that serum Hp concentrations and Hp:SaA values were elevated in cases of chronic rather than acute inflammation. In contrast, Horadagoda et al. (1999) found that Hp, SaA, and α-1-acid glycoprotein...
were greater in cases of acute compared with chronic inflammation. Because those studies examined a large variety of inflammatory diseases, their results may not be comparable to cattle with naturally acquired BRD. The objectives of this experiment were to evaluate serum concentrations of fibrinogen (Fb), Fb:total protein, Hp, and SaA in a large group of feedlot calves receiving various dietary concentrations of energy and starch, and to correlate changes in acute-phase proteins with development of BRD and responses to antimicrobial therapy.

Materials and Methods

All procedures used for this experiment were approved by the Oklahoma State University Animal Care and Use Committee.

Experimental Design

Two hundred forty-five bull and steer calves (initial BW = 205 ± 14 kg) were received at the Willard Sparks Beef Research Center (Stillwater, OK) beginning in January 2001. Two truckloads (n = 167 calves) arrived on January 21, 2001, and an additional truckload (n = 78 calves) arrived on September 15, 2001. Calves were blocked by arrival date into two groups (Groups 2 and 4; Berry et al., 2004). Calves from Group 2 were mingled and randomly assigned to 12 pens (three replicates/treatment; 13 or 14 calves/pen), whereas calves from Group 4 were randomly assigned to eight pens (two replicates/treatment; 9 or 10 calves/pen). Methods pertaining to the processing protocol, dietary treatments, and health data collection were described by Berry et al. (2004). Bulls were left intact until after<br>
measuring the external diameter of each precipitin ring to the nearest 0.01 mm with a supplied plastic scale. Absence of a precipitin ring indicated an Hp concentration of less than 10 μL/mL, which is in the “normal” range of 5.5 to 24.25 for healthy cattle (Saini et al., 1998). The results were plotted on the vertical axis of a semilogarithmic graph, and Hp concentration (μg/mL) was determined from the horizontal axis. A twofold dilution factor was used to develop the reference curve by plotting the ring diameters against two known dilutions. The coefficient of variation for the kit was less than 4% for repeated, identical measurements on the same specimen.

**Serum Amyloid-A.** Serum amyloid-A concentrations (ng/mL) were determined using commercial ELISA kits (Tridelta Phase Range kit, Tridelta Development, Ltd., Wicklow, Ireland). Serum samples were vortexed and added (50 μL diluted 1:500; one sample per well) to each well of a 12 × 8 microtiter strip coated with a monoclonal antibody specific for SaA along with biotinylated anti-SaA monoclonal antibody (50 μL; diluted 1:100 in 1× diluent buffer). Plates were covered and incubated at 37°C for at least 1 h, followed by a complete wash (6×) to remove unbound material. Streptavidin-horseradish peroxygenase diluted 1:4,000 (100 μL) was added to each well and the plate was incubated at room temperature in darkness for 30 min. The plates were washed again (6×) and tapped dry. Substrate (100 μL) was added, and the plates were incubated at room temperature in darkness for an additional 30 min. Stop solution was added, and the plates were read in an automated plate reader (V Max Kinetic Microplate Reader, Molecular Devices, Inc., Sunnyvale, CA) at optical density 490.

**Statistical Analyses.** Acute-phase protein response to dietary treatment was analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) as a randomized complete block with a 2 × 2 factorial treatment structure. Pen was used as the experimental unit. To minimize sex effects, the subsample of calves was assigned within pens such that each pen had the same number of steers and bulls. Main effects were the two levels of energy and two levels of ME contribution from starch. Repeated measures were taken over days, and the model included fixed effects of energy, starch, days, and the appropriate two- and three-way interactions (Littell et al., 1998). The proper covariance structure was determined to be autoregressive 1. In addition, analysis was conducted to determine whether differences in acute-phase protein concentrations could be detected in calves that were never treated for BRD, calves that were treated only once for BRD, or calves that required multiple antimicrobial treatments for BRD. For this analysis, individual animal was used as the experimental unit because the number of times treated was recorded for each animal. The model included fixed effects of number of antimicrobial treatments, day of sampling, and the antimicrobial treatments × day interaction. Least squares means were compared using LSD protected by a significant (P < 0.05) F-value. Regression analysis was conducted using the REG procedure of SAS, with number of times treated (0, 1, >1) as the independent variable and acute-phase protein concentration as a dependent variable. Results are discussed as significant when P < 0.05 and as a tendencies when P > 0.05 and P < 0.10.

**Results and Discussion**

Effects of dietary energy and starch on animal performance and health of newly received feedlot calves were previously reported by Berry et al. (2004). Dietary treatment did not affect morbidity of calves. Of the subset of 120 calves used in the present experiment, 37.5% were never treated, 28.3% received one antimicrobial treatment, 25.0% received two or more antimicrobial treatments, and 9.2% were removed from the experiment due to animal welfare issues (Berry et al., 2004).

**Fibrinogen**

There were no dietary energy × starch or dietary energy × starch × day of sampling interactions for Fb concentration (data not shown). In addition, dietary energy or starch levels did not affect Fb concentration. Fibrinogen concentration was greater (P < 0.05) on d 7 and than on d 14, and was greater on d 14 than on d 28 (Table 1). A similar response was observed when Fb was expressed as a percentage of total blood protein.

**Results of Fb concentrations for calves receiving no treatment (Med0), one (Med1), or more than one (Med-1) antimicrobial treatment are shown in Table 2. Calves receiv-

### Table 1. Effect of day of sampling on acute-phase protein concentrations

<table>
<thead>
<tr>
<th>Item</th>
<th>Day of sampling</th>
<th>SEMa</th>
<th>Probability &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pens</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (Fb), mg/100 mL</td>
<td>581.8c</td>
<td>575.1c</td>
<td>515.7d</td>
</tr>
<tr>
<td>Fb:total protein ratio, mg/100 mg</td>
<td>78.2c</td>
<td>78.9c</td>
<td>71.1d</td>
</tr>
<tr>
<td>Haptoglobin, μg/mL</td>
<td>717.1c</td>
<td>908.9d</td>
<td>343.6a</td>
</tr>
<tr>
<td>Serum amyloid-A, ng/mL</td>
<td>159.1c</td>
<td>139.8c</td>
<td>122.8ed</td>
</tr>
</tbody>
</table>

aStandard error of the least squares means.

cLeast squares means within row with different superscripts differ (P < 0.05).
ing Med0 or Med1 had lower \( (P < 0.05) \) Fb concentrations and lower Fb:total protein compared with calves receiving Med-1. Although these data suggest that Fb concentration and/or Fb:total protein may be useful in identifying calves that will require multiple antimicrobial treatments, regression analysis showed a lack of fit between the number of antimicrobial treatments and Fb concentration \( (r^2 = 0.04; P < 0.04) \) or Fb:total blood protein \( (r^2 = 0.05; P < 0.03) \) on d 0. Similar to our results, Carter et al. (2002) found no effect of number of antimicrobial treatments on serum Fb concentrations, and suggested that serum Fb concentrations might not be useful for predicting illness or responses to antimicrobial treatment in cattle with naturally acquired BRD. Duncan et al. (1994) suggested that Fb:total protein concentration might be more accurate at assessing the acute-phase response than Fb concentration alone. Fibrinogen/total protein concentration was not examined in the study of Carter et al. (2002); however, response of Fb:total protein to BRD was similar to the response of Fb concentration in the present experiment.

**Haptoglobin**

Similar to Fb, no dietary energy × starch × day interactions were detected, and dietary energy or starch levels did not affect serum Hp concentration (data not shown). However, serum Hp concentration was greater \( (P < 0.05) \) on d 7 than on d 0, and Hp concentrations decreased below d-0 concentrations by d 14 (Table 1).

There was an antimicrobial treatment × sampling day interaction \( (P < 0.03) \) for serum Hp concentration (Table 3). Haptoglobin concentration increased \( (P < 0.01) \) as the number of antimicrobial treatments increased on d 0 and 7, whereas on d 14 and 28, Hp concentration was greater for Med-1 compared with Med0 and Med1. Serum Hp concentrations were elevated \( (P < 0.05) \) on d 7 for calves never treated and returned to d 0 levels by d 14. Calves treated for BRD had elevated Hp concentrations on d 0 and 7, and concentrations fell below d 0 concentrations by d 14. These data suggest that correlations could be developed to determine if calves should receive therapeutic treatment at processing in order to maximize the effects of BRD treatment and minimize medical costs. Regression analysis showed that the number of antimicrobial treatments was positively related with Hp concentration \( (r^2 = 0.36; P < 0.001) \) on d 0, and could be predicted as:

\[
\text{Antimicrobial treatments} = 0.452 (\pm 0.085) \\
+ 0.0006 (\pm 0.0001) \times [\text{Hp}, \mu g/mL]
\]

Similarly, from serum harvested on d 7, the number of medical treatments required could be predicted as:

\[
\text{Antimicrobial treatments} = 0.514 (\pm 0.120) \\
+ 0.0005 (\pm 0.0001) \times [\text{Hp}, \mu g/mL] \\
\quad (r_2 = 0.17; P < 0.001).
\]

Carter et al. (2002) found that serum Hp concentrations were greater in calves that were treated more than once for BRD than in calves not treated or treated only once, similar to the present results. In addition, significant correlations between Hp concentration and number of treatments were observed on d 0 \( (r = 0.35) \) and d 7 \( (r = 0.20) \).

Serum Hp concentrations have been previously described in sick and healthy cattle. Similar to the present experiment, Godson et al. (1996) found that serum Hp concentrations corresponded to the severity of respiratory disease in calves experimentally inoculated with bovine herpesvirus-1 and *Mannheimia haemolytica*. Young et al. (1996) measured serum Hp concentrations

Table 2. Number of antimicrobial treatments and acute-phase protein concentrations

<table>
<thead>
<tr>
<th>Item</th>
<th>No. of observations</th>
<th>Med0</th>
<th>Med1</th>
<th>Med&gt;1</th>
<th>SEM</th>
<th>Probability &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of observations</td>
<td>177</td>
<td>136</td>
<td>123</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (Fb), mg/100 mL</td>
<td>483.8</td>
<td>500.4</td>
<td>630.9</td>
<td>23.9</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Fb:total protein ratio, mg/100 mg</td>
<td>66.3</td>
<td>69.3</td>
<td>84.3</td>
<td>3.4</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Serum amyloid-A, ng/mL</td>
<td>123.8</td>
<td>132.9</td>
<td>150.2</td>
<td>35.4</td>
<td>0.63</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \)Med0 = no antibiotic treatment; Med1 = exactly one antibiotic treatment; and Med>1 = more than one antibiotic treatment.

\( ^b \)Standard error of the least squares means.

\( ^c,d \)Means in same row with different superscript differ \( (P < 0.05) \).

\( ^* \)Means in columns within acute-phase protein with different superscripts differ \( (P < 0.05) \).

Table 3. Number of antimicrobial treatments and day of sampling on serum haptoglobin (µg/mL) concentrations

<table>
<thead>
<tr>
<th>Item</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Med0( ^b )</td>
<td>282.6</td>
<td>620.1</td>
<td>120.5</td>
<td>256.1</td>
</tr>
<tr>
<td>Med1( ^b )</td>
<td>724.6</td>
<td>918.7</td>
<td>301.9</td>
<td>193.1</td>
</tr>
<tr>
<td>Med1( ^b )</td>
<td>1187.9</td>
<td>1140.6</td>
<td>603.4</td>
<td>627.9</td>
</tr>
</tbody>
</table>

\( ^a \)Standard error of the least squares means = 164.1. There was a med × day interaction \( (P < 0.03) \).

\( ^b \)Med0 = no antimicrobial treatment; Med1 = exactly one antimicrobial treatment; and Med>1 = more than one antimicrobial treatment.

\( ^c,d \)Means in same row with different superscript differ \( (P < 0.05) \).

\( ^* \)Means in columns within acute-phase protein with different superscripts differ \( (P < 0.05) \).
Table 4. Concentrations of acute-phase proteins when cattle were diagnosed as morbid and determined to be recovered

<table>
<thead>
<tr>
<th>Item</th>
<th>Antimicrobial treatment</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haptoglobin, µg/mL£</td>
<td>1549.3 ± 66.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>688.2 ± 66.8</td>
</tr>
<tr>
<td>LELS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1254.4 ± 70.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>510.3 ± 71.2</td>
</tr>
<tr>
<td>LEHS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1540.7 ± 78.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>686.9 ± 79.3</td>
</tr>
<tr>
<td>HELS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1401.7 ± 69.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>557.1 ± 70.2</td>
</tr>
<tr>
<td>Serum amyloid-A, ng/mL</td>
<td>216.0 ± 29.6</td>
<td>91.2 ± 31.0</td>
</tr>
<tr>
<td>LELS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155.1 ± 29.6</td>
<td>128.6 ± 29.6</td>
</tr>
<tr>
<td>LEHS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>215.7 ± 49.0</td>
<td>186.9 ± 46.2</td>
</tr>
<tr>
<td>HELS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>178.8 ± 28.3</td>
<td>106.4 ± 28.3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Blood sample collected before antibiotic treatment.
<sup>b</sup>Recovery effect (<i>P</i> < 0.05).
<sup>c</sup>LELS = low energy, low starch; LEHS = low energy, high starch; HELS = high energy, low starch; HEHS = high energy, high starch.
<sup>e</sup>Means in same column within an item with different superscript differ (<i>P</i> < 0.05).

Acute-phase protein concentrations generally did not differ among dietary treatments; however, the acute-phase proteins varied in response to morbidity in calves. Serum haptoglobin concentration increased as the number of antimicrobial treatments increased and could potentially be used to predict the number of treatments a calf would require. Fibrinogen and Fb:total protein concentrations also were greater in morbid calves, but the relationship between Fb concentration on d 0 and subsequent antimicrobial treatments was low. Our data suggest that haptoglobin concentration may be useful for producers and veterinarians to make health management decisions based on objective evaluations.

Implications

Acute-phase protein concentrations generally did not differ among dietary treatments; however, the acute-phase proteins varied in response to morbidity in calves. Serum haptoglobin concentration increased as the number of antimicrobial treatments increased and could potentially be used to predict the number of treatments a calf would require. Fibrinogen and Fb:total protein concentrations also were greater in morbid calves, but the relationship between Fb concentration on d 0 and subsequent antimicrobial treatments was low. Our data suggest that haptoglobin concentration may be useful for producers and veterinarians to make health management decisions based on objective evaluations.

Literature Cited


