Backgrounding and finishing diets are associated with inflammatory responses in feedlot steers

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ABSTRACT: The objective of this investigation was to study the effects of feeding backgrounding and finishing diets on selected acute phase proteins in the plasma of feedlot steers. Two groups of 12 steers each, at the backgrounding and finishing stages, were offered either a backgrounding (45% barley grain-based concentrate and 55% barley silage on a DM basis) or a finishing (91% barley grain-based concentrate and 9% barley silage) diet for 12 and 15 wk, respectively. Steers at the backgrounding and finishing stages had initial BW of approximately 250 and 380 kg, respectively, at the beginning of the experiment. Blood samples were obtained from a jugular vein at 3-wk intervals during the experimental period beginning at wk 3 or 0 for the backgrounding and finishing periods, respectively. Plasma samples were analyzed for serum amyloid A (SAA), lipopolysaccharide-binding protein (LBP), haptoglobin, and α1-acid glycoprotein. Steers fed the finishing diet showed peak plasma SAA, LBP, and haptoglobin within 3 wk from the initiation of the diet (20, 23, and 1,940 µg/mL for SAA, LBP, and haptoglobin, respectively). Although plasma α1-acid glycoprotein reached a peak concentration (449 µg/mL) at the beginning of the finishing phase, no diet effect was obtained for this variable. Steers fed the backgrounding diet showed less variation in the concentrations of plasma acute phase proteins measured; plasma haptoglobin reached a peak concentration (1,720 µg/mL) 9 wk after the beginning of this diet. In conclusion, feeding feedlot steers the backgrounding or finishing diet was associated with increased peak concentrations of acute phase proteins in the plasma. More research is warranted to elucidate the mechanisms behind the inflammatory responses observed in feedlot steers and their implications for health issues and the production efficiency of feedlot operations.

Key words: acute phase protein, barley grain, cattle, feedlot

INTRODUCTION

It is a common practice to feed growing beef cattle large proportions of grain to accelerate BW gain during the backgrounding and finishing periods. However, feeding feedlot cattle diets rich in rapidly fermentable carbohydrates results in the decline of rumen pH to acidotic values, major changes in ruminal microbial ecosystems, and the accumulation of large amounts of endotoxin in the rumen fluid (Nagaraja et al., 1978; Andersen et al., 1994; Diez-Gonzalez et al., 1998; Emmanuel et al., 2008). Grain diets also are associated with a greater incidence of several diseases, such as subacute or acute ruminal acidosis, bloat, chronic rumenitis, laminitis, polioencephalomalacia, sudden death syndrome, and liver abscesses (Nagaraja and Chennappa, 1998; Nagaraja and Titgemeyer, 2007). Such diseases are associated with suboptimal performance of growing cattle, with a significant impact on the profitability of feedlot industry.

Many studies have described how rapidly available starch results in many of the aforementioned diseases. It is possible that situations related to prolonged acidotic rumen pH, inflammation or degenerative processes of ruminal mucosa, rumenitis, and parakeratosis as well as the presence of endotoxin may render the rumen epithelium susceptible to injury, resulting in translocation

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of endotoxin into the bloodstream (Kleen et al., 2003; Emmanuel et al., 2008). Inflammation or the presence of endotoxin in the bloodstream is associated with a general, nonspecific immune response known as the acute phase response (Werling et al., 1996). Among the known positive acute phase proteins, serum amyloid A (SAA) and lipopolysaccharide (LPS)-binding protein (LBP) participate directly in the detoxification and removal of endotoxin (Gallay et al., 1994; Cabana et al., 1999). Haptoglobin scavenges hemoglobin to prevent iron utilization by bacteria (Wassell, 2000), whereas α1-acid glycoprotein (α1-AGP) has several functions that are related to easing the inflammatory response (Libert et al., 1994). In this study, we aimed to test the hypothesis that feeding high-grain diets to steers during the backgrounding and finishing stages is associated with activation of an acute phase response, reflected by modulation of plasma concentrations of SAA, LBP, haptoglobin, and α1-AGP.

MATERIALS AND METHODS

The experiment was conducted in accordance with the guidelines of the Canadian Council on Animal Care (1993) and was approved by the institutional Animal Care Committee.

Animals and Diets

A backgrounding and finishing study with 312 beef steer calves purchased from a single source was conducted at the Lethbridge Research Center of Agriculture and Agri-Food Canada. A subset of 12 beef steers was selected for the backgrounding phase, with an initial BW of 248 ± 26 kg (mean ± SD) and a final BW of 323 ± 24 kg. Twelve other steers were selected immediately after the backgrounding phase to be used in the finishing phase, with an initial BW of 382 ± 36 kg and a final BW of 563 ± 62 kg. The DM of steers during the backgrounding phase was 6.24 ± 0.19 kg of DM/d and during the finishing phase was 11.86 ± 1.22 kg of DM/d (mean ± SD).

Diets for backgrounding and finishing beef cattle were based on barley grain and barley silage and were representative of standard feedlot diets used in commercial production systems in western Canada. The ingredients and nutrient composition of both diets are shown in Table 1. The backgrounding diet contained 45% barley grain-based concentrate and 55% silage (DM basis) and was fed for 12 wk, with the first 3 wk used for diet adaptation. Steers fed the finishing diet were adapted to the diet by incrementally increasing the amount of grain fed over a 28-d period, and the measurement period lasted 15 wk. As shown in Table 1, the feedlot finishing diet contained 9% silage and 91% barley grain-based concentrate. The diets contained approximately 12% CP and were formulated using NRC (1996) recommendations to meet or exceed the CP, effective fiber, mineral, and vitamin requirements. The amount of vitamin E (RRR-α-tocopheryl acetate) was such that cattle received 1,000,000 IU before slaughter, as required by the abattoir. All diets contained the ionophore monensin (Elanco, Division of Eli Lilly Canada Inc., Guelph, Ontario, Canada) at concentrations in accordance with the current North American industry standards (22 mg/kg for the backgrounding diet and 33 mg/kg for the finishing diet). The diet was fed as a total mixed ration and was offered once per day at approximately 0800 h in an amount to permit ad libitum consumption (minimum of 5% refusal). Water was freely available throughout the experiment.

Blood Collection and Analyses

Four or 6 blood samples were collected from a jugular vein every 3 wk (wk 3, 6, 9, and 12 during backgrounding and wk 0, 3, 6, 9, 12, and 15 for finishing) at 0800 h shortly before the morning feeding. Blood was collected into 10-mL Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing 143 USP units of sodium heparin (spray-coated) as an anticoagulant. Blood tubes were stored in ice and centrifuged within 20 min at 3,000 × g and 4°C for 20 min to separate plasma. Plasma samples were stored at −20°C until analysis. Concentrations of SAA in plasma were determined by commercially available ELISA kits (Tridelta Development Ltd., Greystones Co., Wicklow, Ireland) according to the instructions of the manufacturer. The monoclonal antibodies and the ELISA were originally described by McDonald et al. (1991). All samples including the standards were tested in duplicate. The inter- and intraassay CV for the SAA analysis were less than 10%. Samples were initially diluted 1:500. Optical density values were read on a microplate spectrophotometer (model Spectra Max 190, Molecular Devices Corporation, Sunnyvale, CA) at 450 nm. According to the manufacturer, the detection limit of the assay was 0.3 µg/mL.

Concentrations of LBP in plasma were determined with a commercially available LBP ELISA kit that cross-reacts with bovine LBP (Cell Sciences Inc., Norwood, MA; Emmanuel et al., 2007). Plasma samples were initially diluted 1:1,500, and samples with optical density values less than the range of the standard curve were diluted to 1:1,200 and reassayed according to the instructions of the manufacturer. The inter- and intraassay CV for LBP analysis were less than 10%. The optical density at 450 nm was measured on a microplate spectrophotometer (model Spectra Max 190, Molecular Devices Corporation). The concentration of LBP was calculated from a standard curve of known amounts of human LBP.

Concentrations of haptoglobin in plasma were determined by ELISA kits (Tridelta Development Ltd., Greystones Co.) as described by Godson et al. (1996) by using a pool of bovine serum (provided by the manufacturer) as the standard. All samples including the standards were tested in duplicate. The inter- and in-
tra assay CV for the analysis of haptoglobin were less than 10%. Optical density values were read with a microplate spectrophotometer (model Spectra Max 190, Molecular Devices Corporation) at 630 nm.

Concentrations of α1-AGP in plasma were measured with radial immunodiffusion assay plates (Tridelta Development Ltd., Greystones Co.). Single radial immunodiffusion assays were prepared to measure plasma concentrations of AGP. Standards and samples were applied to wells in 5.0-µL volumes. Plates were placed in humidified chambers at 37°C and allowed to incubate for 24 h before reading the test results. For the standards, a plot of the diameter squared on the y-axis and the concentration of the antigen on the x-axis gave a linear function as described previously by Mancini et al. (1965). On the basis of this linear function, sample concentrations were calculated.

### Statistical Analyses

All data obtained were subject to ANOVA by using PROC MIXED (SAS Institute Inc., Cary, NC). Steer was the experimental unit and the measurements at different times on the same steer were considered as repeated measures in the model (Wang and Goonewardene, 2004). The model used is shown below:

\[ Y_{ijkl} = \mu + D_i + T_{j(i)} + \text{steer}_{k(i)} + e_{ijkl} \]

where \( \mu \) is the overall mean, \( D_i \) is the fixed effect of dietary treatment \( i \) (\( i = 2 \)), \( T_{j(i)} \) is the fixed effect of blood collection time \( j \) (\( j = 4 \) to 6) nested within dietary treatment \( i \), \( \text{steer}_{k(i)} \) is the random effect of steer \( k \) (\( k = 12 \)) nested within dietary treatment \( i \), and \( e_{ijkl} \) is the residual error.

A Bayesian fit criterion was used to determine the best variance-covariance structure for the repeated measures analyses and, based on a decreased Bayesian fit criterion, an autoregressive [AR(1)] structure provided the best fit to the data. Least squares means and the corresponding SEM were computed. Significance was declared at \( P \leq 0.05 \), whereas a tendency was considered up to \( 0.05 < P \leq 0.10 \).

### RESULTS AND DISCUSSION

An increasing body of evidence indicates that feeding beef cattle diets rich in grain induces a general nonspecific inflammatory response (Berry et al., 2004; Jafari et al., 2006). Moreover, the amount of grain in the diet is associated with multiple diseases, such as subacute or acute ruminal acidosis, bloat, chronic rumenitis, laminitis, polioencephalomalacia, sudden death syndrome, fatty liver, and liver abscesses (Nagaraja and Chengappa 1998; Ametaj et al., 2005; Nagaraja and Titgemeyer, 2007). The precise mechanism by which diets rich in rapidly fermentable carbohydrates initiate multiple inflammatory conditions is not understood at present. In this study, our main goal was to investigate the role of feeding 2 different amounts of barley grain during 12 wk of backgrounding and 15 wk of finishing (45 and 91% of the ration DM, respectively) on selected acute phase proteins in feedlot cattle. Acute phase proteins, including SAA, LBP, haptoglobin, and α1-AGP, are part of the host general nonspecific immune responses to counteract inflammatory conditions and are known markers of the health status of the animals (Petersen et al., 2004).

Results of this study clearly demonstrated that plasma concentration of SAA was greater in the group of

### Table 1. Experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Backgrounding</th>
<th>Finishing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, % of DM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley silage</td>
<td>55.11</td>
<td>9.01</td>
</tr>
<tr>
<td>Barley grain, coarse dry-rolled</td>
<td>38.60</td>
<td>84.47</td>
</tr>
<tr>
<td>Barley grain, ground</td>
<td>3.50</td>
<td>3.70</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.40</td>
<td>1.50</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.70</td>
<td>0.65</td>
</tr>
<tr>
<td>Salt</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Mineral and vitamin premix(^1)</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>Canola oil</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>Molasses</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Monensin,(^2) 200 g/kg</td>
<td>0.011</td>
<td>0.017</td>
</tr>
<tr>
<td>Vitamin E, 500,000 IU/kg</td>
<td>—</td>
<td>0.006</td>
</tr>
</tbody>
</table>

**Nutrient composition, % of DM**

<table>
<thead>
<tr>
<th>Item</th>
<th>Backgrounding</th>
<th>Finishing</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>53.9</td>
<td>81.4</td>
</tr>
<tr>
<td>OM</td>
<td>90.8</td>
<td>95.0</td>
</tr>
<tr>
<td>CP</td>
<td>12.0</td>
<td>12.6</td>
</tr>
<tr>
<td>NDF</td>
<td>48.2</td>
<td>20.3</td>
</tr>
<tr>
<td>ADF</td>
<td>30.8</td>
<td>7.3</td>
</tr>
</tbody>
</table>

\(^1\) Contained 16% calcium carbonate, 102 g/kg of Zn, 47 g/kg of Mn, 26 g/kg of Cu, 1,140 mg/kg of I, 500 mg/kg of Se, 340 mg/kg of Co, 17,167,380 IU/kg of vitamin A, 858,370 IU/kg of vitamin D, and 23,605 IU of vitamin E.

\(^2\) Rumensin Premix (Elanco, Division of Eli Lilly Canada Inc., Guelph, Ontario, Canada).
steers fed the finishing diet \( (P < 0.001; \text{Figure 1}) \). Interestingly, the peak concentration of SAA was reached 3 wk after the beginning of the experiment \( (P < 0.05) \). Although the mechanisms by which feeding a high-grain diet stimulates the release of SAA in plasma are not clear, it is speculated that rumen endotoxin might play a role. In a recent report by Emmanuel et al. (2008), it was shown that feeding dairy cows increasing proportions of barley grain at 0, 15, 30, and 45\% of the ration DM was associated with a 14-fold increase in the amount of endotoxin in the rumen fluid. Moreover, the amount of endotoxin in the rumen correlated positively with concentrations of SAA in the plasma. It is not clear whether endotoxin translocates from rumen fluid into the host blood circulation; however, previous research conducted by our team (Emmanuel et al., 2007) demonstrated that LPS from \textit{Escherichia coli} B:055 was able to permeate both rumen and colon tissues when present on the mucosal side and at concentrations similar to those reported in the rumen fluid of cattle fed high-grain diets \( \sim 500 \mu \text{g/mL}; \text{Nagaraja et al., 1978} \). Recently, endotoxin was reported to stimulate the release of proinflammatory cytokines such as tumor necrosis factor-\( \alpha \), IL-1, or IL-6 by liver macrophages (Jacobsen et al., 2004). The proinflammatory cytokines are known to stimulate the release of SAA from hepatocytes (Hagihara et al., 2004). Serum amyloid A is involved in binding and neutralizing endotoxin as well as in removing endotoxin from blood circulation through hepatocytes (Cabanà et al., 1999).

As shown in Figure 1, plasma SAA responded in a biphasic fashion to the finishing diet, with 2 peak concentrations at 3 and 12 wk after the initiation of the experiment. The first peak, at 3 wk, might be explained by the activation of primary inflammatory conditions in response to endotoxin translocation into the bloodstream when steers were offered the finishing diet. Similarly, Gozho et al. (2006) reported greater peaks of plasma SAA measured at 4 wk after adapting beef steers to a 76\% wheat grain-based diet, and this was associated with increasing concentrations of rumen endotoxin. It is postulated that the second increase of plasma SAA on wk 12 in the present investigation might be related to the development of secondary inflammatory conditions in different organs such as the liver or hoof soft tissues in response to a prolonged exposure to endotoxin (Nagaraja and Chengappa, 1998; Eckersall, 2000).

Lipopolysaccharide-binding protein is another acute phase protein involved in facilitating clearance of endotoxin from blood circulation (Gallay et al., 1994). Results of this study showed that both diet and time affected the amount of LBP in the plasma of steers (Figure 2). Similar to plasma SAA, concentrations of LBP in the plasma of beef steers fed the backgrounding diet were less than those fed the finishing diet \( (P < 0.01) \). In addition, plasma LBP increased at 3 wk after the initiation of the finishing diet \( (P < 0.001) \). Interestingly, plasma SAA and LBP declined to baseline concentrations 6 wk after feeding of the finishing diet, suggesting a common causal agent (i.e., endotoxin) and potential development of a tolerance to endotoxin by 6 wk after the initiation of the diet (Jacobsen et al., 2004).

Haptoglobin is released by hepatocytes during bacterial translocation to scavenge plasma free hemoglobin, which is released during hemolysis of red blood cells, and to prevent utilization of iron contained in the he-
moglobin by bacteria (Wassell, 2000). Iron is an essential trace element required for the growth and multiplication of bacteria. The steers fed the finishing diet, in our experiment, showed an enhanced concentration of haptoglobin at 3 wk after the initiation of the finishing phase (Figure 3). Similar to the peaks obtained for SAA and LBP, plasma haptoglobin also declined to the least concentration 6 wk after the initiation of the finishing diet. Interestingly, there was also a peak of plasma haptoglobin 9 wk after the beginning of the backgrounding diet. The increase in plasma haptoglobin at 9 wk after the initiation of the experiment indicates the presence of an inflammatory condition in steers fed the backgrounding diet. Differences in temporal patterns of plasma haptoglobin vs. SAA and LBP in response to the backgrounding vs. finishing diet can be explained by the different roles these proteins play in the inflammatory processes (Schroedl et al., 2001; Jacobsen et al., 2004) as well as by their different half-lives in the plasma (Gabay and Kushner, 1999).

Haptoglobin is the most widely studied acute phase protein in cattle (Horadagoda et al., 1999), and concentrations greater than 110 µg/mL have been proposed to indicate the presence of an inflammatory condition in beef cattle (Tourlomoussis et al., 2004). In the present study, steers showed greater plasma haptoglobin (>1,500 µg/mL) compared with the proposed inflammatory threshold. Elevated concentrations of haptoglobin in the plasma of steers at wk 9 and 3 of the backgrounding and finishing diets, respectively, suggest translocation of bacteria into the bloodstream of the host. Other research has demonstrated that endotoxin increases bacterial translocation (Deitch et al., 1989). Moreover, it is a well-known observation that feeding steers diets high in grain is associated with an increased incidence of liver abscesses (Nagaraja and Lechtenberg, 2007), and translocation of endotoxin might play a role in the etiology of liver abscesses. Understanding the mechanism behind the increase in haptoglobin concentration 9 wk after feeding of the backgrounding diet as well as its consequences for the general health and production efficiency of growing steers warrants further research.

Data from the present study showed that blood values for α1-AGP were approximately 380 µg/mL during the whole experimental period. There were no differences in plasma concentrations of α1-AGP in relation to the dietary treatments \((P = 0.85\); Figure 4). Interestingly, plasma concentration of α1-AGP tended to be greater at the beginning of the finishing phase and decreased to baseline concentrations on wk 6 of the experiment \((P < 0.10\). Alpha1-acid glycoprotein is another acute phase protein synthesized in the liver, and it has been reported to increase during acute or chronic inflammatory conditions (Hochepied et al., 2003). Although the biological functions of this protein are not very clear, several activities have been described, such as stimulation of the antiinflammatory cytokines IL-1 receptor antagonist and soluble tumor necrosis factor receptor (Hochepied et al., 2003). Alpha1-acid glycoprotein has also been reported to be involved in nonspecific resistance to infection by gram-negative bacteria as well as in protection against LPS (Hochepied et al., 2003). For example, Moore et al. (1997) showed that α1-AGP interacts directly with LPS and that α1-AGP–LPS complexes are removed by macrophages, enhancing clearance of LPS from the body. The lack of differences in plasma α1-AGP in our experiment suggests either 1)
that the methodology of measurement (i.e., radial immunodiffusion assay) was not sensitive enough to detect differences in the amount of α1-AGP in plasma, or 2) that this protein was not a good marker for inflammatory conditions in feedlot steers.

In conclusion, the diet fed to steers during the finishing stage modulated plasma concentrations of SAA, LBP, and haptoglobin, with the greatest concentrations detected at 3 wk after the initiation of the experiment. All 3 acute phase proteins decreased to baseline concentrations 6 wk after the finishing diet was offered. Enhanced plasma concentrations of SAA, LBP, and haptoglobin at wk 3 in steers fed the finishing diet as well as the concentration of haptoglobin beginning at wk 9 in steers fed the backgrounding diet indicate the presence of inflammatory conditions at those stages.

Figure 3. Concentration of haptoglobin in the plasma of steers during a 12-wk period of feeding a backgrounding diet with 45% barley grain-based concentrate and 55% barley silage (DM basis) and during a 15-wk period of feeding a finishing diet with 91% barley grain-based concentrate and 9% barley silage. Error bars are SEM (n = 12). Diet effect (P < 0.01). Time effect nested within the backgrounding (P < 0.001) or finishing phase (P < 0.001).

Figure 4. Concentration of α1-acid glycoprotein (α1-AGP) in the plasma of steers during the 12-wk period of feeding a backgrounding diet with 45% barley grain based-concentrate and 55% barley silage (DM basis) and during a 15-wk period of feeding a finishing diet with 91% barley grain-based concentrate and 9% barley silage. Error bars are SEM (n = 12). Diet effect (P = 0.85). Time effect nested within the backgrounding (P = 0.37) or finishing phase (P = 0.08).
Further research is warranted to better understand the mechanisms by which these types of treatments stimulate inflammatory responses in feedlot steers and their role on the health status and growing efficiency of feedlot steers.

LITERATURE CITED


