Effects of dietary source and intake of energy on immune competence and the response to an infectious bovine rhinotracheitis virus (IBRV) challenge in cattle

LR. Schwertner a, M.L. Galyean b, L.E. Hulbert a,b, J.A. Carroll b, M.A. Ballou a,*

a Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, USA
b Livestock Issues Research Unit, USDA-ARS, Lubbock, TX, USA

ARTICLE INFO

Article history:
Received 10 March 2011
Received in revised form 15 June 2011
Accepted 18 June 2011

Keywords:
Energy
Immune
Receiving cattle
Stress

ABSTRACT

Objectives were to evaluate how dietary energy intake and source affect immune competence and response to an infectious bovine rhinotracheitis virus (IBRV) challenge in cattle. Forty-eight crossbred beef steers were stratified by body weight within two periods and randomized to 1 of 3 dietary treatments (8 steers/treatment within period). Treatments were: a 70% concentrate diet fed ad libitum (70 AD); a 30% concentrate diet fed ad libitum (30 AD); and 70% concentrate diet restricted to the net energy for gain intake of 30AL (70RES). Ex vivo immune responses were evaluated after treatments were applied for 28 d, after which cattle were moved into individual pens (d 28 to 40) and intranasally challenged with IBRV on d 30. On d 34, all cattle were offered a 50% concentrate diet ad libitum until d 50. Both energy source (P < 0.02) and intake level (P < 0.04) affected peripheral blood mononuclear cell synthesis of tumor necrosis factor-α, with cell culture supernatant concentrations averaging 2264, 1887, and 1241 pg/ml for 70 AD, 70RES, and 30 AD, respectively. Neither whole blood killing of Mannheimia haemolytica nor neutrophil oxidative burst in response to M. haemolytica was affected by treatments. Serum neutralizing IBRV antibody titers were not different among treatments either before or after the IBRV challenge. Rectal temperature following IBRV peaked 3 d after the IBRV challenge and returned to baseline by d 6, but it was not affected by treatment. No differences were observed in dry matter intake among treatments while the cattle were individually penned and fed a 50% concentrate diet from d 34 to 40. When cattle were group-penned from d 40 to 50 of the study (d 10 to 20 after the IBRV challenge), the 70RES cattle had greater DMI (P < 0.04) than cattle in the other two groups. Following the IBRV challenge, serum glucose concentrations did not differ among treatments; however, the 70 AD cattle had greater blood urea N concentrations (P < 0.01). There was a treatment x time interaction (P < 0.01) for non-esterified fatty acids, such that cattle fed the 70 AD had increased non-esterified fatty acids on d 3 and 5 after the IBRV challenge. Results indicate that cattle fed diets with a greater energy concentration and to an extent a greater percentage of concentrates had a more pronounced pro-inflammatory response, but other aspects of innate immune responses were not influenced by intake or source of energy.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Morbidity and mortality from bovine respiratory disease complex (BRDC) plagues newly weaned and received cattle. The high incidence of BRDC in these cattle adversely affects animal welfare, as well as the economics of beef production.
Management practices, stress, nutrition, genetics, and microbial exposure play a role in the complex etiology of BRDC. Metaphalactic antibiotic treatment is the most effective management practice to decrease the incidence of the disease in lightweight, stressed cattle (Rivera et al., 2005), but concerns are increasing that antibiotic use in animal agriculture might increase antibiotic-resistant bacterial strains that could affect human disease (Sayah et al., 2004). Therefore, strategies to decrease the use of antibiotics, especially metaphalactic use, need to be identified.

Nutrition is an attractive approach to increase disease resistance as well as limit the adverse effects of disease on animal performance. Our interest in the nutritional effects on BRDC centers on data from rodent models and beef cattle suggesting that dietary energy intake and source influence various aspects of immune competence and disease resistance (Ballou et al., 2009; Jolly, 2004; Pahlavani, 2000; Reuter et al., 2008). Thus, the objective of this study was to evaluate the effects of preconditioning diets varying in energy intake and source of energy on innate immune competence and the response to an infectious bovine rhinotracheitis virus (IBRV) challenge in crossbred beef steers.

2. Materials and methods

2.1. Experimental design, cattle, and diets

The Texas Tech Animal Care and Use Committee reviewed and approved all of the procedures that involved the use of live animals in the current study. The experiment was conducted at the Texas Tech University Burnett Center Research Feedlot, 24 km northeast of Lubbock, TX between May and July 2009.

An outline of the experimental procedures is presented in Fig. 1. Forty-eight crossbred steers (body weight = 284 ± 25.9 kg) were purchased from an order buyer and transported from West Plains, Missouri, USA, to the Texas Tech University Burnett Center research feedlot in New Deal, Texas, USA. On arrival, all steers were processed, which included an individual BW measurement, ear tag, vaccination with Vista 5 SQ (Intervet, Inc., Millsboro, DE; IBRV, BVD, PI-3, BRV modified live virus vaccine) and clostridial bacterin toxoid (Vision 7 with SPUR; Intervet), and treatment with Safe-Guard (Intervet). Either 2 or 4 wk after arrival (the study was conducted in two periods, 2 wk apart), cattle were stratified by BW and assigned randomly to one of three dietary treatments. Treatments included a 70% concentrate diet fed ad libitum (70AL); a 30% concentrate diet fed ad libitum (30AL); and a 70% concentrate diet restricted (70RES) to equal the net energy for gain (NEg) intake of the 30AL for a 28-d preconditioning period (Table 1). For practicality, the same diet was used for 70 AD and 70 RES treatments; thus, the intake of protein (g/d) was not equal among treatments. A booster of the Vista 5 SQ was given to all steers at enrollment and 2 wk later. During the preconditioning period, steers were group penned (n = 4/pen) in outdoor, concrete-slotted floor pens with concrete feed bunkers and automatic water troughs. Quantity of feed offered to each pen was recorded daily and feed bunks were managed to leave minimal orts at the time fresh feed was offered each day.

On d 28, the steers were moved into individual stanchions (0.8 m × 2.1 m) in an enclosed barn that was continuously illuminated. The steers had ad libitum access to water. Individual feed intake was recorded from d 28 to 40. On d 30, all steers were intranasally challenged with IBRV in 2 mL (titer value = 10^8.5 tissue culture infected dose/ml) of sterile, isotonic saline (1 mL per nostril) using a MAD®, Mucosal Atomization Device (Wolfe Tory Medical, Inc., Salt Lake City, UT). To simulate the situation in a commercial feedlot in which cattle would be removed from their original group pen and housed in a hospital pen and feed one diet ad libitum, all steers were switched to a 50% concentrate diet (Table 1) fed ad libitum on d 34. Steers were switched on d 34 because this is when peak rectal temperatures were expected, thereby corresponding to the time when the cattle would likely be moved to a hospital pen in a commercial facility. All steers remained on the 50% concentrate diet for the rest of the study.

2.2. Sampling

Individual body weight (BW) measurements were collected at enrollment and on d 28, 30, and 50 using a calibrated scale (Silencer Hydraulic Scale, Moly MFG. Inc, Lorraine, KS). A peripheral blood sample (20 mL) was collected into heparinized and no additive vacutainers via jugular vein puncture for biochemical and ex vivo immunological analyses on d 28. In addition, peripheral blood samples (10 mL) were collected into vacutainers with no additive via jugular vein puncture on d 30, 33, 35, 37, 40, and 50 for biochemical analyses. Serum was harvested by centrifuged at 1200×g for 15 min and stored at −40 °C for later analysis. Rectal temperatures were collected daily at 0800 h from d 30 to 40 using a calibrated, hand-held thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA).

![Fig. 1. Timeline and sampling dates of the experimental plan. Steers were fed either a 70% concentrate diet ad libitum (70 AD), a 30% concentrate diet ad libitum (30 AD), or the 70% concentrate diet restricted to the NEg intake of the 30 AD (70RES) for 34 d. All steers were switched to a 50% concentrate diet ad libitum on d 34 (50 AD). BW = body weight; BS = blood sample; IR = ex vivo immune responses; IBRV = intranasal challenge with infectious bovine rhinotracheitis virus.](image-url)
The oxidative burst of polymorphonuclear neutrophils in response to Mannheimia haemolytica (ATCC #43270) was analyzed. The M. haemolytica was grown overnight in tryptic soy broth + 5% defibrinated sheep blood and quantified by serial dilution and spread-plating on tryptic soy agar + 5% defibrinated sheep blood. The bacteria were heat-killed at 60°C for 30 min, washed, and resuspended at 10^9 colony forming units/mL in 1× PBS. Bacteria were aliquoted into 1 mL volumes and stored at −80°C. Two hundred microliters of whole blood from each steer was aseptically transferred into the bottom of a 1.7-mL microcentrifuge tube and placed in an ice bath for 10 min. Forty microliters each of a 100 μM working concentration of dihydroxyrhodamine and the M. haemolytica were added to each tube, vortexed thoroughly, and then placed in a 38.5°C re-circulating water bath where they were incubated for 10 min. After completion of incubation, the samples were removed from the water bath and placed immediately in a nice bath for 10 min to suspend the reaction at a constant rate in all samples. Erythrocytes were hypo­tonically lysed and remaining leukocytes washed once with 1× PBS. Leukocyte suspensions were analyzed by single color flow cytometry on a Cell Lab Quanta SC flow cytometer (Beckman Coulter, Fullerton, CA). Data are reported as the percentage of neutrophils, as well as the geometric mean fluorescence intensity of the positive neutrophil population.

2.3. Laboratory analyses

The neutrophil population was determined from the scatter plot of electronic volume and side scatter light characteristics.

The ability of whole blood to kill a live culture of the M. haemolytica used in the oxidative burst assay was evaluated. Briefly, an overnight broth culture of the M. haemolytica was diluted in 1× PBS to an approximate concentration of 25 colony forming units/mL and kept in an ice bath. Whole blood was diluted 1:2 with RPMI 1640 to a final volume of 200 μL. All tubes were placed in an ice bath for 15 min. Twenty microliters of the working M. haemolytica culture were added to each tube of diluted whole blood, vortexed thoroughly, and incubated in a re-circulating water bath at 38.5°C for 10 min, which corresponded with the oxidative burst assay. Following the incubation, cultures were vortexed thoroughly, 50 μL of the culture pipetted and spread plated on tryptic soy agar + 5% defibrinated sheep blood plates in duplicate, and incubated overnight before the number of colony forming units were determined. Data are expressed as the percentage of killing and were calculated from plating the diluted working M. haemolytica culture in 200 μL of RPMI 1640 only.

Peripheral blood mononuclear cell cultures (2 × 10^6 cells/mL) were cultured in RPMI 1640 and 10% autologous serum and 1% penicillin/streptomycin and stimulated at a final concentration of 5 ng/mL recombinant interferon-γ (Invitrogen, Carlsbad, CA) and either 0, 0.01, or 5 μg/mL of lipopolysaccharide (LPS; Escherichia coli O111:B5; Sigma, St. Louis, MO) for 24 h after which the supernatant was collected and stored at −80°C until analysis of tumor necrosis factor-α concentrations using a commercially available ELISA (Thermo Scientific, Waltham, MA).

The innate immune responses evaluated in the current study were chosen because they reflect key responses in the recognition (isolated peripheral blood mononuclear cell culture TNF-α secretion), and elimination of bacteria (oxidative burst and whole blood killing capacities to M. haemolytica).

Serum neutralizing antibody titers against the IBRV were determined on d 30 and 40, which corresponded with immediately before the IBRV challenge and 10 d post-challenge, respectively. All laboratory procedures were performed by the Texas Vet Labs, San Angelo, TX. Serum glucose and urea nitrogen were analyzed by commercially available enzymatic, colorimetric kits (Wako Diagnostics, NEFA-HR(2); Wako Diagnostics, Richmond, VA) as described by Bailou et al. (2009). All colorimetric and enzymatic assays were analyzed on a SpectraMax 340PC microplate reader (Molecular Devices, Sunnyvale, CA).

2.4. Statistical analyses

Ex vivo immunological analyses on d 28 were analyzed by ANOVA using the general linear model procedure of SAS (v.9.2, SAS Inst. Inc., Cary, NC, USA) with treatment as the main effect. All repeated, continuous data were analyzed by restricted maximum likelihood ANOVA using the MIXED procedure of SAS (v.9.2, SAS Inst. Inc., Cary, NC, USA). A linear, mixed model with the fixed effects of treatment, sampling time, and the interaction of treatment × sampling time was fitted. The ante-regression covariance structure for the
within-subject measurement was used. Steer nested within treatment was the random effect. For the biochemical responses following the IBRV challenge, samples collected on d28 were used as a covariate in the model. Means separations were performed at each time using a sliced–effect multiple comparison approach with a Tukey–Kramer adjustment. All data were tested for normality of the residuals by evaluating the Shapiro-Wilk statistic using the UNIVARIATE procedure of SAS (v9.2, SAS Inst. Inc., Cary, NC, USA). Contrasts were performed on all data to determine the effects of energy source (30AL vs. 70AL and 70RES) and energy intake (70AL vs. 30AL and 70RES). The interactions of period x sampling time, period x treatment, and period x sampling time x treatment were evaluated and found to be non-significant (P>0.10); therefore, period was not included in the model. Least squares means (±SEM) are reported throughout. A treatment difference at P ≤ 0.05 was considered significant, and 0.05 < P ≤ 0.10 was considered a tendency.

3. Results

3.1. Preconditioning performance and serum constituents

During the preconditioning period, predicted differences in average daily gain (ADG), dry matter intake (DMI), and NE\textsubscript{g} intake were observed (P<0.001; Table 2). Following along with the design of the treatments, cattle on the 70 AD had greater ADG and NE\textsubscript{g} intake than cattle in either the 30 AD or 70RES treatments. The cattle in the 30 AD had greater DMI than cattle fed either the 70 AD or 70RES. There were no differences in either serum glucose or urea nitrogen concentrations among treatments at the end of the preconditioning period (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Item</th>
<th>70 AD</th>
<th>30 AD</th>
<th>70RES</th>
<th>SEM</th>
<th>Trt Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pens, n</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Initial body weight, kg</td>
<td>283</td>
<td>284</td>
<td>285</td>
<td>6.5</td>
<td>-</td>
</tr>
<tr>
<td>Body weight</td>
<td>317</td>
<td>310</td>
<td>308</td>
<td>5.2</td>
<td>-</td>
</tr>
<tr>
<td>Average daily gain, kg</td>
<td>1.2</td>
<td>0.91</td>
<td>0.82</td>
<td>0.069</td>
<td>0.01 1</td>
</tr>
<tr>
<td>Dry matter intake, kg</td>
<td>8.9</td>
<td>9.6</td>
<td>5.9</td>
<td>0.26</td>
<td>0.0001 1.2</td>
</tr>
<tr>
<td>Net energy gain intake, Mcal/d</td>
<td>10.7</td>
<td>7.1</td>
<td>7.1</td>
<td>0.30</td>
<td>0.0001 1.2</td>
</tr>
<tr>
<td>Serum glucose, mg/dL</td>
<td>101.6</td>
<td>92.4</td>
<td>92.0</td>
<td>5.92</td>
<td>0.42</td>
</tr>
<tr>
<td>Serum urea nitrogen, mg/dL</td>
<td>7.1</td>
<td>7.8</td>
<td>6.5</td>
<td>0.76</td>
<td>0.43</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Treatments included a 70% concentrate diet fed ad \textit{libitum} (70 AD), a 30% concentrate diet fed ad \textit{libitum} (30 AD), and the 70% concentrate diet restricted to the NE\textsubscript{g} intake of the 30 AD treatment (70RES).

\textsuperscript{b} Contrasts: (1) Energy Intake: 70% concentrate fed ad \textit{libitum} vs. 30% concentrate fed ad \textit{libitum} and 70% concentrate diet fed in a quantity restricted to equal the NE\textsubscript{g} intake of the 30% concentrate treatment. For the biochemical responses following the IBRV challenge, samples collected on d28 were used as a covariate in the model. Means separations were performed at each time using a sliced–effect multiple comparison approach with a Tukey–Kramer adjustment. All data were tested for normality of the residuals by evaluating the Shapiro-Wilk statistic using the UNIVARIATE procedure of SAS (v9.2, SAS Inst. Inc., Cary, NC, USA). Contrasts were performed on all data to determine the effects of energy source (30AL vs. 70AL and 70RES) and energy intake (70AL vs. 30AL and 70RES). The interactions of period x sampling time, period x treatment, and period x sampling time x treatment were evaluated and found to be non-significant (P>0.10); therefore, period was not included in the model. Least squares means (±SEM) are reported throughout. A treatment difference at P ≤ 0.05 was considered significant, and 0.05 < P ≤ 0.10 was considered a tendency.

3.2. Preconditioning immune responses

Following the 28 d preconditioning period, many ex \textit{vivo} immune responses were evaluated from peripheral leukocytes including the killing ability of whole blood against \textit{M. haemolytica}, the oxidative burst capacity of neutrophils to \textit{M. haemolytica}, and the ability of mononuclear cells to produce TNF-\alpha when co-cultured with LPS. There were no differences observed among treatments for either whole blood killing or neutrophil oxidative burst capacities when co-cultured with \textit{M. haemolytica} (Table 3). The secretion of TNF-\alpha by peripheral blood mononuclear cell cultures was influenced by both source and intake of energy (P<0.05; Table 3). Mononuclear cells isolated from steers fed the 30 AD, as well as those fed the 70RES treatment, secreted less TNF-\alpha when stimulated with LPS than steers fed 70 AD.

### Table 3

<table>
<thead>
<tr>
<th>Item</th>
<th>70 AD</th>
<th>30 AD</th>
<th>70RES</th>
<th>SEM</th>
<th>Trt Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steers, n</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Monohaeimelico haemolytica killing, %</td>
<td>81.9</td>
<td>76</td>
<td>77.5</td>
<td>5.8</td>
<td>0.49</td>
</tr>
<tr>
<td>Oxidative burst neutrophils, %</td>
<td>96.6</td>
<td>94.8</td>
<td>89.2</td>
<td>13.70</td>
<td>0.92</td>
</tr>
<tr>
<td>Tumor necrosis factor-\alpha</td>
<td>2264</td>
<td>1241</td>
<td>1887</td>
<td>269.8</td>
<td>0.03 1.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Treatments included a 70% concentrate diet fed ad \textit{libitum} (70 AD), a 30% concentrate diet fed ad \textit{libitum} (30 AD), and the 70% concentrate diet restricted to the NE\textsubscript{g} intake of the 30 AD treatment (70RES).

\textsuperscript{b} Contrasts: (1) Energy Intake: 70% concentrate fed ad \textit{libitum} vs. 30% concentrate fed ad \textit{libitum} and 70% concentrate diet fed in a quantity restricted to equal the NE\textsubscript{g} intake of the 30% concentrate treatment; (2) Energy Source: 30% concentrate fed ad \textit{libitum} vs. 70% concentrate fed ad \textit{libitum} and 70% concentrate diet fed in a quantity restricted to equal the NE\textsubscript{g} intake of the 30% concentrate treatment.

\textsuperscript{c} There was no effect of concentration of lipopolysaccharide in culture. Data are reported as the mean of the 0.01 and 5 \mu g/mL of lipopolysaccharide in the culture media.
in DMI during the remaining 7 d the steers were individually penned in the stanchions. Dry matter intake decreased among all treatments when cattle were moved back into their original group pen on d 40. From d 43 to 47, cattle previously fed 70RES diet had a lesser decrease in DMI and subsequently recovered more quickly to pre-group penning either before or after the IBRV challenge, but were increased in OMI during the remaining 7 d the steers were individually penned in the 30% concentrate treatment (70RES: n = 16). Serum urea nitrogen concentrations were influenced by treatment effects could be the result of similar concentrations of blood glucose. Newbold (1973) observed that when serum glucose concentrations increased, the phagocytic actions of blood leukocytes increased. Current data contrast those of Sun et al. (2001), who observed that mice fed 40% calorie restriction for 6 mo had decreased macrophage phagocytic function; however, it should be noted that the energy restricted mice in the study of Sun et al. (2001) did not grow over the 6-mo study period, which contrasts with the current study. In Holstein cows, plasma non-esterified fatty acid concentrations greater than 400 μmol/L 1 wk before parturition were associated with large reductions in myeloperoxidase activity of neutrophils (Hammon et al., 2006). Taken together, these data suggest that reduced energy intake of animals, no growth in young animals or negative energy balance in adult animals, decreases neutrophil functions, whereas decreased energy intake in young, growing animals does not influence neutrophil or bactericidal responses.

Secretion of the pro-inflammatory cytokine, TNF-α, by peripheral blood mononuclear cells stimulated with LPS was decreased in cattle fed either 30 AD or 70RES compared with 70 AD. In agreement with this finding, mice in the study of Sun et al. (2001) had decreased mRNA expressions of pro-inflammatory cytokine genes as well as secretion of interleukin-6 when macrophages were stimulated ex vivo with LPS. In that same study, pro-inflammatory response was evaluated following cecal ligation and puncture to induce polymicrobial sepsis. Following the cecal ligation and puncture, mice on the calorie-restricted diet displayed increased concentrations of systemic TNF-α, and survival analysis indicated a more rapid mortality in the calorie-restricted mice. These data suggest that calorie restriction decreases the pro-inflammatory response of macrophages in response to gram-negative bacteria, which might allow for growth of the pathogen once it has evaded the physical barriers of the immune system, thereby resulting in a greater degree of sepsis.

Fig. 2. Least square means of steer dry matter intake following the infectious bovine rhinotracheitis virus (IBRV) challenge. Dietary treatments were 70% concentrate diet fed ad libitum (70 AD; n = 16), 30% concentrate diet fed ad libitum (30 AD; n = 16), and 70% concentrate diet fed in a quantity restricted to equal the NEg intake of the 30% concentrate treatment (70RES; n = 16). *P < 0.05. Error bars represent ± SEM.

4. Discussion

The influence of energy source and level during the preconditioning period on various aspects of innate immune competence and the response to an IBRV challenge were investigated. Greater NEg intake by cattle in the 70 AD treatment increased ADG by these steers. Reuter et al. (2008) fed similar diets as in the current study, and reported similar differences in performance among treatments. Feeding diets with a greater proportion of concentrates is known to improve performance (Lofgreen et al., 1975); however, little is known about how energy intake or source influences the innate immune responses of preconditioning cattle.

No differences were observed among treatments in either the oxidative burst capacity of neutrophils or the bactericidal activity of whole blood to M. haemolytica. The lack of treatment effects could be the result of similar concentrations of blood glucose. Newbold (1973) observed that when serum glucose concentrations increased, the phagocytic actions of blood leukocytes increased. Current data contrast those of Sun et al. (2001), who observed that mice fed 40% calorie restriction for 6 mo had decreased macrophage phagocytic function; however, it should be noted that the energy restricted mice in the study of Sun et al. (2001) did not grow over the 6-mo study period, which contrasts with the current study. In Holstein cows, plasma non-esterified fatty acid concentrations greater than 400 μmol/L 1 wk before parturition were associated with large reductions in myeloperoxidase activity of neutrophils (Hammon et al., 2006). Taken together, these data suggest that reduced energy intake of animals, no growth in young animals or negative energy balance in adult animals, decreases neutrophil functions, whereas decreased energy intake in young, growing animals does not influence neutrophil or bactericidal responses.
Fig. 3. a. Least square means of plasma glucose concentrations following the infectious bovine rhinotracheitis virus (IBRV) challenge. Dietary treatments were 70% concentrate diet fed ad libitum (70 AD: n = 16), 30% concentrate diet fed ad libitum (30 AD: n = 16), and 70% concentrate diet fed in a quantity restricted to equal the NEg intake of the 30% concentrate treatment (70RES: n = 16). No differences between treatments were detected. *(P < 0.05). Error bars represent ± SEM. b. Least square means of plasma urea nitrogen concentrations following the infectious bovine rhinotracheitis virus (IBRV) challenge. Dietary treatments were 70% concentrate diet fed ad libitum (70 AD: n = 16), 30% concentrate diet fed ad libitum (30 AD: n = 16), and 70% concentrate diet fed in a quantity restricted to equal the NEg intake of the 30% concentrate treatment (70RES: n = 16).*(P < 0.05); # = (P < 0.10). Error bars represent ± SEM. c. Least square means of steer plasma non-esterified fatty acids concentrations following the infectious bovine rhinotracheitis virus (IBRV) challenge. Dietary treatments were 70% concentrate diet fed ad libitum (70 AD: n = 16), 30% concentrate diet fed ad libitum (30 AD: n = 16), and 70% concentrate diet fed in a quantity restricted to equal the NEg intake of the 30% concentrate treatment (70RES: n = 16).* (P < 0.05). Error bars represent ± SEM.
of serum TNF-α following an intravenous LPS challenge in cattle fed lower-energy (70RES) and higher-roughage (30 AD) diets. The reason for the discrepancies observed between the current data and that observed by Reuter et al. (2008) is not known, but could reflect the use of different experimental models. Reuter et al. (2008) used an in vivo LPS challenge, whereas isolated peripheral mononuclear cells stimulated with LPS ex vivo in the current study. The ex vivo model might reflect the sensitivity of the monocyte/macrophages to LPS, whereas the in vivo challenge may be more indicative of how an animal will respond once they have become septicemic. The role that either the results from in vivo or ex vivo models plays in defining resistance to disease of cattle is not known and should be addressed with future research.

Decreased pro-inflammatory responses observed in cattle fed lower energy intakes might have negative effects on resistance to disease in cattle that are exposed to pathogens. The decreased secretion of TNF-α could potentially decrease the ability of the cattle's immune system to recognize, sequester, and eliminate the pathogen. In dairy cattle, data indicate that the severity of Escherichia coli mastitis is inversely related to the speed of neutrophil recruitment into the mammary gland (Hill, 1981). Decreased secretions of TNF-α could result in less neutrophils and other effector leukocytes being recruited to tissue sites of infection, which could allow growth of the pathogen. If pathogen growth continues, there will be more pathogen-derived immunogens for a subsequent systemic inflammatory response. This effect was likely observed in the study by Sun et al. (2001), in which ex vivo pro-inflammatory cytokine responses were lower in energy restricted mice; however, those mice had greater cytokine responses and higher mortality following a cecal ligation and puncture septicemia model. Therefore, an ideal acute-phase response within an animal would be a rapid and robust response to sequester and eliminate the pathogen, followed by a rapid down-regulation of the response to prevent excessive host tissue damage. These data indicate that cattle fed higher energy and to an extent higher concentrate diets may have a more desirable pro-inflammatory immune response phenotype.

There were no differences observed in serum neutralizing IBRV titers among treatments either before or 10 d after the IBRV challenge. Therefore, energy level or source did not influence the humoral response to vaccination, which is consistent with that observed by Reuter et al. (2008). However, these data contrast with results from Whitney et al. (2006); whereas calves fed a higher roughage diet had increased IgG concentrations following vaccination. The current data indicate that differences in energy level or source would not influence the response to vaccinations during the pre-conditioning period. The lack of a treatment effect on serum neutralizing IBRV titers either before or after the challenge indicate any treatment differences in the response to the IBRV challenge were not due to differences in antibody titers.

During the IBRV challenge, no differences were observed among treatments in rectal temperatures; however, the challenge caused a mild febrile response from d 33 to 35. Therefore, using vaccinated cattle challenged intranasally with the MAD®, Mucosal Atomization Device (Wolfe Tory Medical, Inc., Salt Lake City, UT) caused a mild acute-phase response. Similar to the rectal temperatures, DMI was not influenced following the IBRV challenge while cattle were individually penned. Nonetheless, once cattle were moved back to their original group pen on d 40, DMI was decreased in all treatments, possibly because of the stress associated with handling and/or redefining the social dominance structure in the pen. From d 43 to 47, DMI in the 70RES cattle was greater than by cattle in other two treatments. The reason(s) for the difference in DMI observed during this period is not known, but it could be the result of cattle fed restricted quantities of feed being conditioned to cope with the social stress because they were "programmed" to have to compete for available resources. This is an area that warrants further research, as it might be an effective management strategy to keep cattle on feed during periods of stress.

Serum concentrations of glucose decreased following the IBRV challenge. Activation of innate immune cells increases whole body glucose utilization, which could explain the decreased plasma glucose in these cattle following the IBRV challenge (Gamelli et al., 1996). The effects that individual penning had on plasma glucose concentrations, however, cannot be ascertained because of the experimental design of the current study.

Serum urea nitrogen concentrations were elevated on d 33 to 40 in steers fed the 70 AD. Orr et al. (1988) observed an increase in the concentration of serum urea nitrogen after cattle were challenged with IBRV. The greater concentrations of serum urea nitrogen in 70 AD could have resulted from elevated catabolism of whole body protein pool (Nielsen et al., 2005). Whole body nitrogen balance is decreased during infection, and the greater response in 70 AD steers could be a reflection of a stronger response. Although intake of crude protein was not equal among treatments due to the 70RES consuming less dry matter during the pre-conditioning period, the intake of crude protein among steers fed 70 AD were not influenced by time during the IBRV challenge (data not shown). Therefore, it is unlikely that the elevated serum urea nitrogen concentrations observed in these steers on d 33 to 40 were associated with changes in the intake of crude protein. Similar to the serum urea nitrogen concentrations, on d 35 the steers fed the 70 AD had increased concentrations of serum non-esterified fatty acids. Whitney et al. (2005) reported similar findings when they challenged steers with IBRV. The diets fed by Whitney et al. (2005) were three high-roughage diets and a 70% concentrate diet. Steers fed the 70% concentrate diet had greater non-esterified fatty acid concentrations than steers fed the high-roughage diets. The increased non-esterified fatty acids observed in cattle fed 70 AD could be connected to production of pro-inflammatory cytokines, as they can stimulate hormone-sensitive lipase (Coppack, 2001). The present data are consistent with the ex vivo sensitivity of monocyte/macrophages to LPS. As noted previously, we cannot completely rule out that the differences observed in the 70 AD cattle following the IBRV were caused by the individual penning of the steers from d 28 to 40 or that all cattle were switched to a 50% concentrate diet on d 34.

Our data are consistent with the suggestion made by Rivera et al. (2005) that feeding higher concentrate diets might produce a more aggressive acute-phase response in cattle when exposed to a pathogen. The observations of Lofgren et al.
that cattle fed lower energy diets had decreased morbidity might reflect lower expression of pro-inflammatory cytokines. Cattle with a lower pro-inflammatory cytokine response could display fewer of the usual clinical signs that accompany disease and are known as sickness behaviors. Therefore, BRDC in cattle fed higher-roughage diets might go undetected. Other data support the fact that the feedlot industry needs more sensitive measures of morbidity, as Gardner et al. (1999) reported that 68% of cattle not treated for BRDC during the finishing period had pulmonary lesions at slaughter indicative of a previous incidence of BRDC.

5. Conclusion

In conclusion, a higher-energy diet (lower in roughage) might be the most appropriate diet to feed preconditioning and stressed, newly received cattle. The more aggressive acute-phase response could benefit cattle by allowing the rapid recognition, recruitment, and elimination of a potential pathogen. In addition, the observation that steers previously fed 70RES were able to return to feed more quickly when being group penned needs to be further investigated, as this could be a management strategy employed during the preconditioning period that could help cattle cope with the social stress on entering a feedlot.

Acknowledgements

The authors thank Clayton and Colton Cobb of Texas Tech University for their assistance with animal husbandry. This research was partially funded by the Texas Cattle Feeders Association in Amarillo, TX, USA.

References


