Effects of dietary source and intake of energy on immune competence and the response to an Infectious Bovine Rhinotracheitis Virus (IBRV) challenge in cattle

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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 2 periods and randomized to 1 of 3 dietary treatments (8 steers/treatment within period). Treatments were: a 70% concentrate diet fed *ad libitum* (70AD); a 30% concentrate diet fed *ad libitum* (30AD); 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 2,264, 1,887, and 1,241 pg/mL for 70AD, 70RES, and 30AD, respectively. Neither whole blood killing of *Mannheimia haemolytica* nor neutrophil oxidative burst in response to *M. haemolytica* was affected by treatments. 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 4 to 10 after the IBRV challenge. 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 2 groups. Following the IBRV challenge, serum glucose concentrations did not differ among treatments; however, the 70AD 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 70AD 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.

**Key words:** energy, immune, receiving cattle, stress

**IMPLICATIONS**

A higher energy diet could be the most appropriate diet to feed to stressed cattle. The higher acute-phase immune response could benefit cattle by allowing the rapid recognition, recruitment of other immune cells to the site of infection, and elimination of the potential pathogen. The more aggressive acute-phase immune response could also allow the cattle to more clearly show clinical signs of disease, which would allow producers to recognize the animals and administer appropriate therapeutic treatments, thereby improving animal welfare.

**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 (Duff and Galyean, 2007). Currently, metaphalactic antibiotic treatment is the most effect 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., 2005).
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 (Pahlavani, 2000; Jolly, 2004; Duff and Galyean, 2007; 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.

MATERIALS AND METHODS

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 Figure 1. Forty-eight crossbred steers (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, BRSV 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 2 periods, 2 wk apart), cattle were stratified by BW and assigned randomly to 1 of 3 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 70AD 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 bunk were managed to leave minimal ors at the time fresh feed was offered each day.

On d 28, the steers were moved into individual stanchions (0.8 m x 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$_{50}$/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 animals would be removed from their pen and housed in a hospital pen and fed 1 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.

**Sampling**
Individual BW measurements were collected at enrollment and on d 28, 40, 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 venipuncture 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 venipuncture on d 30, 33, 35, 37, 40, and 50 for biochemical analyses. Serum was harvested by centrifuged at 1,200 x 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).

Laboratory Analyses

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 10⁹ colony forming units/mL in 1X 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 dihydrorhodamine 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 an ice 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 1X 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. 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 1X PBS to an approximate concentration of 25 colony forming units/µL 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 x 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 (*Escherichia coli* 0111: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).

Serum glucose and urea nitrogen were analyzed by commercially available enzymatic, colorimetric kits (Stanbio Laboratory, Boerne, TX). In addition, serum non-esterified fatty acids were analyzed using commercially available reagents from Wako Diagnostics (NEFA-HR(2); Wako Diagnostics, Richmond, VA) as described by Ballou et al. (2009). All colorimetric and enzymatic assays were analyzed on a SpectraMax 340PC microplate reader (Molecular Devices, Sunnyvale, CA).

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 x 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 (v.9.2, 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 ($\pm$SEM) are reported throughout. A treatment difference at $P \leq 0.05$ was considered significant, and $0.05 < P \leq 0.10$ was considered a tendency.

RESULTS

Preconditioning Performance and Serum Constituents

During the preconditioning period, predicted differences in average daily gain (ADG), dry matter intake (DMI), and NE$_g$ intake were observed ($P < 0.001$; Table 2). Following along with the design of the treatments, cattle on the 70AD had greater ADG and NE$_g$ intake than cattle in either the 30AD or 70RES treatments. The cattle in the 30AD had greater DMI than cattle fed either the 70AD 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).

Preconditioning Immune Responses

Following the 28 d preconditioning period, many *ex vivo* immune responses were evaluated from peripheral leukocytes including the killing ability of whole blood against *M. haemolytica*, the oxidative burst capacity of neutrophils to *M. haemolytica*, and the ability of mononuclear cells to produce TNF-\(\alpha\) when co-cultured with LPS. No differences were observed among treatments for either whole blood killing or neutrophil oxidative burst capacities when co-cultured with *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 30AD, as well as those fed the 70RES treatment, secreted less TNF-\(\alpha\) when stimulated with LPS than steers fed 70AD.
Infectious Bovine Rhinotracheitis Virus Challenge

Intranasal inoculation with 2mL of IBRV (10^{8.5} tissue culture infected dose_{50}/mL) using the MAD® device (Wolfe Tory Medical, Inc., Salt Lake City, UT) on vaccinated calves caused an acute-phase response as evidenced by increased rectal temperatures (Figure 2); however, no differences were observed in the febrile responses among treatments. Dry matter intake data are presented in Figure 3. When cattle were moved from group pens into the individual stanchions on d 28, DMI decreased on both 70AD and 30AD treatments, but cattle in these treatments had returned to preconditioning intakes by d 30. As a result of the experimental design, steers on the 70RES had lower DMI from d30 to 33; however, once all steers were offered the 50% concentrate diet *ad libitum* on d 34, there were no differences in DMI during the remaining 7 d the steers were individually penned in the stanchions. When cattle were moved back into their original group pen on d 40, DMI decreased in all treatment groups. 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 DMI (*P* < 0.05; Figure 3).

Serum concentrations of glucose decreased following the IBRV challenge (*P* < 0.01; Figure 4a). There were no differences among treatments except for a tendency (*P* < 0.10) for steers fed the 30AD to have higher glucose concentrations on d 40. Serum urea nitrogen concentrations were influenced by treatment (*P* < 0.05; Figure 4b). Cattle fed the 70AD had greater concentrations of serum urea nitrogen than those fed either 30AD or 70RES on d 33, 35, and 37. Similarly, steers fed the 70AD had elevated (*P* < 0.05) serum concentrations of non-esterified fatty acids on d 35.

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. Higher NE\textsubscript{g} intake by cattle in the 70AD treatment increased the 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 \textit{M. haemolytica}. The lack of treatment effects could be the result of similar concentrations of blood glucose. Newbould (1973) observed that when serum glucose concentrations increased, the phagocytic actions of blood leukocytes increased. The current data contrast those of Sun et al. (2001), who observed that mice fed 40% calorie restriction for 6 mo had lower 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 cattle fed the lower energy diets in the current study. In Holstein cows, plasma non-esterified fatty acid concentrations greater than 400 \textmu Eq/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 low 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-\textalpha, by peripheral blood mononuclear cells stimulated with LPS was lower in cattle fed either 30AD or 70RES compared with 70AD.
In agreement with this finding, mice in the study of Sun et al. (2001) had lower 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 septicemia. 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 septicemia (Sun et al., 2001). In contrast to our data, Reuter et al. (2008) observed an increased concentration of serum TNF-α following an intravenous LPS challenge in cattle fed lower-energy (70RES) and higher-roughage (30AD) diets. The reason for the discrepancies observed between the current data and that observed by Reuter et al. (2008) is not known, but could be 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). Lower secretion 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.

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 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 2 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 70AD. 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 70AD 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 higher response in 70AD 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 preconditioning period, the intake of crude protein among steers fed 70AD 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 was associated with changes in the intake of crude protein. Similar to the serum urea nitrogen concentrations, on d 35 the steers fed the 70AD 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 3 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 70AD 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 70AD 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 Lofgreen et al. (1975) 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.

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. Moreover, a more visual display of signs associated with infection and disease would allow the cattle to be detected, removed from their pen, and
administered appropriate medical treatments. 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

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REFERENCES


Table 1. Description of experimental diets (%DM).

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<th>Item</th>
<th>70</th>
<th>30</th>
<th>50</th>
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<tr>
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<td>Steam-flaked Corn</td>
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<td>Cottonseed Meal</td>
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<td>8.5</td>
<td>8.5</td>
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<td>Molasses</td>
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<td><strong>Supplement</strong></td>
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<td>2.5</td>
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**Analyzed composition**

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**Calculated composition**

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<td>0.52</td>
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1 Supplement contained (DM basis): 66.382% cottonseed meal; 0.500% Endox (antioxidant; Kemin Industries, Des Moines, IA); 0.647% dicalcium phosphate; 10.000% potassium chloride; 0.333% manganous oxide; 4.167% ammonium sulfate; 15.000% salt; 0.0022% cobalt carbonate; 0.196% copper sulfate; 0.0833% iron sulfate; 0.0031% ethylenediamine dihydroiodide; 0.125% selenium premix (0.2% Se); 0.986% zinc sulfate; 0.0099% vitamin A (1,000,000 IU/g); 0.157% vitamin E (500 IU/g); 0.844% Rumensin (Elanco Animal Health, Greenfield, IN); and 0.56% Tylan (Elanco Animal Health). Concentration values in parentheses are expressed on a 90% DM basis.

2 Diets were sampled once weekly and composited by period prior to analyses by a commercial laboratory.

3 Composition calculated from the tabular values in NRC (1996).
Table 2. Performance during the 28-day preconditioning period

<table>
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<tr>
<th>Item</th>
<th>70AD</th>
<th>30AD</th>
<th>70RES</th>
<th>SEM</th>
<th>Trt</th>
<th>Contrasts&lt;sup&gt;1&lt;/sup&gt;</th>
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<td>-</td>
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<td>285</td>
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<td>ADG, kg</td>
<td>1.2</td>
<td>0.91</td>
<td>0.82</td>
<td>0.069</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td>DMI, kg</td>
<td>8.9</td>
<td>9.6</td>
<td>5.9</td>
<td>0.26</td>
<td>0.0001</td>
<td>1, 2</td>
</tr>
<tr>
<td>NEg intake, Mcal/d</td>
<td>10.7</td>
<td>7.1</td>
<td>7.1</td>
<td>0.30</td>
<td>0.0001</td>
<td>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>
<td>-</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>
<td>-</td>
</tr>
</tbody>
</table>

<sup>1</sup>Treatments included a 70% concentrate diet fed *ad libitum* (70AD), a 30% concentrate diet fed *ad libitum* (30AD), and the 70% concentrate diet restricted to the NE<sub>g</sub> intake of the 30AD treatment (70RES).

<sup>2</sup>Contrasts: (1) Energy Intake: 70% concentrate fed *ad libitum* vs. 30% concentrate fed *ad libitum* and 70% concentrate diet fed in a quantity restricted to equal the NE<sub>g</sub> intake of the 30% concentrate treatment; (2) Energy Source: 30% concentrate fed *ad libitum* vs. 70% concentrate fed *ad libitum* and 70% concentrate diet fed in a quantity restricted to equal the NE<sub>g</sub> intake of the 30% concentrate treatment.
Table 3. Immune competence measurements on d 28 of the preconditioning period

<table>
<thead>
<tr>
<th>Item</th>
<th>70AD</th>
<th>30AD</th>
<th>70RES</th>
<th>SEM</th>
<th>Trt</th>
<th>Contrasts¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steers, n</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mannheimia haemolytica</em> killing, %</td>
<td>72</td>
<td>69</td>
<td>69</td>
<td>6</td>
<td>0.90</td>
<td>-</td>
</tr>
<tr>
<td>Oxidative burst neutrophils, %</td>
<td>81.9</td>
<td>76</td>
<td>77.5</td>
<td>5.8</td>
<td>0.49</td>
<td>-</td>
</tr>
<tr>
<td>Oxidative burst neutrophils, geometric mean fluorescence</td>
<td>96.6</td>
<td>94.8</td>
<td>89.2</td>
<td>13.70</td>
<td>0.92</td>
<td>-</td>
</tr>
<tr>
<td>Tumor necrosis factor-α, pg/mL³</td>
<td>2264</td>
<td>1241</td>
<td>1887</td>
<td>269.8</td>
<td>0.03</td>
<td>1,2</td>
</tr>
</tbody>
</table>

¹Treatments included a 70% concentrate diet fed *ad libitum* (70AD), a 30% concentrate diet fed *ad libitum* (30AD), and the 70% concentrate diet restricted to the NEg intake of the 30AD treatment (70RES).
²Contrasts: (1) Energy Intake: 70% concentrate fed *ad libitum* vs. 30% concentrate fed *ad libitum* and 70% concentrate diet fed in a quantity restricted to equal the NEg intake of the 30% concentrate treatment; (2) Energy Source: 30% concentrate fed *ad libitum* vs. 70% concentrate fed *ad libitum* and 70% concentrate diet fed in a quantity restricted to equal the NEg intake of the 30% concentrate treatment.
³There was no effect of concentration of lipopolysaccharide in culture. Data is reported as the mean of the 0.01 and 5 µg/mL of lipopolysaccharide in the culture media.
Figure 2

Day of study

Temperature, °C

[Graph showing temperature changes over days from 28 to 40.]
Figure 3

Dry matter intake, kg/d vs. Day of study for groups of individually and group penned animals.

- 70AD
- 30AD
- 70RES

**Individually Penned**

**Group Penned**

IBRV
Figure 4a

Individually Penned  Group Penned

IBRV

Day of study

Serum glucose, mg/dL

70AD
30AD
70RES

70AD
30AD
70RES
Figure 4b

Serum urea nitrogen, mg/dL

Time of study, day

Individually Penned
Group Penned

IBRV

* #

70AD
30AD
70RES
Figure 4c

Serum non-esterified fatty acids, uEq/L

Day of study

Individually Penned  Group Penned

IBRV

*
Figure 1: Timeline and sampling dates of the experimental plan. Steers were fed either a 70% concentrate diet *ad libitum* (70AD), a 30% concentrate diet *ad libitum* (30AD), or the 70% concentrate diet restricted to the NE$_g$ intake of the 30AD (70RES) for 34 d. All steers were switched to a 50% concentrate diet *ad libitum* on d 34 (50AD). BW = body weight; BS = blood sample; IR = *ex vivo* immune responses; IBRV = intranasal challenge with infectious bovine rhinotracheitis virus.

Figure 2: Least square means of all steer’s (n = 48) rectal temperature measured daily at 0800 h following the IBRV challenge. No differences were detected among treatments. All animals displayed a moderate febrile response in response to the viral challenge ($P < 0.05$). Error bars represent ± SEM.

Figure 3: Least square means of steer dry matter intake following the IBRV challenge. Dietary treatments were 70% concentrate diet fed *ad libitum* (70AD; closed circle, n = 16), 30% concentrate diet fed *ad libitum* (30AD; open circle, n = 16), and 70% concentrate diet fed in a quantity restricted to equal the NE$_g$ intake of the 30% concentrate treatment (70RES; closed triangle, n = 16). * = ($P < 0.05$). Error bars represent ± SEM.

Figure 4a: Least square means of plasma glucose concentrations following the IBRV challenge. Dietary treatments were 70% concentrate diet fed *ad libitum* (70AD; closed circle, n = 16), 30% concentrate diet fed *ad libitum* (30AD; open circle, n = 16), and 70% concentrate diet fed in a quantity restricted to equal the NE$_g$ intake of the 30% concentrate diet.
concentrate treatment (70RES; closed triangle, n = 16). No differences between treatments were detected. # = (P ≤ 0.10). Error bars represent ± SEM.

**Figure 4b:** Least square means of plasma urea nitrogen concentrations following the IBRV challenge. Dietary treatments were 70% concentrate diet fed *ad libitum* (70AD; closed circle, n = 16), 30% concentrate diet fed *ad libitum* (30AD; open circle, n = 16), and 70% concentrate diet fed in a quantity restricted to equal the NEg intake of the 30% concentrate treatment (70RES; closed triangle, n = 16). * = (P < 0.05); # = (P ≤ 0.10). Error bars represent ± SEM.

**Figure 4c:** Least square means of steer plasma non-esterified fatty acids concentrations following the IBRV challenge. Dietary treatments were 70% concentrate diet fed *ad libitum* (70AD; closed circle, n = 16), 30% concentrate diet fed *ad libitum* (30AD; open circle, n = 16), and 70% concentrate diet fed in a quantity restricted to equal the NEg intake of the 30% concentrate treatment (70RES; closed triangle, n = 16). * = (P < 0.05). Error bars represent ± SEM.