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

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24 **ABSTRACT**

25 Objectives were to evaluate how dietary energy intake and source affect immune
26 competence and response to an infectious bovine rhinotracheitis virus (IBRV) challenge in cattle.
27 Forty-eight crossbred beef steers were stratified by body weight within 2 periods and randomized
28 to 1 of 3 dietary treatments (8 steers/treatment within period). Treatments were: a 70%
29 concentrate diet fed *ad libitum* (70AD); a 30% concentrate diet fed *ad libitum* (30AD); and 70%
30 concentrate diet restricted to the net energy for gain intake of 30AL (70RES). *Ex vivo* immune
31 responses were evaluated after treatments were applied for 28 d, after which cattle were moved
32 into individual pens (d 28 to 40) and intranasally challenged with IBRV on d 30. On d 34, all
33 cattle were offered a 50% concentrate diet *ad libitum* until d 50. Both energy source ($P < 0.02$)
34 and intake level ($P < 0.04$) affected peripheral blood mononuclear cell synthesis of tumor
35 necrosis factor- α , with cell culture supernatant concentrations averaging 2,264, 1,887, and 1,241
36 pg/mL for 70AD, 70RES, and 30AD, respectively. Neither whole blood killing of *Mannheimia*
37 *haemolytica* nor neutrophil oxidative burst in response to *M. haemolytica* was affected by
38 treatments. Rectal temperature following IBRV peaked 3 d after the IBRV challenge and
39 returned to baseline by d 6, but it was not affected by treatment. No differences were observed
40 in dry matter intake among treatments while the cattle were individually penned and fed a 50%
41 concentrate diet from d 4 to 10 after the IBRV challenge. When cattle were group-penned from
42 d 40 to 50 of the study (d 10 to 20 after the IBRV challenge), the 70RES cattle had greater DMI
43 ($P < 0.04$) than cattle in the other 2 groups. Following the IBRV challenge, serum glucose
44 concentrations did not differ among treatments; however, the 70AD cattle had greater blood urea
45 N concentrations ($P < 0.01$). There was a treatment x time interaction ($P < 0.01$) for non-
46 esterified fatty acids, such that cattle fed the 70AD had increased non-esterified fatty acids on d 3

47 and 5 after the IBRV challenge. Results indicate that cattle fed diets with a greater energy
48 concentration and to an extent a greater percentage of concentrates had a more pronounced pro-
49 inflammatory response, but other aspects of innate immune responses were not influenced by
50 intake or source of energy.

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

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53 **IMPLICATIONS**

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

60

61 **INTRODUCTION**

62 Morbidity and mortality from bovine respiratory disease complex (BRDC) plagues newly
63 weaned and received cattle. The high incidence of BRDC in these cattle adversely affects animal
64 welfare, as well as the economics of beef production. Management practices, stress, nutrition,
65 genetics, and microbial exposure play a role in the complex etiology of BRDC (Duff and
66 Galyean, 2007). Currently, metaphalactic antibiotic treatment is the most effect management
67 practice to decrease the incidence of the disease in lightweight, stressed cattle (Rivera et al,
68 2005), but concerns are increasing that antibiotic use in animal agriculture might increase
69 antibiotic-resistant bacterial strains that could affect human disease (Sayah et al., 2005).

70 Therefore, strategies to decrease the use of antibiotics, especially metaphalactic use, need to be
71 identified.

72 Nutrition is an attractive approach to increase disease resistance as well as limit the
73 adverse effects of disease on animal performance. Our interest in the nutritional effects on
74 BRDC centers on data from rodent models and beef cattle suggesting that dietary energy intake
75 and source influence various aspects of immune competence and disease resistance (Pahlavani,
76 2000; Jolly, 2004; Duff and Galylean, 2007; Reuter et al., 2008). Thus, the objective of this study
77 was to evaluate the effects of preconditioning diets varying in energy intake and source of energy
78 on innate immune competence and the response to an infectious bovine rhinotracheitis virus
79 (IBRV) challenge in crossbred beef steers.

80

81 **MATERIALS AND METHODS**

82 *Experimental Design, Cattle, and Diets*

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

87 An outline of the experimental procedures is presented in Figure 1. Forty-eight crossbred
88 steers (284 ± 25.9 kg) were purchased from an order buyer and transported from West Plains,
89 Missouri, USA, to the Texas Tech University Burnett Center research feedlot in New Deal,
90 Texas, USA. On arrival, all steers were processed, which included an individual BW
91 measurement, ear tag, vaccination with Vista 5 SQ (Intervet, Inc., Millsboro, DE; IBRV, BVD,
92 PI-3, BRSV modified live virus vaccine) and clostridial bacterin toxoid (Vision 7 with SPUR;

93 Intervet), and treatment with Safe-Guard (Intervet). Either 2 or 4 wk after arrival (the study was
94 conducted in 2 periods, 2 wk apart), cattle were stratified by BW and assigned randomly to 1 of 3
95 dietary treatments. Treatments included a 70% concentrate diet fed *ad libitum* (70AL); a 30%
96 concentrate diet fed *ad libitum* (30AL); and a 70% concentrate diet restricted (70RES) to equal
97 the net energy for gain (NEg) intake of the 30AL for a 28-d preconditioning period (Table 1).
98 For practicality, the same diet was used for 70AD and 70 RES treatments; thus, the intake of
99 protein (g/d) was not equal among treatments. A booster of the Vista 5 SQ was given to all
100 steers at enrollment and 2 wk later. During the preconditioning period, steers were group penned
101 (n = 4/pen) in outdoor, concrete-slotted floor pens with concrete feed bunkers and automatic
102 water troughs. Quantity of feed offered to each pen was recorded daily and feed bunk were
103 managed to leave minimal orts at the time fresh feed was offered each day.

104 On d 28, the steers were moved into individual stanchions (0.8 m x 2.1 m) in an enclosed
105 barn that was continuously illuminated. The steers had *ad libitum* access to water. Individual
106 feed intake was recorded from d 28 to 40. On d 30, all steers were intranasally challenged with
107 IBRV in 2 mL (titer value = $10^{8.5}$ tissue culture infected dose₅₀/mL) of sterile, isotonic saline (1
108 mL per nostril) using a MAD®, Mucosal Atomization Device (Wolfe Tory Medical, Inc., Salt
109 Lake City, UT). To simulate the situation in a commercial feedlot in which animals would be
110 removed from their pen and housed in a hospital pen and fed 1 diet *ad libitum*, all steers were
111 switched to a 50% concentrate diet (Table 1) fed *ad libitum* on d 34. Steers were switched on d
112 34 because this is when peak rectal temperatures were expected, thereby corresponding to the
113 time when the cattle would likely be moved to a hospital pen in a commercial facility. All steers
114 remained on the 50% concentrate diet for the rest of the study.

115 ***Sampling***

116 Individual BW measurements were collected at enrollment and on d 28, 40, and 50 using
117 a calibrated scale (Silencer Hydraulic Scale, Moly MFG. Inc, Lorraine, KS). A peripheral blood
118 sample (20 mL) was collected into heparinized and no additive vacutainers via jugular
119 venipuncture for biochemical and *ex vivo* immunological analyses on d 28. In addition,
120 peripheral blood samples (10 mL) were collected into vacutainers with no additive via jugular
121 venipuncture on d 30, 33, 35, 37, 40, and 50 for biochemical analyses. Serum was harvested by
122 centrifuged at 1,200 x g for 15 min and stored at -40°C for later analysis. Rectal temperatures
123 were collected daily at 0800 h from d 30 to 40 using a calibrated, hand-held thermometer (GLA
124 M700 Digital Thermometer, San Luis Obispo, CA).

125 ***Laboratory Analyses***

126 The oxidative burst of polymorphonuclear neutrophils in response to *Mannheimia*
127 *haemolytica* (ATCC #43270) was analyzed. The *M. haemolytica* was grown overnight in tryptic
128 soy broth + 5% defibrinated sheep blood and quantified by serial dilution and spread-plating on
129 tryptic soy agar + 5% defibrinated sheep blood. The bacteria were heat-killed at 60°C for 30
130 min, washed, and resuspended at 10^9 colony forming units/mL in 1X PBS. Bacteria were
131 aliquoted into 1 mL volumes and stored at -80°C. Two hundred microliters of whole blood from
132 each steer was aseptically transferred into the bottom of a 1.7-mL microcentrifuge tube and
133 placed in an ice bath for 10 min. Forty microliters each of a 100 μ M working concentration of
134 dihydrorhodamine and the *M. haemolytica* were added to each tube, vortexed thoroughly, and
135 then placed in a 38.5°C re-circulating water bath where they were incubated for 10 min. After
136 completion of incubation, the samples were removed from the water bath and placed
137 immediately in an ice bath for 10 min to suspend the reaction at a constant rate in all samples.
138 Erythrocytes were hypotonically lysed and remaining leukocytes washed once with 1X PBS.

139 Leukocyte suspensions were analyzed by single color flow cytometry on a Cell Lab Quanta SC
140 flow cytometer (Beckman Coulter, Fullerton, CA). Data are reported as the percentage of
141 neutrophils, as well as the geometric mean fluorescence intensity of the positive neutrophil
142 population. The neutrophil population was determined from the scatter plot of electronic volume
143 and side scatter light characteristics.

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

156 Peripheral blood mononuclear cell cultures (2×10^6 cells/mL) were cultured in RPMI
157 1640 and 10% autologous serum and 1% penicillin/streptomycin and stimulated at a final
158 concentration of 5 ng/mL recombinant interferon- γ (Invitrogen, Carlsbad, CA) and either 0, 0.01,
159 or 5 μ g/mL of lipopolysaccharide (*Escherichia coli* 0111:B5; Sigma, St. Louis, MO) for 24 h
160 after which the supernatant was collected and stored at -80°C until analysis of tumor necrosis

161 factor- α concentrations using a commercially available ELISA (Thermo Scientific, Waltham,
162 MA).

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

169 ***Statistical Analyses***

170 *Ex vivo* immunological analyses on d 28 were analyzed by ANOVA using the general
171 linear model procedure of SAS (v.9.2, SAS Inst. Inc., Cary, NC, USA) with treatment as the
172 main effect. All repeated, continuous data were analyzed by restricted maximum likelihood
173 ANOVA using the MIXED procedure of SAS (v.9.2, SAS Inst. Inc., Cary, NC, USA). A linear,
174 mixed model with the fixed effects of treatment, sampling time, and the interaction of treatment
175 x sampling time was fitted. The ante-regression covariance structure for the within-subject
176 measurement was used. Steer nested within treatment was the random effect. For the
177 biochemical responses following the IBRV challenge, samples collected on d28 were used as a
178 covariate in the model. Means separations were performed at each time using a sliced-effect
179 multiple comparison approach with a Tukey-Kramer adjustment. All data were tested for
180 normality of the residuals by evaluating the Shapiro-Wilk statistic using the UNIVARIATE
181 procedure of SAS (v.9.2, SAS Inst. Inc., Cary, NC, USA). Contrasts were performed on all data
182 to determine the effects of energy source (30AL vs. 70AL and 70RES) and energy intake (70AL
183 vs. 30AL and 70RES). The interactions of period x sampling time, period x treatment, and

184 period x sampling time x treatment were evaluated and found to be non-significant ($P > 0.10$);
185 therefore, period was not included in the model. Least squares means (\pm SEM) are reported
186 throughout. A treatment difference at $P \leq 0.05$ was considered significant, and $0.05 < P \leq 0.10$
187 was considered a tendency.

188

189 **RESULTS**

190 *Preconditioning Performance and Serum Constituents*

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

197 *Preconditioning Immune Responses*

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

207 *Infectious Bovine Rhinotracheitis Virus Challenge*

208 Intranasal inoculation with 2mL of IBRV ($10^{8.5}$ tissue culture infected dose₅₀/mL) using
209 the MAD® device (Wolfe Tory Medical, Inc., Salt Lake City, UT) on vaccinated calves caused
210 an acute-phase response as evidenced by increased rectal temperatures (Figure 2); however, no
211 differences were observed in the febrile responses among treatments. Dry matter intake data are
212 presented in Figure 3. When cattle were moved from group pens into the individual stanchions
213 on d 28, DMI decreased on both 70AD and 30AD treatments, but cattle in these treatments had
214 returned to preconditioning intakes by d 30. As a result of the experimental design, steers on the
215 70RES had lower DMI from d30 to 33; however, once all steers were offered the 50%
216 concentrate diet *ad libitum* on d 34, there were no differences in DMI during the remaining 7 d
217 the steers were individually penned in the stanchions. When cattle were moved back into their
218 original group pen on d 40, DMI decreased in all treatment groups. From d 43 to 47, cattle
219 previously fed 70RES diet had a lesser decrease in DMI and subsequently recovered more
220 quickly to pre-group penning DMI ($P < 0.05$; Figure 3).

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

228

229 **DISCUSSION**

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

237 No differences were observed among treatments in either the oxidative burst capacity of
238 neutrophils or the bactericidal activity of whole blood to *M. haemolytica*. The lack of treatment
239 effects could be the result of similar concentrations of blood glucose. Newbould (1973)
240 observed that when serum glucose concentrations increased, the phagocytic actions of blood
241 leukocytes increased. The current data contrast those of Sun et al. (2001), who observed that
242 mice fed 40% calorie restriction for 6 mo had lower macrophage phagocytic function; however,
243 it should be noted that the energy restricted mice in the study of Sun et al. (2001) did not grow
244 over the 6-mo study period, which contrasts with the cattle fed the lower energy diets in the
245 current study. In Holstein cows, plasma non-esterified fatty acid concentrations greater than 400
246 $\mu\text{Eq/L}$ 1 wk before parturition were associated with large reductions in myeloperoxidase activity
247 of neutrophils (Hammon et al., 2006). Taken together, these data suggest that low energy intake
248 of animals, no growth in young animals or negative energy balance in adult animals, decreases
249 neutrophil functions, whereas decreased energy intake in young, growing animals does not
250 influence neutrophil or bactericidal responses.

251 Secretion of the pro-inflammatory cytokine, TNF- α , by peripheral blood mononuclear
252 cells stimulated with LPS was lower in cattle fed either 30AD or 70RES compared with 70AD.

253 In agreement with this finding, mice in the study of Sun et al. (2001) had lower mRNA
254 expressions of pro-inflammatory cytokine genes as well as secretion of interleukin-6 when
255 macrophages were stimulated *ex vivo* with LPS. In that same study, pro-inflammatory response
256 was evaluated following cecal ligation and puncture to induce polymicrobial septicemia.
257 Following the cecal ligation and puncture, mice on the calorie-restricted diet displayed increased
258 concentrations of systemic TNF- α , and survival analysis indicated a more rapid mortality in the
259 calorie-restricted mice. These data suggest that calorie restriction decreases the pro-
260 inflammatory response of macrophages in response to Gram-negative bacteria, which might
261 allow for growth of the pathogen once it has evaded the physical barriers of the immune system,
262 thereby resulting in a greater degree of septicemia (Sun et al., 2001). In contrast to our data,
263 Reuter et al. (2008) observed an increased concentration of serum TNF- α following an
264 intravenous LPS challenge in cattle fed lower-energy (70RES) and higher-roughage (30AD)
265 diets. The reason for the discrepancies observed between the current data and that observed by
266 Reuter et al. (2008) is not known, but could be reflect the use of different experimental models.
267 Reuter et al. (2008) used an *in vivo* LPS challenge, whereas isolated peripheral mononuclear
268 cells stimulated with LPS *ex vivo* in the current study. The *ex vivo* model might reflect the
269 sensitivity of the monocyte/macrophages to LPS, whereas the *in vivo* challenge may be more
270 indicative of how an animal will respond once they have become septicemic. The role that either
271 the results from *in vivo* or *ex vivo* models plays in defining resistance to disease of cattle is not
272 known and should be addressed with future research.

273 Decreased pro-inflammatory responses observed in cattle fed lower energy intakes might
274 have negative effects on resistance to disease in cattle that are exposed to pathogens. The
275 decreased secretion of TNF- α could potentially decrease the ability of the cattle's immune

276 system to recognize, sequester, and eliminate the pathogen. In dairy cattle, data indicate that the
277 severity of *Escherichia coli* mastitis is inversely related to the speed of neutrophil recruitment
278 into the mammary gland (Hill, 1981). Lower secretion of TNF- α could result in less neutrophils
279 and other effector leukocytes being recruited to tissue sites of infection, which could allow
280 growth of the pathogen. If pathogen growth continues, there will be more pathogen-derived
281 immunogens for a subsequent systemic inflammatory response. This effect was likely observed
282 in the study by Sun et al. (2001), in which *ex vivo* pro-inflammatory cytokine responses were
283 lower in energy restricted mice; however, those mice had greater cytokine responses and higher
284 mortality following a cecal ligation and puncture septicemia model. Therefore, an ideal acute-
285 phase response within an animal would be a rapid and robust response to sequester and eliminate
286 the pathogen, followed by a rapid down-regulation of the response to prevent excessive host
287 tissue damage. These data indicate that cattle fed higher energy and to an extent higher
288 concentrate diets may have a more desirable pro-inflammatory immune response phenotype.

289 During the IBRV challenge, no differences were observed among treatments in rectal
290 temperatures; however, the challenge caused a mild febrile response from d 33 to 35. Therefore,
291 using vaccinated cattle challenged intranasally with the MAD[®], Mucosal Atomization Device
292 (Wolfe Tory Medical, Inc., Salt Lake City, UT) caused a mild acute-phase response. Similar to
293 the rectal temperatures, DMI was not influenced following the IBRV challenge while cattle were
294 individually penned. Nonetheless, once cattle were moved back to their original pen on d 40,
295 DMI was decreased in all treatments, possibly because of the stress associated with handling
296 and/or redefining the social dominance structure in the pen. From d 43 to 47, DMI in the 70RES
297 cattle was greater than by cattle in other 2 treatments. The reason(s) for the difference in DMI
298 observed during this period is not known, but it could be the result of cattle fed restricted

299 quantities of feed being conditioned to cope with the social stress because they were
300 “programmed” to have to compete for available resources. This is an area that warrants further
301 research, as it might be an effective management strategy to keep cattle on feed during periods of
302 stress.

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

308 Serum urea nitrogen concentrations were elevated on d 33 to 40 in steers fed the 70AD.
309 Orr et al. (1988) observed an increase in the concentration of serum urea nitrogen after cattle
310 were challenged with IBRV. The greater concentrations of serum urea nitrogen in 70AD could
311 have resulted from elevated catabolism of whole body protein pool (Nielsen et al., 2005). Whole
312 body nitrogen balance is decreased during infection, and the higher response in 70AD steers
313 could be a reflection of a stronger response. Although intake of crude protein was not equal
314 among treatments due to the 70RES consuming less dry matter during the preconditioning
315 period, the intake of crude protein among steers fed 70AD were not influenced by time during
316 the IBRV challenge (data not shown). Therefore, it is unlikely that the elevated serum urea
317 nitrogen concentrations observed in these steers on d 33 to 40 was associated with changes in the
318 intake of crude protein. Similar to the serum urea nitrogen concentrations, on d 35 the steers fed
319 the 70AD had increased concentrations of serum non-esterified fatty acids. Whitney et al. (2005)
320 reported similar findings when they challenged steers with IBRV. The diets fed by Whitney et
321 al. (2005) were 3 high-roughage diets and a 70% concentrate diet. Steers fed the 70%

322 concentrate diet had greater non-esterified fatty acid concentrations than steers fed the high-
323 roughage diets. The increased non-esterified fatty acids observed in cattle fed 70AD could be
324 connected to production of pro-inflammatory cytokines, as they can stimulate hormone-sensitive
325 lipase (Coppack, 2001). The present data are consistent with the *ex vivo* sensitivity of
326 monocyte/macrophages to LPS. As noted previously, we cannot completely rule out that the
327 differences observed in the 70AD cattle following the IBRV were caused by the individual
328 penning of the steers from d 28 to 40 or that all cattle were switched to a 50% concentrate diet on
329 d 34.

330 Our data are consistent with the suggestion made by Rivera et al. (2005) that feeding
331 higher concentrate diets might produce a more aggressive acute-phase response in cattle when
332 exposed to a pathogen. The observations of Lofgreen et al. (1975) that cattle fed lower energy
333 diets had decreased morbidity might reflect lower expression of pro-inflammatory cytokines.
334 Cattle with a lower pro-inflammatory cytokine response could display fewer of the usual clinical
335 signs that accompany disease and are known as sickness behaviors. Therefore, BRDC in cattle
336 fed higher-roughage diets might go undetected. Other data support the fact that the feedlot
337 industry needs more sensitive measures of morbidity, as Gardner et al. (1999) reported that 68%
338 of cattle not treated for BRDC during the finishing period had pulmonary lesions at slaughter
339 indicative of a previous incidence of BRDC.

340 In conclusion, a higher-energy diet (lower in roughage) might be the most appropriate
341 diet to feed preconditioning and stressed, newly received cattle. The more aggressive acute-
342 phase response could benefit cattle by allowing the rapid recognition, recruitment, and
343 elimination of a potential pathogen. Moreover, a more visual display of signs associated with
344 infection and disease would allow the cattle to be detected, removed from their pen, and

345 administered appropriate medical treatments. In addition, the observation that steers previously
346 fed 70RES were able to return to feed more quickly when being group penned needs to be
347 further investigated, as this could be a management strategy employed during the
348 preconditioning period that could help cattle cope with the social stress on entering a feedlot.

349

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402

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

Item	% Concentrate		
	70	30	50
Alfalfa Hay	15	35	25
Cottonseed Hulls	15	35	25
Steam-flaked Corn	52.5	12.7	32.6
Cottonseed Meal	8.5	8.5	8.5
Urea	0.5	0.3	0.4
Molasses	4	4	4
Supplement ¹	2.5	2.5	2.5
Fat - Yellow grease	2	2	2
Analyzed composition ²			
CP, %	13.15	13.52	12.77
ADF, %	17.48	39.10	35.64
NDF, %	25.26	49.44	47.80
Ca, %	0.54	0.73	0.60
P, %	0.32	0.27	0.27
Mg, %	0.21	0.23	0.21
K, %	1.10	1.85	1.72
Calculated composition ³			
NE _m , Mcal/kg	1.97	1.24	1.35
NE _g , Mcal/kg	1.27	0.52	0.64

403

404 ¹Supplement contained (DM basis): 66.382% cottonseed meal; 0.500% Endox (antioxidant;
 405 Kemin Industries, Des Moines, IA); 0.647% dicalcium phosphate; 10.000% potassium chloride;
 406 0.333% manganous oxide; 4.167% ammonium sulfate; 15.000% salt; 0.0022% cobalt carbonate;
 407 0.196% copper sulfate; 0.0833% iron sulfate; 0.0031% ethylenediamine dihydroiodide; 0.125%
 408 selenium premix (0.2% Se); 0.986% zinc sulfate; 0.0099% vitamin A (1,000,000 IU/g); 0.157%
 409 vitamin E (500 IU/g); 0.844% Rumensin (Elanco Animal Health, Greenfield, IN); and 0.56%
 410 Tylan (Elanco Animal Health). Concentration values in parentheses are expressed on a 90% DM
 411 basis.

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

414 ³Composition calculated from the tabular values in NRC (1996).

415

416

Table 2. Performance during the 28-day preconditioning period¹

Item	70AD	30AD	70RES	SEM	Trt	Contrasts ¹
Pens, n	4	4	4	-	-	-
Initial BW, kg	283	284	285	6.5	-	-
BW d 28, kg	317	310	308	5.2	-	-
ADG, kg	1.2	0.91	0.82	0.069	0.01	1
DMI, kg	8.9	9.6	5.9	0.26	0.0001	1,2
NEg intake, Mcal/d	10.7	7.1	7.1	0.30	0.0001	1,2
Serum glucose, mg/dL	101.6	92.4	92.0	5.92	0.42	-
Serum urea nitrogen, mg/dL	7.1	7.8	6.5	0.76	0.43	-

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 NE_g 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 NE_g 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_g intake of the 30% concentrate treatment.

Table 3. Immune competence measurements on d 28 of the preconditioning period¹

Item	70AD	30AD	70RES	SEM	Trt	Contrasts ¹
Steers, n	16	16	16	-		
<i>Mannheimia haemolytica</i> killing, %	72	69	69	6	0.90	-
Oxidative burst neutrophils, %	81.9	76	77.5	5.8	0.49	-
Oxidative burst neutrophils, geometric mean fluorescence	96.6	94.8	89.2	13.70	0.92	-
Tumor necrosis factor- α , pg/mL ³	2264	1241	1887	269.8	0.03	1,2

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 NE_g 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 NE_g 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_g 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 μ g/mL of lipopolysaccharide in the culture media.

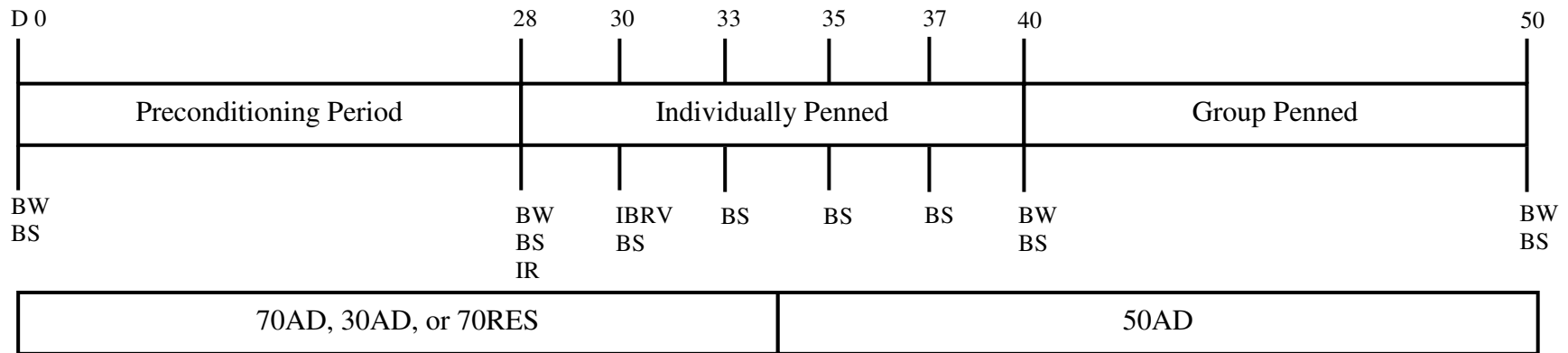


Figure 2

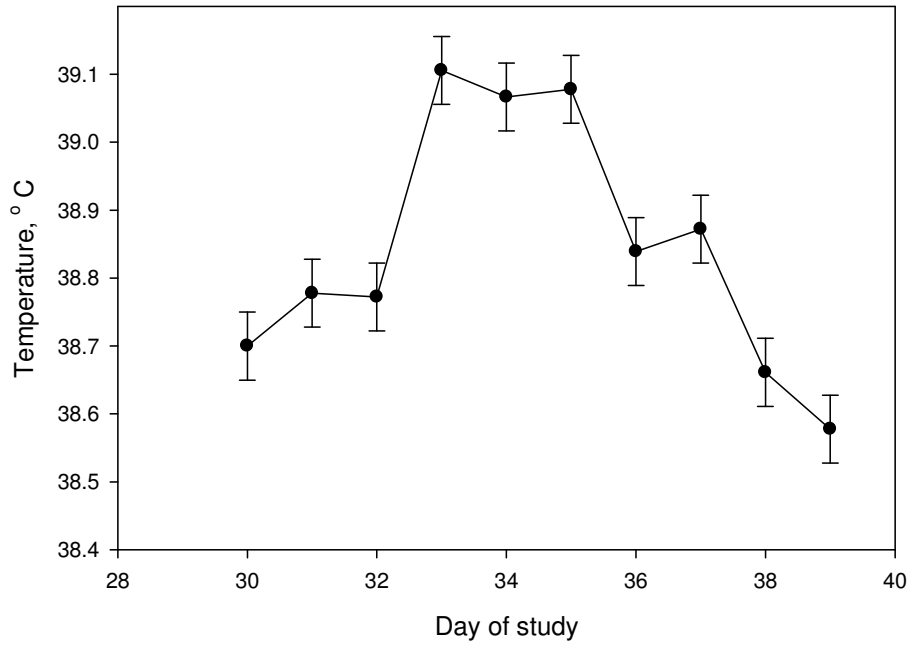


Figure 3

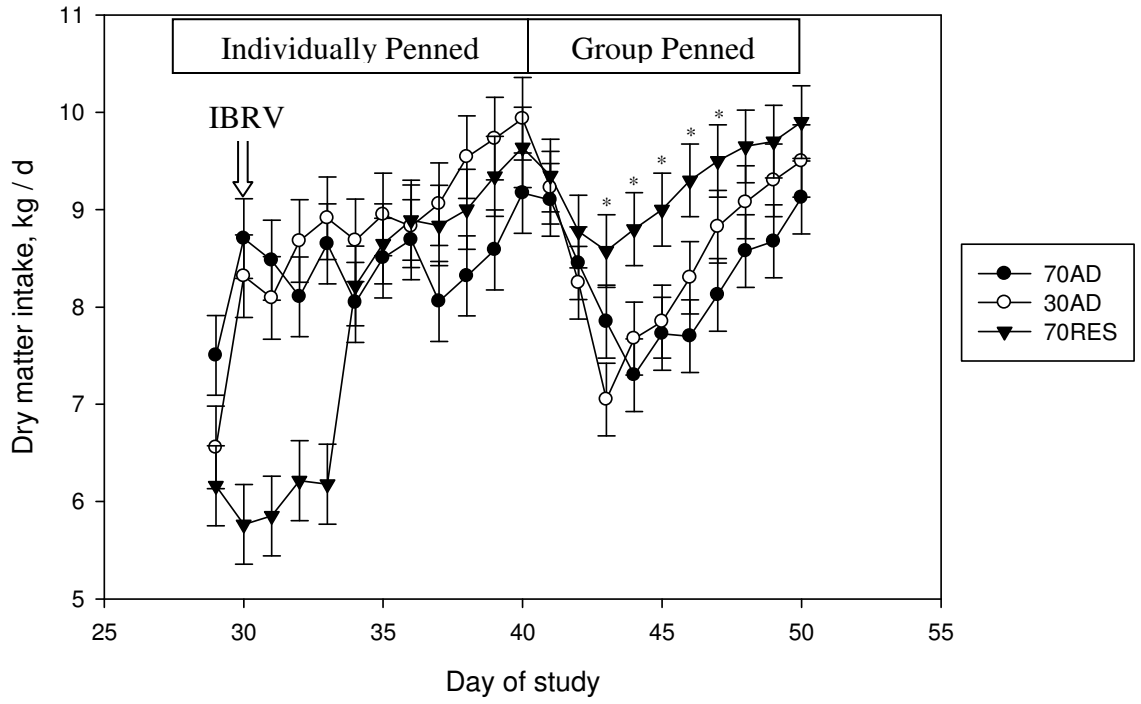


Figure 4a

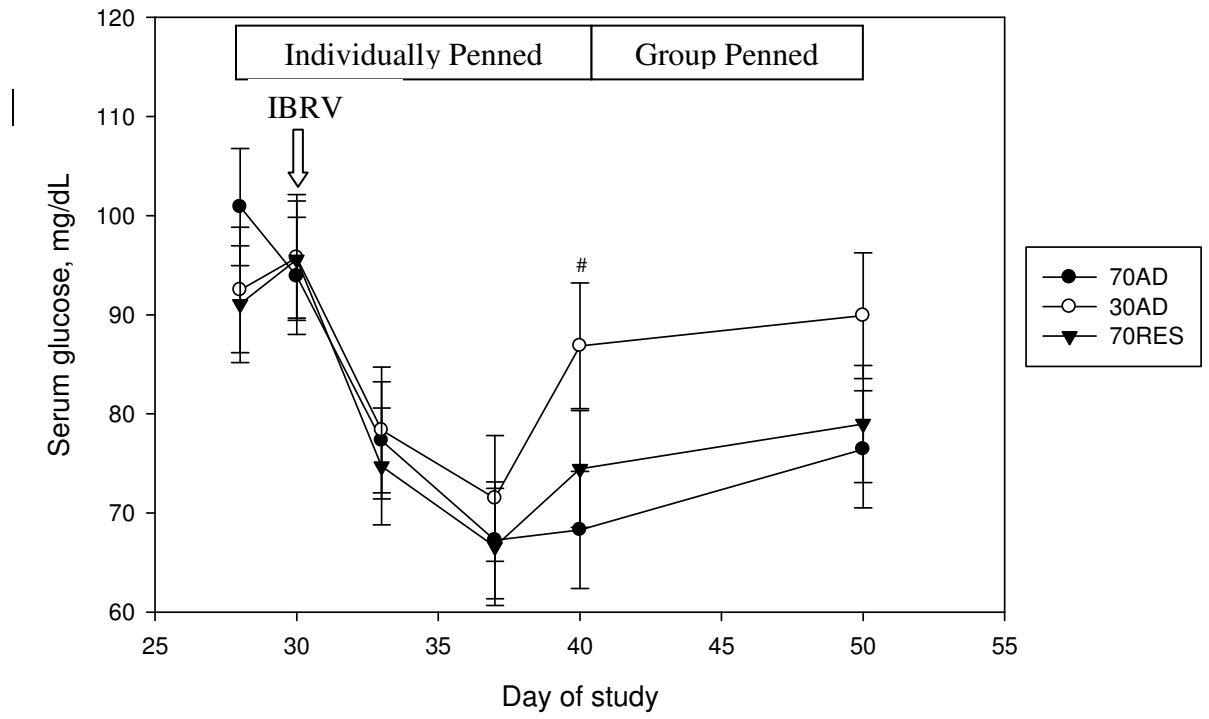


Figure 4b

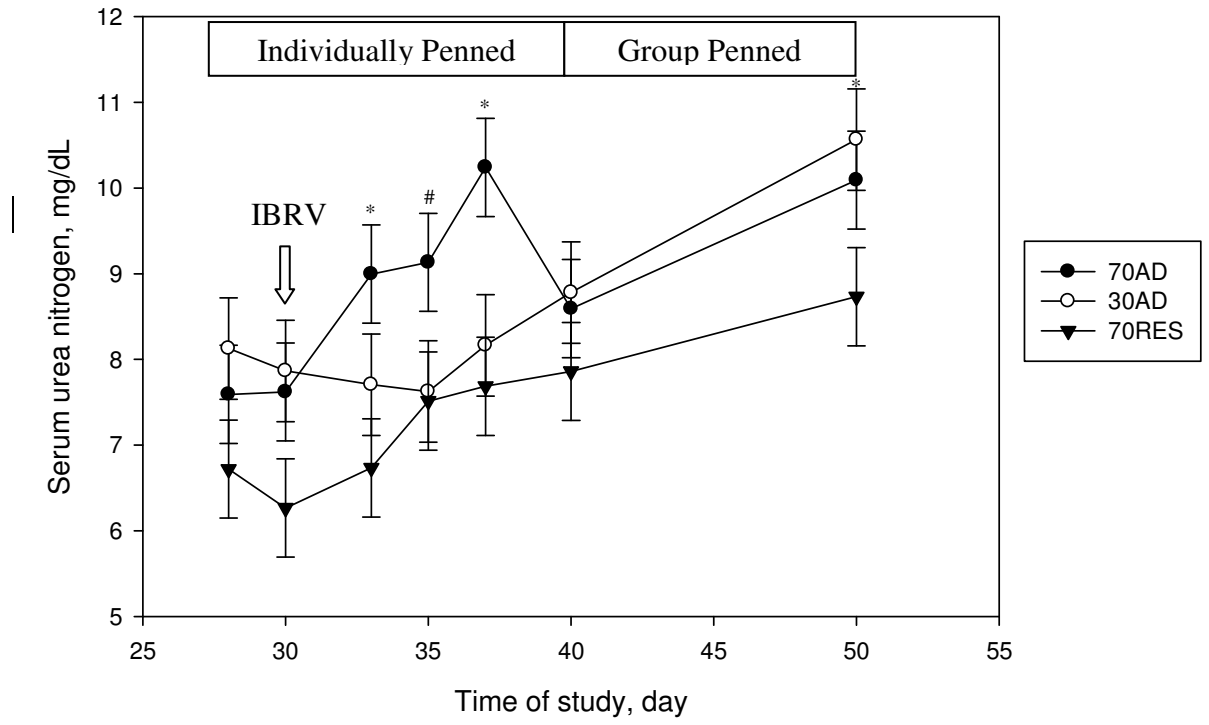


Figure 4c

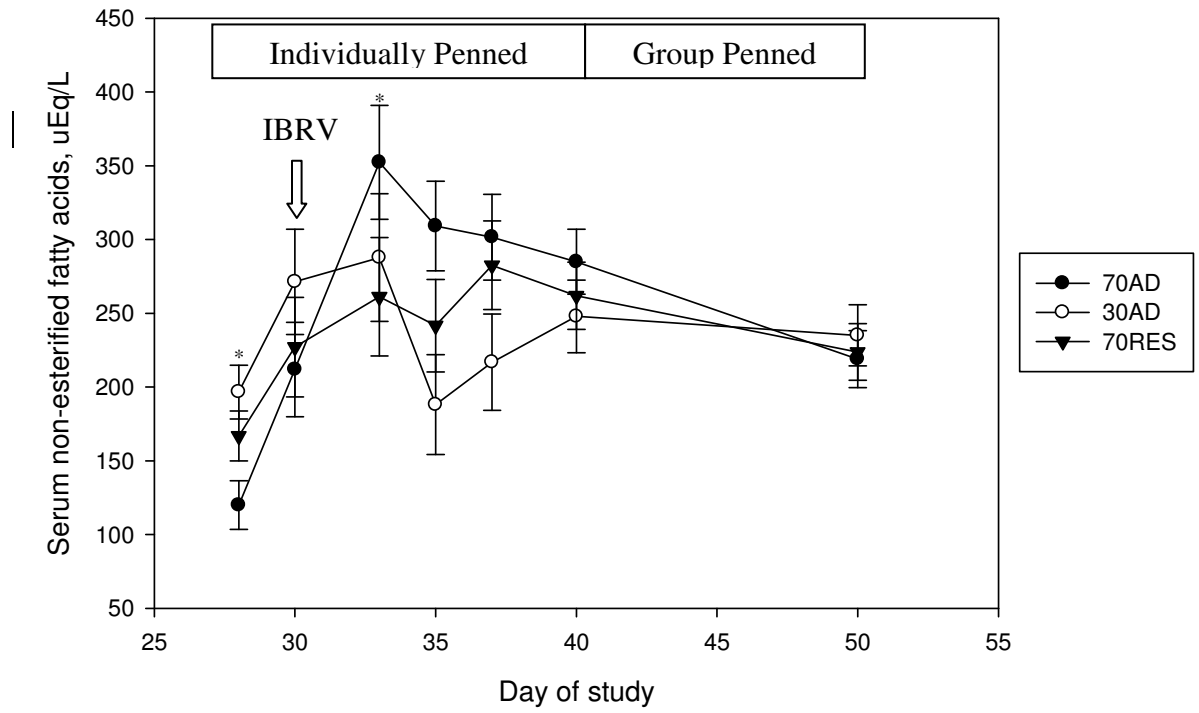


Figure 1: Timeline and sampling dates of the experimental plan. Steers were fed either a 70% concentrate diet *ad libitum* (70AD), a 30% concentrate diet *ad libitum* (30AD), or the 70% concentrate diet restricted to the NE_g intake of the 30AD (70RES) for 34 d. All steers were switched to a 50% concentrate 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 \pm 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 \pm 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 treatment (70RES; closed triangle, n = 16). No differences between treatments were detected. # = ($P \leq 0.10$). Error bars represent \pm 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). * = ($P < 0.05$); # = ($P \leq 0.10$). Error bars represent \pm 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 \pm SEM.