Effects of an Intratracheal *Mannheimia haemolytica* Challenge on Intake and Nitrogen Balance in Fed or Fasted Steers

Burciaga-Robles, L.O., D.L. Step, B.P. Holland, M. Montelongo, A.W. Confer, J.N. Gilliam, C.L. Goad and C.R. Krehbiel

Story in Brief

Bovine Respiratory Disease (BRD) is the most common disease in stocker and feedlot cattle. Although many efforts have been made to decrease the economic losses associated with BRD, in the last several years an incremental increase in mortality associated with this disease has been reported. In addition to losses associated with morbidity and mortality, the greatest concern for the beef industry might be related to decreased performance and carcass quality once an animal has had a BRD event. The objective of this study was to understand the metabolic changes associated with BRD and fasting. To achieve our objectives, twenty-four multi-catheterized steers were intratracheally challenged with *Mannheimia haemolytica*, one of the most common bacterial pathogens involved in BRD. The intratracheal disease challenge with *M. haemolytica* was an effective model to simulate the onset of BRD based on an increase of antibody concentration for the whole bacteria and for the Leukotoxin produced by this microorganism. During the first 4 d after challenge, the animals that were fasted and challenged had a decreased DMI compared to their healthy/non-fasted counterparts, and this difference was still detectable 14 d post challenge. Nitrogen retention was affected by the dietary treatment whereas the fasted animals had lower nitrogen retention compared to the fed group. In addition, animals challenged with *M. haemolytica* tended to have lower nitrogen retention during the course of the first week of the experiment compared with controls. The increased protein synthesis required by the animals in the form of acute phase proteins and antibodies, decreased dry matter intake, and lower nitrogen balance associated with fasting and BRD results in a greater metabolic stress for sick compared to healthy animals.

Introduction

Bovine Respiratory Disease is the result of a complex, multifactorial interaction of stressors, animal susceptibility, and respiratory pathogens. The immune response to a pathogen not only decreases feed intake (Klasing et al., 1987), but also alters the digestion, absorption, metabolism, and excretion of nutrients (Kuotsos and Klasing, 2002). Therefore, the availability of nutrients for production purposes in food animals is decreased in animals with BRD. In beef cattle, the economic losses associated with BRD have been well documented; Gardner et al. (1998) reported that the net return of a healthy steer compared to a sick steer during the finishing phase was $73.78, of which 21% was associated with medicine expenses and the remainder due to lower (8.4%) carcass weight. Based on the available literature, emphasis has been placed on strategies to prevent (McKever and Rege,1999; Potter et al., 2004), diagnose (Cole et al., 1997; McKeever and Rege, 1999; Coghe et al., 2000), and/or treat (Thomson, 1998; Doherty et al., 2001; Nowakowski et al., 2004) BRD. However, according to Loneragan et al. (2004), the incidence and mortality associated with this disease increased 9% each year from 1994 to 1997 and accounted for a higher percent of the total animals dead in feedlots (52% to 67%, respectively) from 1994 to 2003. Although many pharmaceutical companies have developed products to treat BRD, this continued trend is most likely the result of our lack of understanding

of the pathophysiological events occurring during and after a BRD event. The results reported in this research report are a portion from an experiment designed to model the immunological, physiological, and metabolic changes associated with BRD.

Materials and Methods

Animals. This study was conducted at the Nutrition Physiology Research Center at Oklahoma State University. A total of 22 animals (initial BW=319.7+ 24.3 kg) with chronic indwelling catheters to measure blood flow, nutrient flux, and immune response components across the portal drained viscera (PDV) and liver were used for this study. The experiment consisted of two periods with 12 animals each. During the experimental adaptation period (28 d), a receiving diet formulated to meet or exceed nutrient requirements (NRC, 1996) was fed. Three days prior to the sampling period, animals were placed in metabolism crates where blood, feces, and urine were collected. In each period, animals were allotted to one of the following treatments:

1) Fed/Challenge - Animals were allowed ad libitum access to feed and were challenged (d 0) with a solution of 10 mL of $1x10^9$ CFU/mL of *Mannheimia haemolytica* followed by 20 mL PBS solution via a tracheal tube.

2) Fed/Control - Animals were allowed ad libitum access to feed. On d 0, 20 mL of the PBS solution without *M. haemolytica* was given via a tracheal tube.

3) Fasted/Challenge – Twelve hours before the challenge, feed was removed from animals in the fasted groups. At 8:00 AM on Day 0, steers were challenged with a solution of 10 mL of $1x10^9$ CFU/mL of *M. haemolytica* followed by 20 mL PBS solution via a tracheal tube. Animals were fed 60 h post challenge.

4) Fasted/Control - Feed was removed for 72 h as described for the Fasted/Challenge group. In addition, 20 mL of the PBS solution without *M. haemolytica* was given via a tracheal tube.

During each experimental period, animals were housed in metabolism crates on d -3, -2,-1, 0, 1, 2, 3, 4, 6, 7, 8, 14, 15, and 16. During these days, total fecal output was weighed, sampled, and frozen until further analysis. For total urine output, 500 mL of HCl (6N) were added daily to the urine containers; after the 24-h collection period the containers were weighed, pH was recorded, and a subsample was collected and frozen until further analysis.

Fecal samples were weighed and freeze dried to determine total fecal DM output. Subsequently, samples were ground using a Wiley mill to pass a 2-mm screen and OM (AOAC, 1990) and N (model FP-2000, Leco Corp., St. Joseph, MI) were analyzed. Urine samples were thawed and 2 mL of sample were used to determine N with the Kjeldahl procedure (AOAC, 1990). All lab values were adjusted to the daily composite outputs for the appropriate calculations.

Before the disease challenge was performed, a peripheral blood sample was collected from the mesenteric arterial catheter and was kept overnight at 4°C before centrifugation and harvest of serum. The sample was frozen at -40°C and was used to measure basal antibody concentrations to *M. haemolytica* whole bacterial cell and *M. haemolytica* Leukotoxin. Blood was also obtained on d 4 and 15 to evaluate the immune response of animals to our disease challenge. The

antibodies for *M. haemolytica* whole cell and Leukotoxin were determined using an enzyme-linked immunosorbent assay.

Response Variables. Response variables measured in this experiment were: dry matter intake (DMI), nitrogen intake (NI), urinary nitrogen excretion (UNE), fecal nitrogen excretion (FNE). Nitrogen retention (NR) was calculated as the difference between NI and the sum of UNE+FNE. All values are expressed in grams.

Statistical Analysis. Each experimental period was divided into three phases:

a) Pre-challenge - Data from d -2 and -1 of the collection period were combined to determine the baseline for each of the response variables.

b) Acute response to treatments was determined from samples taken on d 0, 1, 2, 3, and 4.

c) Three-week response to treatments was determined from sample composites from d 1, 2, and 3 (Wk 1), d 6, 7, and 8 (Wk 2), and d 14, 15, and 16 (Wk 3).

All baseline determinations were analyzed and no significant diet, disease, or interaction effects were observed. Repeated measures analysis was performed for the daily measurements using mixed model methods to model the covariance structure (SAS/MIXED, SAS Institute, 2003). Repeated measures over the 3-wk period were analyzed similarly. A first-order autoregressive correlation structure was adopted for all response variables in both the daily (acute) and 3-wk (long term) time period models.

Results and Discussion

Challenge Response. The intratracheal disease challenge with *M. haemolytica* was an effective model to simulate the onset of BRD based on an increase of antibody concentration for the whole bacteria (Figure 1) and for the leukotoxin (Figure 2) produced by this microorganism.

Dry Matter Intake. Animals in the challenged groups generally had lower DMI than Fed/Control steers during the acute model of the disease. After the 72-h fasting period the Fasted/Control steers consumed the same amount of DM as fed steers. However, an erratic pattern of intake was observed during the acute phase of the experimental period for the fasted groups that can also lead to decreased performance in a commercial feedlot setting (Figure 3). Fed/Challenged steers had lower ($P<.05$) DMI than Fed/Control steers on d 0, 1, 2, and 3 of the acute phase model.

In the three-wk model (Figure 4) a diet*week*disease interaction was observed ($P=.002$). The Fed/Control animals generally had constant intake as a reflection of no health or metabolic alterations during the experiment compared with the Fasted/Control and Fed/Challenged. However, no statistical difference was observed during wk 2 and 3 of the experiment. Fasted/Challenged animals had lower DMI during wk 2 in comparison with the remaining treatments.

Nitrogen Retention. During the acute phase of the experiment, N retention of fasted animals was lower ($P=.006$) compared with fed animals (Figure 5). In addition, steers challenged with a BRD pathogen tended ($P=.10$) to have a decreased amount of N retained during the experimental

period. During the acute phase of BRD challenge (Figure 6) a diet*day*disease interaction (P=.02) was observed for N retention. This interaction most likely resulted due to the Fasted/Challenged steers having lower (P<.05) N retention than Fed/Control animals on d 4. For the long-term effect no diet*day*disease interaction was observed (P=.20). A summary of the results for the acute (Table 1) and long-term responses (Table 2) are presented.

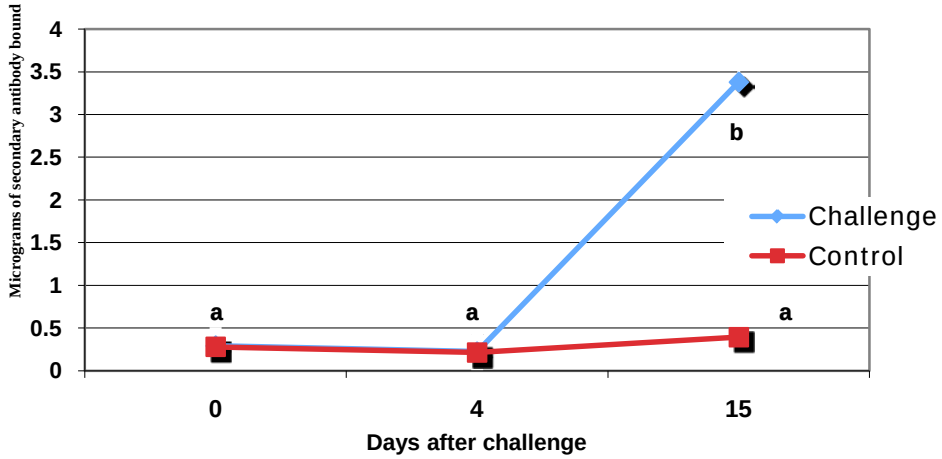

Figure 1. *M. haemolytica* whole cell antibody production in response to the intratracheal challenge performed to simulate a BRD event.

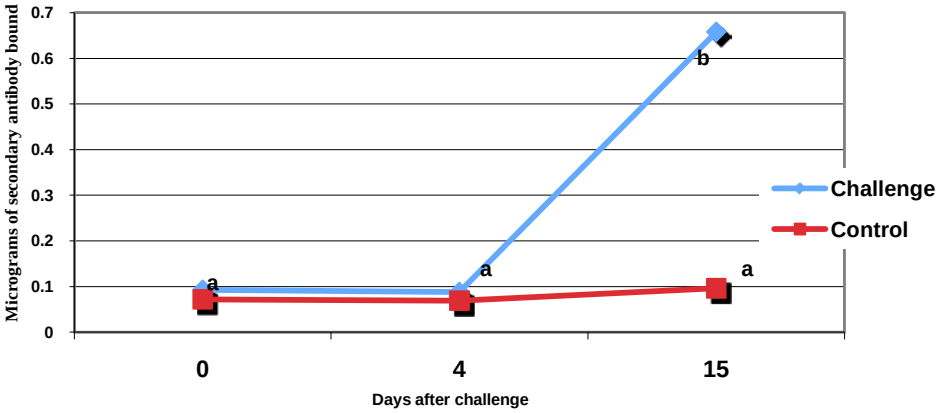

Figure 2. *M. haemolytica* leukotoxin antibody production as an indicator of bacterial replication in the lungs of animals challenged during the experiment.

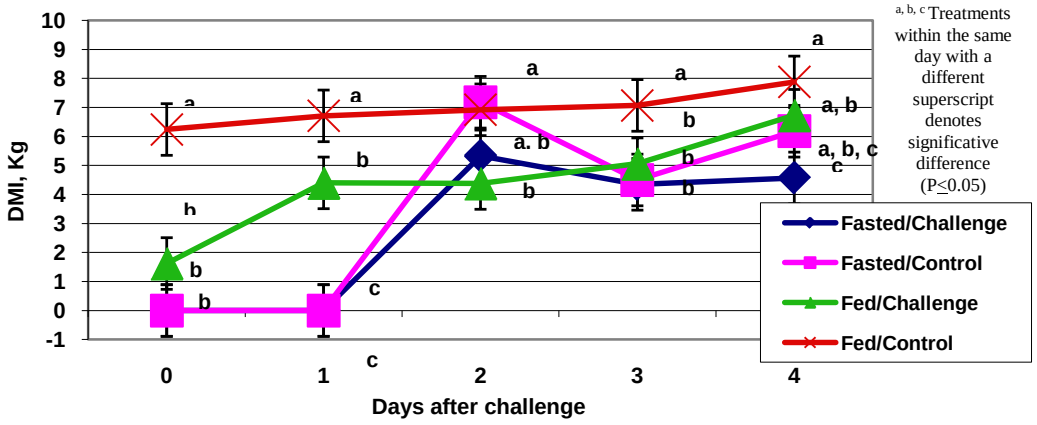

Figure 3. A diet*disease*day interaction (P=0.02) was observed for DMI during the acute response of the experiment.

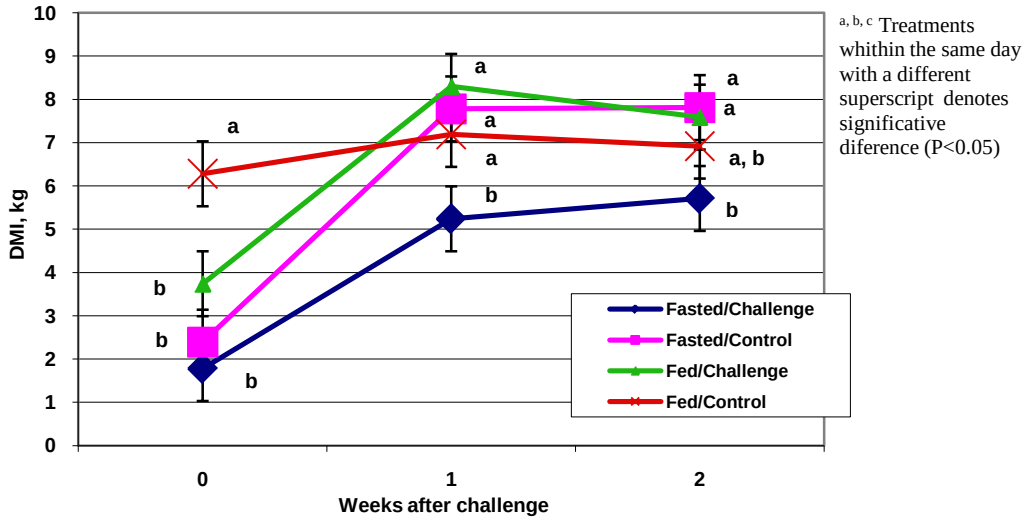

Figure 4. In the long-term period, a diet*disease*week interaction (P=0.02) was observed for DMI. During the second week after the challenge, animals that were fasted and challenged did not reach the same level of DMI as the remaining groups.

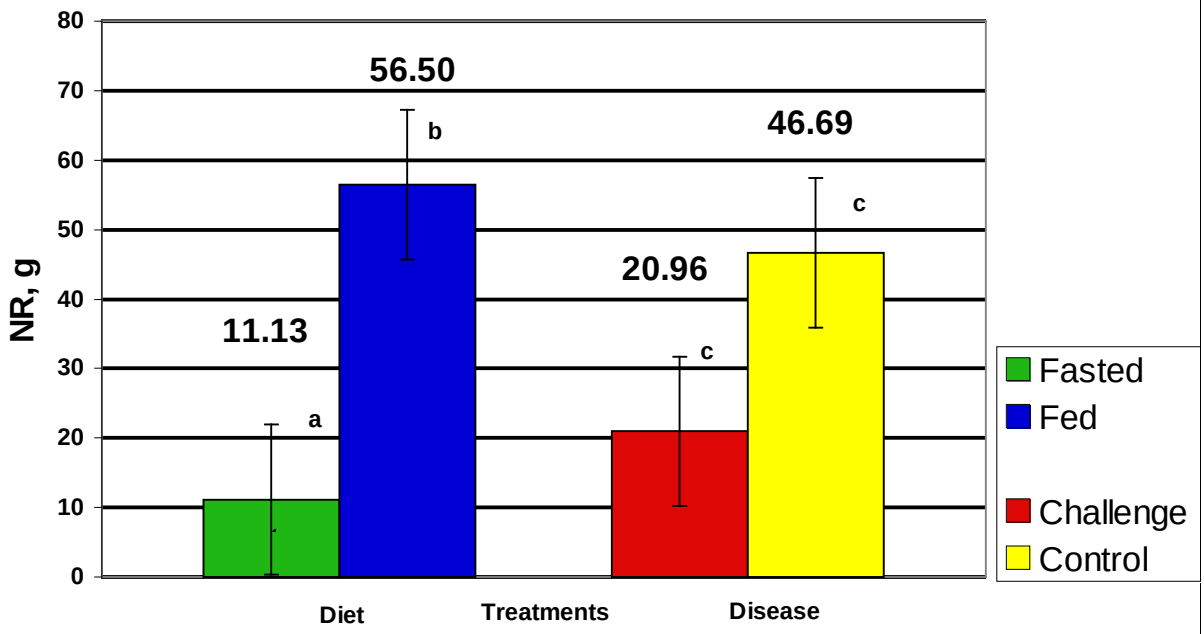

Figure 5. Nitrogen retention of fed vs fasted (P=0.006) and control vs challenged (P=0.10) during the acute phase of the experimental period

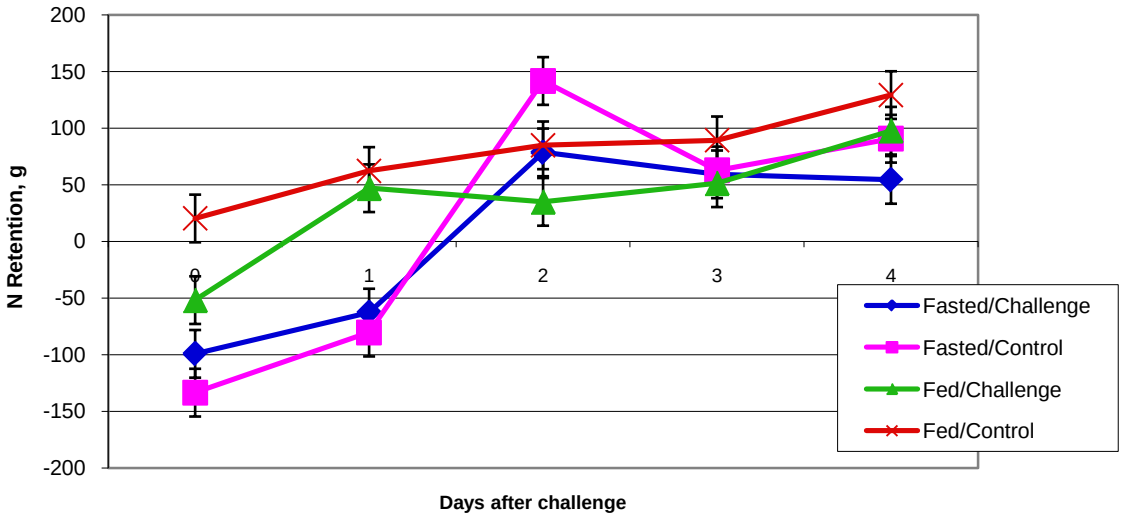

Figure 6. A diet*disease*day interaction (P=0.02) was observed for N retention during the acute response of the experimental period.

Table 1. Simple effect least squares means in response to the experimental treatments during the 4-d acute response

ITEM	DIET				DISEASE				INTERACTION P-VALUE	
	Fasted	Fed	SEM	P-value	Challenge	Control	SEM	P-value	Diet*Disease	Diet*Disease*day
DMI, g	3214	5813	490	.02	3649	5378	498	.02	.16	.02
NI, g	87.9	156	14.2	.002	99.8	144	14.2	.04	.23	.04
UNE, g	49.4	60.9	2.87	.009	52.1	58.2	2.87	.14	.61	.64
FNE, g	27.4	38.5	3.77	.048	26.8	39.1	3.77	.03	.22	.47
NR, g	11.1	56.5	10.8	.006	21.0	46.7	10.8	.10	.32	.09

DMI=dry matter intake; NI=nitrogen intake; UNE=Urinary Nitrogen excretion; FNE=Fecal Nitrogen excretion; and NR=Nitrogen Retention.

Table 2. Simple effect least squares means during the long term (3 wk) response of animals to experimental treatments

ITEM	DIET				DISEASE				INTERACTION P-VALUE	
	Fasted	Fed	SEM	P-value	Challenge	Control	SEM	P-value	Diet*Disease	Diet*Disease*day
DMI, g	5017	6713	400	.005	5349	6381	400.0	.07	.37	.002
NI, g	137	178	11.2	.02	146	169	11.2	.16	.24	.01
UNE, g	62.6	68.6	3.97	.30	59.4	71.8	3.97	.04	.06	.07
FNE, g	40.4	48.2	3.68	.14	37.6	51.0	3.68	.02	.67	.02
NR, g	34.2	61.5	7.40	.01	49.9	46.5	7.40	.79	.58	.20

DMI=dry matter intake; NI=nitrogen intake; UNE=Urinary Nitrogen excretion; FNE=Fecal Nitrogen excretion; and NR=Nitrogen Retention.

Conclusion

Our data suggest that cattle that are fasted and challenged with a BRD pathogen tend to have lower NR over the initial 14 d following the insult. Due to lower DMI, loss of performance in fasted and challenged steers may not be compensated for, at least within the first 3 wk following

the challenge. Therefore, once an animal is infected with BRD its growth potential is most likely compromised compared with healthy animals.

Literature Cited

Coghe, J. et al. 2000. The Veterinary Journal. 160: 139-146.

Cole, D.J. et al. 1997. Veterinary Medicine, May. Pp. 470-478.

Doherty, M.L. et al. 2001. Irish Veterinary Journal. 54:232-238.

Gardner, B.A. et al. 1998. Oklahoma State University Research Report.

Klasing, K.C. et al. 1987. J. Nutr. 117:1629-1637.

Koutsos E.A. and C.R Klasing. 2002. Maryland Nutrition Conference for feed manufacturers. USA.

Loneragan, G. et al. 2004. Proceedings of the Academy of Veterinary Consultants. Colorado Springs, CO. USA.

McKeveer, D.J. and J.E.O. Rege. 1999. Livestock Prod. Sci. 59:257-264.

Nowakowski, M.A. et al. 2004. Vet. Ther. 5:60-74.

Potter, A.A. et al. 2004. World Buiatric Congress Proceedings. Vancouver, Canada.

Thomson, P.N. et al. 1998. Onderstepoort Journal of Veterinary Research. 65:105-112.

Authors

Burciaga-Robles, Luis - Graduate Student.

Step, Douglas - Associate Professor, Veterinary Clinical Sciences

Holland, Ben - Graduate Student

Montelongo, Marie – Mgr. Res. Lab, Veterinary Pathobiology

Confer, Anthony - Professor, Veterinary Pathobiology

Gilliam, John - Assistant Professor, Veterinary Clinical Sciences

Goad, Carla - Associate Professor, Statistics Department

Krehbiel, Clinton - Associate Professor, Animal Science.