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Short communication

# Acute phase proteins in naturally occurring respiratory disease of feedlot cattle

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ABSTRACT

The aim of this study was to evaluate three acute phase proteins (APP) [haptoglobin (HPT), lipopolysaccharide binding protein (LBP) and transferrin (Tf)] in feedlot cattle with naturally occurring respiratory disease diagnosed by a calf health scoring chart (CHSC). Seventy-seven beef calves were observed for signs of Bovine Respiratory Disease (BRD) during the first 28 days after arrival at the feedlot. Fourteen cases and pen matched controls were selected based on the CHSC. BRD cases were defined as a score of $\geq$5, while controls were defined as a score $\leq$4. The mean CHSC score in cases was 6.9 which was significantly greater than the controls 2.8 ($P<0.01$). Mean plasma LBP and HPT concentrations were significantly greater in cases than controls ($P<0.01$). Our study results show that measurement of HPT and LBP could be useful in detecting respiratory disease in feedlot conditions. Transferrin concentrations between the two groups were not statistically different.



## 1. Introduction

Bovine respiratory disease complex (BRDC) is the most common disease among feedlot cattle in the United States, accounting for approximately 75% of feedlot morbidity and 50–70% of all feedlot deaths (Edwards, 2010). BRD causes between $800 million and $900 million annually in economic losses (Edwards, 2010; Wittum et al., 1996; USDA USDoA, 2007). Economic losses stem from prevention costs, treatment, death, associated costs and from diminished average daily gain, and decreased feed efficiency. The development of BRD is multifactorial and is influenced by a combination of host, environment and pathogen factors. Susceptibility to viral and bacterial pathogens is influenced by anatomy, physiology and management of beef cattle (Taylor et al., 2010). Respiratory disease diagnosis can be confirmed using an assortment of methods. Necropsy and detection of BRD pathogens remain the gold standard tests to diagnose BRD, however use of clinical scoring systems is widespread and useful for lay people to systematically evaluate and classify sick cattle. There have been six clinical scoring systems for BRD described in the literature which relies on evaluation of a variety of clinical signs and assignment of scores based on the evaluator's impression (Thomas et al., 1977; McGuirk, 2008; Panciera and Confer, 2010; Love et al., 2014).

Bacterial infections usually lead to a strong systemic acute phase response (APR) (Alsemgeest et al., 1994), due to the marked activation of monocytes and macrophages and release of inflammatory mediators, such as histamine, leukotriene's, prostaglandins, and pro-inflammatory cytokines. TNF-α, IL-1 and IL-6 play a pivotal role in activating hepatocyte receptors (HepG2 or Hep3B cells) to initiate the synthesis of various APPs (Heinrich et al., 1990, 1998; Gruys et al., 2005). Down-regulation of the hepatic APR is achieved by rapid hepatic removal of circulating cytokines (Heinrich et al., 1990, 1998) and release

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of IL-10 by the Kupffer cells which results in suppression of local IL-6 production (Knolle et al., 1995; Baumann et al., 1994). The initial APR is down regulated once the infective organisms are isolated, cellular debris is removed, and macrophages initiate tissue repair (Serhan and Savill, 2005).

HPT is a positive APP, and is the principal scavenger of free hemoglobin in blood (Murata et al., 2004). HPT elicits bacteriostatic effects by binding free Hemoglobin, thus making iron unavailable to proliferating bacteria. Iron utilization is a conserved process that has been identified in multiple bacterial pathogens, including Vibrio sp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella flexneri*, and *Bacillus subtilis*. (Skaar, 2010). Hpt also plays a key role in the recruitment of neutrophils in the early phase of inflammation (Riollet et al., 2000).

LBP is a soluble polypeptide that binds to bacterial lipopolysaccharide (LPS) and presents the LPS to pattern recognition receptors CD14 and TLR4, found on monocytes, macrophages and granulocytes. The presentation of LPS via LBP enhances the pro-inflammatory activity of these innate immune cells by 100 to 1000 fold (Fierer et al., 2002). LBP can also bind Lipoteichoic acid (LTA), a pathogen recognition molecule exposed on the cell wall of Gram-positive bacteria. The interaction between LBP and LTA triggers a pro-inflammatory cascade via TLR-2 activation (Mogensen, 2009).

Transferrin (Tf) is an iron-binding blood plasma glycoprotein found in mucosa. Tf has a single polypeptide chain of about 700 amino acids and contains two specific high-affinity $Fe^{3+}$ binding sites, for the transport of iron in the circulation (Ceron et al., 2005; Oliveira et al., 2014). Tf concentrations fall during an acute phase response, making Tf a negative APP (Nguyen, 1999). Acute phase response to infection or inflammation can lead to marked anemia (Feldman et al., 1981a,b). Tf elicits indirect bactericidal effects by binding to free iron, thus making iron unavailable to proliferating bacteria, in a response that may be mediated by lipocalin (Flo et al., 2004).

Previous studies using challenge models have shown that all three of these APPs may be useful biomarkers for bacterial pneumonia in calves, however no studies have been done to assess APP levels in naturally occurring pneumonia in a feedlot setting (Gånheim et al., 2003; Conner et al., 1989; Schroedl et al., 2001; Dowling et al., 2002; Heegaard et al., 2000; McNair et al., 1998). The purpose of this study was to evaluate three acute phase proteins (Haptoglobin, Lipopolysaccharide binding protein and Transferrin) in naturally occurring respiratory disease of feedlot cattle diagnosed by a calf health scoring chart (CHSC). The hypothesis was that there would be a significant difference between the acute phase protein levels evaluated, between the clinical cases and their matched healthy controls, as diagnosed by a CHSC.

## 2. Materials and methods

### 2.1. Animals

77 mixed breed beef steer calves of similar age and weight were purchased at three area livestock auction barns for enrollment in a nutritional study. Animals were transported 30–230 mile to and housed at the University of Missouri Beef Research and Teaching Farm (BRTF). At arrival the animals were weighed, ear tagged [both Allflex visual identification (VID) & Radio-frequency identification (RFID)], vaccinated with Bovi-Shield Gold® 5 and One Shot Ultra® 8 (Zoetis, Florham Park, NJ, USA) and treated with Cydectin® Pour-On (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA). All procedures were approved by the University of Missouri Institutional 116 Animal Care and Use Committee. The calves were part of a post-weaning feedlot performance and feed efficiency tests using the GrowSafe automated feeding system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). All calves were housed six per pen. Pens were of open construction, measuring 4.9 × 8.84 m. Frost-free waterers were shared between two pens, and feed bunks and slabs were under sloped roof shades (4.9 × 6.7 m).

**Table 1**
Adapted from McGuirk SM. 2008.

| **Calf health scoring chart** | | | |
|---|---|---|---|
| 0 | 1 | 2 | 3 |
| **Rectal temperature** | | | |
| 100–100.9 °F | 101–101.9 °F | 102–102.9 °F | ≥103 °F |
| **Cough** | | | |
| None | Induce single cough | Induced repeated coughs or occasional spontaneous cough | Repeated spontaneous cough |
| **Nasal discharge** | | | |
| Normal serous discharge | Small amount of unilateral cloudy discharge | Bilateral, cloudy or excessive mucus discharge | Copious bilateral mucopurulent discharge |
| **Eye scores** | | | |
| Normal | Small amounts of discharge | Moderate amount of bilateral discharge | Heavy ocular discharge |
| **Ear scores** | | | |
| Normal | Ear flick or head shake | Slight unilateral droop | Head tilt or bilateral droop |

### 2.2. Clinical examination

Bovine respiratory disease was diagnosed by an experienced animal technician based on a CHSC health scoring chart developed at the University of Wisconsin. The CHSC assigns each calf the sum of the nasal discharge, rectal temperature, cough scores and the greater one of the two scores from the ocular discharge and head/ear carriage (Table 1, McGuirk, 2008). Calves whose total score was ≥5 were categorized "BRD positive", while controls were defined as a score ≤4. The cattle were monitored closely on a daily basis and the scoring based on the CHSC was done in the mornings to mimic common practices in commercial in feedlots. Fourteen steers were selected as cases based on their score and were not diagnosed with any other diseases during the study. Control animals ($n = 14$) were selected from contemporary pen mates and sampled at the same time point.