A. PROPOSAL SIGNATURE COVER PAGE

Title: Transport, nutrient restriction, and effects on health and performance of cattle

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Signatures

PI:____________ Date: 2/21/2011

Department:____________ Date: 2/21/2011

B. PROJECT ABSTRACT

The impact of stress on health parameters is a major subject within livestock research, particularly because stressors are inevitably present in livestock production systems. As an example, the stressors associated with the transfer of beef calves from the ranch of origin to commercial feedlots are major predisposing causes for bovine respiratory disease (BRD), an infectious complex that costs the US cattle industry approximately $ 1 billion annually. Road transport, considered a major stressor during the aforementioned instance, has been shown to elicit inflammatory and acute-phase responses in overtly healthy cattle. These stress-induced immune responses, although imperative for early pathogenic control, are highly demanding and have been shown to be detrimental to subsequent cattle performance and health by increasing the incidence of BRD. Therefore, alternatives to modulate immune reactions elicited by stressful but routine management procedures are warranted to promote animal welfare and productivity. Preliminary data from our research group demonstrated that feed and water deprivation for 24 h stimulates an acute-phase protein reaction in healthy cattle, which may explain why cattle uninterruptedly transported for long distances experience such stress-induced immune responses. Therefore, the goal of the proposed research is to compare the effects of 24-h road transport or 24-h water/feed restriction on inflammatory, acute-phase, and subsequent performance responses of beef cattle. We expect to demonstrate that feed and water deprivation is a major cause for the unwarranted immune reactions elicited by road transportation, and with this resultant knowledge promote management alternatives to enhance animal health and productivity within cattle operations.

C. PROJECT PROPOSAL NARRATIVE

Introduction and Justification:

The importance of stress on health parameters emerged as one of the main subjects within animal and human research in the last decades (Glaser and Kiecolt-Glaser, 2005). The classical definition of stress response - sum of all reactions of an individual to factors that potentially influence its homeostasis - has been commonly used by scientists independent of research species (Moberg, 2000). The foundation for this concept emerged more than 70 years ago, when stress was first employed in the medical community by Hans Selye, who also proposed that an organism responds similarly to different types of stressors in an effort to maintain homeostasis (Selye, 1973). Indeed, although the physiologic consequences of stress are still not fully elucidated (Pacak and Palkovits, 2001), it has been demonstrated that stressors impact the immune system, as well as different responses within the body, mainly via the hypothalamic-pituitary-adrenal (HPA) axis (Elenkov, et al., 2000).

Cattle are inevitably exposed to stress during their productive life (Carroll and Forsberg, 2007). These include psychologic, physiologic, and physical stressors associated with management procedures currently practiced within beef and dairy production systems. A classical example occurs during transfer of beef calves from the ranch of origin to commercial feedyards. During this "feedlot transfer phase", calves are exposed to several stressors within a short period of time (Araujo et al., 2010). These include separation from the dam, social fear during commingling with different animals, and exposure to novel environments (psychologic stressors), injury, thermal stress and road transport (physical stress), as well as the resultant disruption in endocrine or neuroendocrine function (physiologic stress) characterized by activation of the HPA axis (Carroll and Forsberg, 2007). The combination of some or all of the aforementioned stressors is a major predisposing cause for the bovine respiratory disease (BRD) complex (Duff and Galyean, 2007), demonstrating the substantial and direct implication of stress on animal health. This complex, often comprised of viral, bacterial, and mycoplasmal infections (Ellis, 2001), is the most common and costly disease of feedlot cattle in the United States (NASS, 2006). In fact, BRD costs the US cattle industry approximately $ 1 billion annually (Griffin, 1997). These economical losses include, besides cattle mortality, costs associated with wasted feed resources, purchase of pharmaceuticals, and reduced performance of morbid cattle (Loerch and Fluharty, 1999). **Therefore, the long-term goal of our research program is to elaborate strategies that prevent stress-related illnesses elicited by routine cattle management procedures and, consequently, promote cattle welfare and productivity.**

Previous Work and Present Outlook:

Elevated cortisol is one of the main outcomes of the HPA reaction, independently if the stressor is from psychological, physiological, or physical nature (Crookshank et al., 1979; de Kloet et al., 2005; Carroll et al., 2009a). During episodes of chronic stress, sustained increases in circulating cortisol promote an anti-inflammatory and immunosuppressive response, mainly by decreasing synthesis of proinflammatory cytokines by immune cells (Kelley, 1988). Conversely, sharp increases in circulating cortisol, such as during acute stress, have been shown to elicit a temporary immune response, more specifically an inflammatory reaction. **Our research group, with financial support from the USDA/NIFA AHD program,** was the first to report transient increases in circulating cortisol and proinflammatory cytokines in overtly healthy beef cattle during the feedlot transfer phase (Cooke et al., 2011), as well as in beef cattle receiving intravenous infusion of corticotrophin-release hormone (Cooke and Bohnert, 2011).

Our results indicate that, besides their recognized immunosuppressive potential, stressors can also elicit an immune response in animals without the presence of a pathogen. Further, this response is characterized by proinflammatory cytokines, which in turn elicit a major component of innate immune system; the acute-phase response (Murata et al., 2004). Accordingly, physical, psychological, and physiological acute stressors are associated with hepatic synthesis and consequent circulating concentrations of acute-phase proteins in cattle (Arthington et al., 2003; Arthington et al., 2005). Although both proinflammatory cytokine and acute-phase responses are imperative components of the innate immune system and indispensable for early homeostatic restoration following pathogenic infection (Kushner, 1982), these reactions may be unnecessary when induced by stressors and have detrimental effects on health parameters by contributing toward a subsequent immunosuppressive state.

Research has demonstrated that increased circulating levels of cytokines and acute-phase proteins are positively linked with the incidence of various diseases and certain cancers in humans and animals (Young et al. 1996; Harris et al., 1999) including the BRD complex in feedlot cattle (Berry et al., 2004), although it is still unknown if the nature of such relationship is based on cause and/or effect. Alternatively, it is well-known that both proinflammatory and acute-phase responses demand a significant amount of body resources, increase maintenance requirements, and decrease nutrient intake (Elsasser et al., 1997; Johnson, 1997). Indeed, research from our group reported a negative correlation ($r \leq -0.44$; Araujo et al., 2010) between circulating concentrations of acute-phase proteins with feed intake and growth rates of overtly healthy beef calves, indicating that this stress-induced response is also highly detrimental to animal productivity. Therefore, the **central theory** of our research group is that transient stress-induced proinflammatory and acute-phase responses expend significant amount of biological resources required to maintain robust innate and adaptive immune systems, increasing the vulnerability of the animal to subsequent opportunistic infectious diseases. Based on this theory, **one of the current objectives of our research group**, which is the next step towards the accomplishment of our long-term goal, is to properly characterize the biological mechanisms responsible for stress-induced immune responses, and develop feasible alternatives that prevent such reactions during routine management practices that often result in inevitable stress to cattle.

As commented previously, road transport is one of the most stressful events in the productive life of a feeder calf, and has been shown to elicit inflammatory and acute-phase responses (Araujo et al., 2010; Cooke et al., 2011). When cattle are transported for long distances or periods of time, these immune reactions and subsequent detriments to health and performance parameters are further intensified. This is particularly important to beef cattle in Oregon, given that many beef calves are shipped to feedlots in the Midwest, whereas those that are destined to commercial feedlots in Oregon often have to cope with long travel due to the topography, weather, and road conditions within the state. More specifically, cattle experience a series of stressors that potentially elicit inflammatory and acute-phase responses during road transport. One of these stressors is also frequently observed in many other cattle production systems or management practices; inadequate feed and water intake. In fact, preliminary data from our research group indicated that water and feed deprivation for 24 h increased circulating concentrations of acute-phase proteins in overtly healthy beef steers (Cappellozza et al., 2011; Figure 1). This acute-phase protein response was comparable to that observed in cattle following a 24-h road transport; indicating that feed and water deprivation may be a major stimulant of inflammatory and acute-phase responses during road transport. However, this preliminary study was conducted with reduced number of animals, did not evaluate key health, physiologic, and performance parameters, and did not directly compare such responses to those of cattle assigned to road transport. Therefore, proper assessment of the impacts of feed and water restriction on inflammatory, acute-phase, and subsequent performance responses in cattle will serve as foundation for alternatives to alleviate stress-induced immune responses during road transport. This include assurance that cattle has adequate feed and water prior to truck loading and immediately after arrival, or even encourage stops during long transports for water and feed consumption. Further, this resultant knowledge can be applied to other segments within beef and dairy production systems, demonstrating that lack of proper feed and water availability, even if temporarily, can substantially detriment cattle health, performance, and consequent profitability of cattle producers.

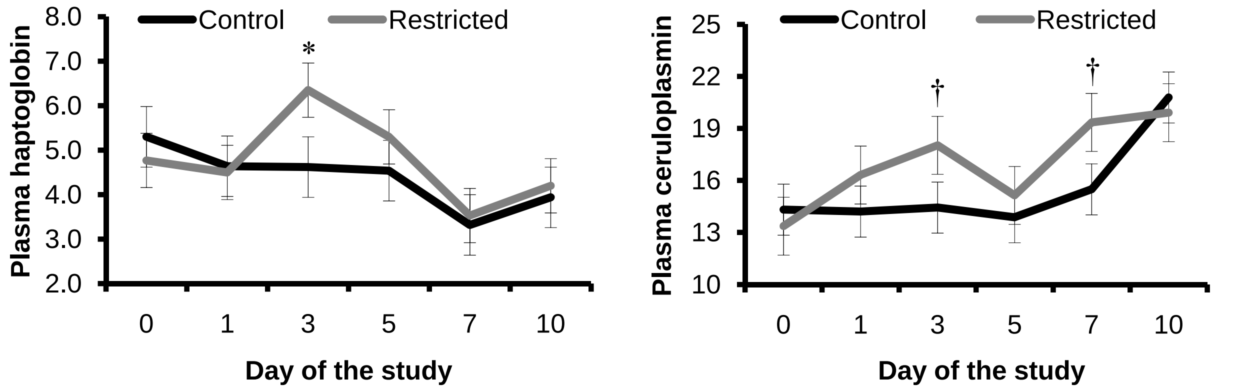

Figure 1. Concentrations of haptoglobin (abs @ 450 nm x 100) and ceruloplasmin (mg/dL) in beef steers assigned (restricted) or not (control) to a 24-h feed and water deprivation (d 0 to d 1). Treatment comparison within days: † $P = 0.15$, * $P = 0.05$.

Objective(s):

Evaluate the effects of 24-h road transport or 24-h water and feed restriction on inflammatory, acute-phase, and subsequent performance responses of weaned beef steers.

Procedures and Methods:

The proposed experiment will be conducted at the OSU-Eastern Oregon Agricultural Research Center, Burns. All procedures will be submitted for approval by the OSU-Institutional Animal Care and Use Committee. The EOARC-Burns has all of the equipment, facilities, personnel, and cattle necessary to complete this research in a timely fashion.

Fifty four Angus × Hereford steers, weaned at 7 mo of age (d -45) and vaccinated against common infectious diseases including BRD complex, will be ranked by body weight and age on d -30 of the study and allocated to 18 drylot pens. All pens will receive the same diet (75:25 forage and concentrate ratio) ad libitum from d -30 to d 0 of the study (preconditioning phase). On d 0, pens will be randomly assigned to 1 of 3 treatments: 1) no transport and full access to feed and water, 2) no transport but feed and water deprivation for 24 h, and 3) continuous road transport for 24 h. Treatments will be concurrently applied, from day 0 to 1. On d 1, transported and feed/water deprived steers will return to their assigned pens and all three treatments will be offered a feedlot receiving diet (50:50 forage and concentrate ratio) ad libitum from d 1 to 30 (feedlot phase). This schedule will simulate the feedlot receiving period, which in commercial situations, is the period where cattle are most susceptible to opportunistic diseases and performance dictates their overall productivity through harvest (Arthington et al., 2005). The transport and feed/water restriction length will simulate the stress challenge of commercial long transport (Arthington et al. 2008; Cooke et al. 2010).

During preconditioning and feedlot phases (d -30 to 30), feed intake will be evaluated daily by measuring offer and refusals. Steer body weight will be collected during consecutive days at the beginning (d -32, -31, and 30), immediately prior and following treatment application (d 0 and 1), and at the end of the study (d 30, 31, and 32) for body weight gain calculation. Blood samples will be collected on d 0 (prior to treatment applications), and d 1, 4, 7, 10, 14, 21, and 29. Steer rectal temperature will be recorded every minute from d 0 to d 1 (during treatment application; Reuter et al., 2010), and concurrently with each blood collection using a GLA M750 digital thermometer (GLA Agricultural Electronics). Whole blood samples will be analyzed for hematocrit (evaluate dehydration) using microhematocrit capillary tubes (VWR International; Radnor, PA). Serum will be harvested and analyzed for concentrations of: A) cortisol (Cayman Chemical; Ann Harbor, MI) – assess HPA response, B) blood urea nitrogen (Pointe Scientific Inc.; Canton, MI), creatitine (Pointe Scientific Inc.), and non-esterified fatty acids (Wako Chemicals: Dallas, TX) - evaluate body tissue degradation and nutritional status, and C) haptoglobin and ceruloplasmin (Arthington et al. 2008) – assess acute-phase protein response. Samples collected on d 0, 1, 3, and 7 will also be analyzed for prostaglandin E2 (Cayman Chemical) and proinflammatory cytokines (IL-1, IL-6, TNF-α, and IFN-γ; collaboration with Dr. Jeff Carroll from USDA-ARS LIRU, Lubbock, TX) to evaluate inflammatory responses.

The sample size planned for this experiment is adequate to result in statistical significances regarding treatment effects for all measurements proposed, according to the G*power 3 software (Faul et al., 2007) and to our preliminary data. Data will be analyzed with the MIXED procedure of SAS using pen as the experimental. For all measurements evaluated, the model statement will contain the effects of treatment, in addition to day of collection and the resultant interaction when appropriate. Values from d 0 will be used as covariate if necessary. Pen(treatment) and steer(pen) will serve as random variables. When required, specified term for the repeated statement will be day of collection, with steer(pen) included as subject, and the covariance structure utilized will depend on the Akaike information criterion.

Personnel Resources:

Program Leader: Reinaldo Cooke (Assistant Professor – 0.25 FTE SY)
Program Co-Leader: David Bohnert (Associate Professor – 0.20 FTE SY)

Institutional Units Involved:

The proposed study will be conducted at the EOARC – Burns, by its faculty and staff.

Cooperation:

Dr. Jeffrey Carroll from USDA-ARS LIRU (Lubbock, TX) will cooperate in the study by providing the analysis of proinflammatory cytokines. This collaborative effort is part of the multistate project W_TEMP3241 (former W-1173): Impacts of stress factors on performance, health, and well-being of farm animals.

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