

EFFECTS OF ENERGY CONCENTRATION AND SOURCE IN RECEIVING
RATIONS FED TO
NEWLY RECEIVED STRESSED CALVES

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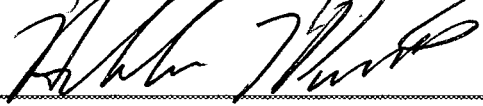
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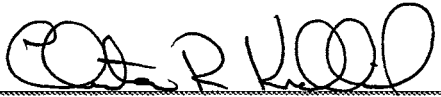
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CHAPTER I

INTRODUCTION

Feeder calves encounter numerous physical and psychological stressors during and immediately following the marketing cycle. Weaning, shipping, feed and water deprivation, inclement weather, threatening encounters, and infectious agents are a few of the stimuli that adversely affect cattle well being. These stressors generally are additive and result in anorexia, physical exhaustion, nutrient loss, altered nutrient metabolism, hormonal changes, behavioral changes, and a depressed immune system.

Bovine respiratory disease (BRD, shipping fever or pneumonia) is the most commonly recognized problem in newly received stressed feedlot calves and may occur as a primary infection resulting from the stress factors that often occur within the first thirty days in the feedlot or secondary to a viral or other infectious agent. With these factors in mind, it is important for producers to manage respiratory disease in newly received calves in two ways. First, a science-based vaccination program should be developed and secondly, stress should be kept to a minimum. Although vaccines are available for a wide variety of infectious agents, there is no vaccine for all pathogens. Also, some pathogens have numerous strains and a vaccine for one strain may not be effective in producing immunity to an alternate strain. In addition, an animal's ability to respond to a vaccine depends on a number of factors including age, nutrition, stress, and previous vaccination.

The nutritional program for newly received stressed calves must restore calves to a positive nutrient balance without causing additional negative effects, and decrease or

prevent negative metabolic changes such tissue losses associated with shrink. Success is often complicated by low feed intake during the initial two weeks in the feedlot. Nonetheless, increased nutrient density of re-alimentation feeds may partially compensate for decreased intake.

In most cases, energy is the first limiting nutrient in diets of newly received calves as a result of low intake. This occurs at a time when calves need have ample energy to mount an immune response to the various pathogens encountered upon entry to the feedlot. High concentrate diets, however, have been reported to increase morbidity in stressed cattle, presumably due to added digestive and metabolic stress. This may be alleviated by the inclusion of ingredients lower in readily available carbohydrate.

This experiment was designed to determine the effects of diets with two energy concentrations and two starch levels on stressed calves during a 42-d receiving period. The goal was to determine if higher energy diets would producer greater morbidity, and if that morbidity could be reduced by the reduction of starch content of the diet. Total mixed rations were fed to stressed calves during the receiving period with dietary effects based on growth, feed efficiency, percent morbidity, prevalence of upper-respiratory pathogens, and antibody concentrations.

As part of this study, acute phase protein concentrations were evaluated over time and in response to disease. Plasma and serum samples were collected from a subset of calves at regular time intervals and when calves were diagnosed with respiratory disease and when they had recovered. Characterization of changes in hepatic proteins may prove beneficial in diagnosis of respiratory disease in cattle.

CHAPTER II

REVIEW OF LITERATURE

Stress

Stressors – In General

Stress can be defined as an internal or external environmental stimulus that initiates an adaptive change or a stress response in an animal (Breazile, 1987). Stress is not inherently harmful to an animal, and in fact may be beneficial. Eustress is brought on by stimuli that initiate responses beneficial to an animal's well being. These stimuli function in the maintenance of a homeostatic state. Neutral stress produces responses that are neither harmful nor beneficial to the animal. Distress, which will be the topic of interest for this section, causes harmful responses interfering with the comfort or well-being of an animal and may evoke pathological changes in an animal. Common stressors of calves include weaning, transportation, introduction to new feedstuffs, exposure to pathogens, and adaptation to a new environment.

Weaning

Weaning of mammals naturally occurs during the transition to adulthood. However, in the quest for more efficient beef production systems, producers often couple the stress of weaning with transportation and marketing, long before weaning would occur naturally. Ohio researchers (Fluharty et al., 2000) measured differences in weight gain of calves weaned at 100 vs. 205 d of age. Early-weaned calves had greater weight gains and reached market weight at 385 d as compared with 418 d for normal-weaned

calves. In most systems, the calf is denied not only its dam's milk but also the freedom to socialize with her and the remainder of the adult herd (Stookey et al., 1997). Although no long-term effects have been detected, the distress of separation may initiate an extended period of vocalization (Stookey et al., 1997). Grandin (1997) designated prolonged vocalization as an indicator of discomfort. In addition, this excessive vocalization in response to distress may increase susceptibility to respiratory tract infection (Loerch and Fluharty, 1999).

Weaning effects on various hormones and blood metabolites have been quantified. Crookshank et al. (1979) measured alkaline phosphatase, cortisol, creatine phosphokinase (CPK), lactic dehydrogenase (LDH), glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), cholesterol, creatinine, glucose, urea nitrogen, uric acid, total protein, calcium, copper, iron, magnesium, inorganic phosphorus, potassium, and zinc levels in the serum, as well as body temperature and weight changes prior to and subsequent to weaning. Of the measured parameters, only weight change, cortisol, CPK, LDH, serum GOT, and serum GPT differed subsequent to weaning. In contrast, Lefcourt and Elsasser (1995) found no elevation in cortisol as a result of weaning; however, these researchers reported an increase in both epinephrine and norepinephrine concentrations.

Transport

Marketing and transportation of calves may involve many stressors (Grandin, 1997). As previously discussed, many calves are removed from their dams when they are placed on the truck destined for a sale barn, preconditioning lot, or wheat pasture.

Calves may experience long periods of time without feed and water during the marketing process. Once in the feedlot, they are supplied with unfamiliar water and feed sources, vaccinated against various diseases and treated for parasites, and commingled with other calves in new surroundings. In addition, calves may be marketed when weather is not ideal, exacerbating an already stressful situation.

Many studies have been conducted examining the effect of transportation and fasting on hormones and blood metabolites. Elevated SGOT concentrations have been reported in fasted and transported steers when compared to control steers (Galyean et al., 1981; Crookshank et al., 1979). Ewbank et al. (1983) measured effects of transportation on blood constituents and noted that corticosteroids responded the most dramatically, which was in agreement with Crookshank et al. (1979). Mixed results have been reported when cortisol concentrations were measured following transport. New Mexico researchers (Galyean et al., 1981) found that cortisol concentration was not elevated in steers when transportation was the only known stressor. In contrast, Agnes et al. (1990) and Locatelli et al. (1989) noted elevated cortisol concentrations associated with transport.

The ability of newly weaned and marketed calves to adapt to a new diet is a result of many factors. Galyean et al. (1981) measured the effects of fasting and transportation on rumen fluid temperature and osmolality, rumen pH, total volatile fatty acid (VFA) concentrations, rumen ammonia, total rumen protozoa, and total rumen bacterial counts. Fasted steers and steers fasted followed by a 32-h transport had increased rumen pH and lower rumen protozoa compared with control steers. Total VFA concentrations and rumen ammonia concentrations were lower in fasted vs. fasted and transported steers.

Rumen fluid temperature and osmolality were only slightly affected. Total rumen bacterial counts declined during fasting and transit, slowly returning to normal levels by 104-h. These researchers concluded that since fasted and transported steers had a decline in ruminal bacteria and protozoa concentrations and lower ruminal DM at 32 h compared to transported steers, the increase in VFA was probably due to a decreased rumen motility or rumen volume resulting from transit rather than increased VFA production. Similarly, Cole and Hutcheson (1981) measured microbial activity (RFA) and the ability of ruminal microbes to ferment added substrate (RFC) in calves for 5 to 7 d subsequent to a 48-h starvation period and found that RFC was reduced by as much as 75% during deprivation. In contrast to the data reported by Galyean et al. (1981), RFA and RFC were still diminished 5 d after deprivation. More recently, Fluharty et al. (1994) conducted experiments to determine the effect of feed and water deprivation on ruminal characteristics of feedlot-adapted calves. These researchers measured *in situ* DM digestibility (DMD) of orchardgrass hay in order to determine the ability of ruminal microorganisms to ferment NDF in calves immediately following weaning, fasting, and trucking. No changes in DMD were noted over time, leading the researchers to conclude that the ruminal microbial population was not affected.

Physiology

Stress is perceived in many areas of the brain, from the cortex through the brainstem. Major stressors activate both corticotropin-releasing hormone (CRH) neurons and adrenergic neurons in the hypothalamus. This activation has a compound effect because norepinephrine increases CRH release, and CRH release increases adrenergic release. CRH release ultimately elevates plasma cortisol levels while adrenergic

stimulation elevates plasma catecholamine levels. Together these hormones increase glucose production. Glucose production is increased rapidly by epinephrine activation of glycogenolysis, and more slowly by cortisol providing amino acid substrate for gluconeogenesis in the liver. Glucose utilization is repartitioned toward the central nervous system and away from peripheral tissue. Epinephrine also rapidly increases the free fatty acid supply to the heart and to muscles, and cortisol facilitates the lipolytic response. If distress involves tissue trauma or invasion, high cortisol levels eventually act to suppress the initial inflammatory and immune responses so that they are less likely to cause irreparable damage.

Immunology

The mammalian immune system includes both innate immunity and acquired immunity. Innate immunity consists primarily of physical, chemical, and mucosal barriers and phagocytic cells. These mechanisms are less specific and generally less powerful than those of acquired immunity, but are nonetheless the most important methods employed by mammals for protection from invasion of pathogens. In cattle, most significant diseases result from a pathogen crossing mucosal barriers located in the gastrointestinal, reproductive, or respiratory tracts (Perino, 1992).

Acquired immunity may be further categorized into humoral immunity and cell-mediated immunity. Humoral immunity is mediated by B-lymphocytes (B-cells), which are produced in bone marrow and generate immune responses to extracellular pathogens by producing antibodies, which aid in destruction by phagocytes. T-lymphocytes (T-cells) are also produced in the bone marrow, however these cells traverse to the thymus

prior to maturation. These leukocytes regulate B-cell activation and function in the activation of destructive mechanisms that rid the host animal of altered-self cells such as viruses and tumor cells. Another class of T-cells (cytotoxic T-cells) are part of the innate immune system to non-specifically destroy altered cells.

Acute-Phase Response.

The acute-phase response (APR) in mammals is a reaction to tissue infection or injury occurring within hours of inflammatory stimuli. This results in a concentration change of multiple plasma proteins, the acute-phase proteins (APP) (Alsemgeest, 1995). These proteins are primarily synthesized by hepatocytes in response to receptor activation by cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) released by macrophages and monocytes in the presence of bacteria (Ludden and Kerley, 1997). These proteins primarily act as opsonins, coating pathogens to aid in phagocytosis. These hepatic proteins may present an opportunity for early detection of BRD. There seems to be some species differences in the acute phase response. In cattle, the concentration acute-phase proteins serum amyloid-A (SAA), a 14 kD apolipoprotein, and haptoglobin (Hp), a complex of greater than 1000 kD, rise most dramatically during the acute-phase response. Netherlands researchers (Alsemgeest et al., 1994) measured SAA and Hp in a group of 60 mixed breed and mixed age cows deemed healthy or with acute, sub-acute, or chronic inflammation. Cattle with inflammation had different SAA and Hp plasma concentrations, and Hp/SAA ratios. In addition, animals with acute inflammatory diseases had different plasma Hp concentrations than cattle with chronic inflammatory disease. In humans SAA and C-reactive protein (CRP) increases most in

response to stimuli (up to 1000-fold), while Hp may only increase by a factor of 2 to 4 (Kushner and Mackiewicz, 1987). Bacterial infections seem to cause the greatest elevation of APP in humans. In an observational study conducted in 545 humans, 33 of the 52 patients with elevated CRP levels were found to have bacterial infections (Kushner and Mackiewicz, 1987).

Regulation of metabolism

Receptors for the proinflammatory cytokines are found on many cells outside of the immune system, allowing for direct regulation of metabolism (Spurlock, 1997). In addition, cytokines may activate immune modulators such as glucocorticoids, prostaglandins, and catecholamines, further affecting cell growth and metabolism. Spurlock (1997) hypothesized that proinflammatory cytokines produce a homeorhetic response during an immune challenge in which nutrients are partitioned away from tissue growth in support of the animal mounting an immune response. During an immune challenge, uptake of glucose is repartitioned from skeletal muscle, heart, and liver by IL-1, IL-6, and TNF- α (Ling et al., 1994; Lang et al., 1992). Insulin resistance was evident in peripheral tissue because: 1) administration of cytokine or endotoxin reduced the rate of glucose infusion necessary to maintain euglycemia during hyperinsulinemic conditions, and 2) typical suppression of hepatic gluconeogenesis by insulin was blocked by cytokines. TNF seems to directly affect insulin function (Stephens and Pekala, 1991, 1992). Chronic exposure to TNF- α by 3T3-L1 adipocytes results in these cells becoming refractory to insulin stimulation.

A repartitioning of amino acid metabolism also occurs during an immune challenge. Homeorhetic and catabolic activities ensure that liver supplies of amino acids are adequate to meet increased requirements of glucogenic amino acids and synthesis of acute phase proteins. Enhanced liver uptake of amino acids is activated by the action of cytokines and glucocorticoids. Conversely, skeletal muscle amino acid uptake may be suppressed during immune challenge by these same hormones.

Bovine respiratory disease (BRD) is a common result of stress in newly received calves. Disease may result because distress compromises the immune system, or secondarily because of failure to respond to vaccines because of immuno-suppression. The combination of these two stress related failures make BRD the most commonly recognized problem in newly received calves.

Bovine Respiratory Disease

Economic Significance

BRD is the leading cause of morbidity and mortality in feedlots (USDA, 1999). The USDA National Animal Health Monitoring Systems (NAHMS) collected feedlot data from a random sample of 49 states during the 1995 calendar year (USDA, 1996). Respiratory disease was the primary cause of death in cattle (28%) resulting in a \$478 million dollar loss. Approximately two-thirds of the cattle that died were calves, rather than yearlings. Loneragan et al. (2001) measured trends in mortality among cattle in feedlots. These researchers calculated yearly and monthly mortality ratios of approximately 21.8 million cattle entering 121 feedlots from 1994 to 1999. Averaged over time, the mortality ratio was 1.26 deaths/100 cattle entering the feedlots. Compared

to cattle entering the feedlot in 1994, cattle received in 1999 had a significantly increased risk (relative risk, 1.46) of dying of respiratory disease. In addition, respiratory tract disorders accounted for 57.1% of all deaths.

Perino (1992) categorized economic losses associated with BRD into: 1) therapeutic treatment costs; 2) costs associated with lost production and/or salvage of chronic cattle; and 3) cost of death loss. If disease prevalence escalates significantly, the ability to effectively manage the disease in the feedlot may become severely compromised as personnel and facilities become limiting.

Growth rate of feedlot cattle experiencing BRD is reduced. During 1992, 28% of cattle received in the Texas Ranch to Rail program became ill with BRD, and of those, 2.88% died (Carperter and McNeil, 1995). Cattle diagnosed with BRD during the feeding phase gained 7% less, had a 3% lower final pay-weight, cost 18.5% more to feed, and had 25% fewer carcasses grading USDA-Choice.

Etiology

Bovine respiratory disease has a multifactorial etiology and develops as a result of complex interactions between environmental factors, host factors, and pathogens. Environmental factors (e.g. weaning, transport, commingling, and crowding) serve as stressors that adversely affect the immune mechanisms of the host. In addition, certain environmental factors (e.g., crowding and commingling) enhance the transmission of infectious agents among animals.

Viral Infections

Viral infections predispose cattle to bacterial pneumonia (Roth and Perino, 1998). In a challenge experiment, cattle were preinfected with bovine herpes virus (BHV1), bovine viral diarrhea virus (BVDV), parainfluenza-3 (PI3), or bovine respiratory syncytial virus (BRSV) and challenged a few days later with an aerosol of *Mannheimia haemolytica* or *Pasteurella multocida*. Cattle infected with viruses developed severe bacterial pneumonia while nonvirus-infected cattle successfully cleared the bacteria from the respiratory tract (Roth, 1984). The viruses associated with BRD may have a number of immunosuppressive effects on the lung, including damage to mucosal surfaces allowing greater susceptibility of airways to bacteria by hampering the clearing effects of the ciliated escalator in the upper respiratory tract, suppression of phagocytic cell function, and interference of lymphocyte function (Roth and Perino, 1998; Roth, 1984).

Bovine herpes virus causes necrosis of the epithelium of the upper respiratory tract, compromising ciliary clearance of pathogens in the upper respiratory tract. This leads to an increase in bacterial colonization and growth preceding bacterial infection (Kapil and Basaraba, 1997). BHV1 also impairs macrophage, polymorphonuclear neutrophil (PMN), and lymphocyte function, thus leading to more frequent occurrences of respiratory infections. PMNs are vital in destruction and clearance of infection from the lower respiratory tract; therefore, local defense mechanisms of the lung may be overwhelmed in cattle infected with BHV1. It is important to note that subsequent to recovery from herpesvirus, an animal is latently infected with the virus for the remainder

of its life. The virus exists in ganglia of nerves in an inactive form and if the animal is stressed later in life, it can become active and be shed (Roth and Perino, 1998).

There are three defined disease syndromes associated with BVDV: bovine viral diarrhoea (primary postnatal infections), mucosal disease, and fetal disease (Potgieter, 1997). Little data can be found in refereed literature establishing BVD's role as a primary pathogen in respiratory disease in cattle. However, BVDV is recovered from pneumonic lungs of cattle with great frequency (Richer and Lamontagne., 1988). Some evidence exists suggesting BVD may have a secondary role in BRD, thus exacerbating the effects of other infectious diseases. Concurrent infections with BVDV and BHV1 have resulted in severe clinical disease affecting the respiratory tract, ocular tissue, and alimentary tract (Greig et al., 1981). Often, apparent opportunistic infections with *M. haemolytica* increase the severity of respiratory tract lesions (Reggiardo, 1979).

There is no single strain of BVDV capable of stimulating antibody production against all potential virulent BVDV isolates that an animal may encounter (Roth and Perino, 1998). This is because, like most RNA viruses, the BVDV has a high mutation rate resulting in great antigenic diversity. It has been suggested, however, that the majority of highly virulent strains of BVDV belong to a distinct and specific genome (Potgieter, 1997).

Parainfluenza virus type 3 (PI3) infects ciliated epithelium located in the upper and lower airways of the respiratory tract, as well as alveolar epithelium and macrophages (Tsai and Thomson, 1965; Bryson et al., 1983). The method in which PI3 predisposes cattle to BRD is largely unknown, however, it is commonly isolated from cattle in which *M. haemolytica* is a co-pathogen (Kapil and Basaraba, 1997). Research

suggests that PI3 may cause viral-mediated alterations in the ability of macrophages to clear bacteria (Hesse and Toth, 1983).

Bovine respiratory syncytial virus (BRSV) infections resulting in severe clinical disease typically occur in calves less than six months of age (Baker et al., 1997). The active immunity that develops during primary infection does not provide the animal with complete immunity from re-infection, thus, cattle often experience secondary BRSV infections resulting in mild disease (Baker et al., 1986). Maternal antibody appears to provide inadequate protection, but it does interfere with active immunization of the calf as assayed by antibody production (Kimman and Westenbrink, 1990). This is problematic because BRSV generally occurs in calves too young to be vaccinated effectively (Roth and Perino, 1998).

Bacterial Infections

The majority of important clinical signs and death loss associated with the BRDC can be attributed to bacterial pneumonia caused by *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), *Pasteurella multocida*, or *Haemophilus somnus* (Roth and Perino, 1998). In healthy cattle, these bacteria are common to the nasopharyngeal regions of the upper respiratory tract. If a calf is immunosuppressed, harmful bacteria may migrate into the lower respiratory tract causing bacterial pneumonia.

M. haemolytica. A breakdown of mucosal barrier integrity caused by distress or viral infection may allow *M. haemolytica* to colonize in the upper respiratory tract, leading to inhalation of microcolonies deep into the lung (Roth and Perino, 1998). These microcolonies may successfully evade the local defense mechanisms in the alveolus and

produce severe pneumonia. The exact method by which these bacteria are able to successfully avoid the immune system are unknown, however, *M. haemolytica* leukotoxin has been shown to cause degeneration and death of both neutrophils and macrophages *in vitro* (Shewen and Wilkie, 1982) and has been suggested to provide chemotactic attraction for neutrophils in the lung. Leukotoxins released during a *M. haemolytica* infection are potentially more damaging than the bacteria itself. The toxins are cytotoxic to ruminant leukocytes (Clinkenbeard et al., 1989). *M. haemolytica* A1 capsular polysaccharide may be involved in an adhesin-receptor interaction with alveolar surfactant allowing the bacteria to attach to epithelial cell surfaces (Brogden et al., 1998). Preincubation of alveolar macrophages with A1 capsular polysaccharide has been shown to impair phagocytosis and killing of *M. haemolytica* (Czuprynski et al., 1991).

Haemophilus somnus. *H. somnus* has large numbers of potentially virulent components (Roth and Perino, 1998). These include endotoxin, antibody binding proteins, surface nucleotides, and hemolysin (Corbeil et al., 1991). This bacteria has been isolated from calves with fibrinous and bronchial pneumonia, however, the relative significance of *H. somnus* in BRD has yet to be demonstrated. The role of the cell-mediated or humoral immune systems in defending calves is uncertain, however, there is some evidence that gamma interferon, which is produced during a cell-mediated response, can help protect calves against *H. somnus* induced pneumonia (Roth and Perino, 1998).

Nutrition and Health/Immunity Interactions

Bioenergetics

Energy may be defined as the ability to work. In animals, energy is required to perform the biological functions necessary for life. The nutritionist's primary concern lies in the prediction of energetic requirements of animals and the ability of feedstuffs to satisfy those requirements (Blaxter, 1962). In order to fully understand these needs and solve this practical task, one must rely on approaches based in the fields of chemistry, biochemistry, physiology, physics, and anatomy (Blaxter, 1962).

A logical starting point would seem to be to recall a few basic theories on which the study of bioenergetics is based. An excellent explanation of these theories is provided in Kleiber's (1961) bioenergetics book, The Fire of Life, and the discussion here will primarily be based on that publication. The *law of constant heat sums*, also known as the *law of Hess* states that the total quantity of heat produced or consumed when a chemical system changes from the initial to final state is independent of the manner in which the change occurred. For example, note that as one mole of glucose is oxidized to water and carbon dioxide, 673 Kcal are released. The oxidation of glucose by a cell via glycolysis and the Krebs cycle also releases 673 Kcal/mole. The first and second *laws of thermodynamics* are also of some importance when considering animal energetics. The first law, the *law of conservation of energy*, states that energy can be converted among forms but not created or destroyed while the second law states that a process can occur spontaneously only if the sum of the entropies of the system and its surroundings increase. Mathematically, these laws can be expressed in terms of free energy (Stryer, 1995). The equation used to express these relationships is stated as the

change of free energy of a system undergoing a transformation at a constant pressure (P) is equal to the change in entropy times the temperature subtracted from the change in heat content of the system. This equation is limited on an applied basis however, because entropy of chemical reactions is difficult to quantify. Therefore, heat production (ΔH) is typically used to assess food energy. Heat production is equal to the difference in energy from the onset of the process to the end of the process (ΔE) less the change in volume (ΔV) multiplied by pressure. Since ΔV is small for biochemical reactions, ΔH is nearly equal to ΔE .

The fasting animal. An animal receiving no food, doing no external work, and yielding no product must still carry on a variety of internal processes including respiration, circulation, maintenance of muscle tone, and maintenance of osmotic integrity. These functions, in the absence of food energy, must be supported by nutrients supplied from catabolism of body stores. The metabolic reactions involved in this process are called basal catabolism, and measured at its minimum value is termed the basal metabolic rate (BMR). Conditions that must be satisfied in order to measure BMR are: 1) the animal must be in good nutritive condition; 2) the animal must be in a thermoneutral environment; 3) the animal must be in a relaxed condition; and 4) the animal must be in a post-absorptive state (Blaxter, 1962). Only the first two conditions can be met with any certainty in most animals. For example, ruminants do not sleep as most animals do. They never reach complete muscle relaxation. Moreover, a true post-absorptive state is difficult to reach in ruminants due to a prolonged retention of food in the rumen. A fasting period of three days seems to be sufficient. Thus, in ruminants, a fasting metabolic state is used instead of BMR.

By application of the *law of Hess*, an animal's fasting metabolism may be quantified by a measurement of heat produced. The heat production represents oxidation of body nutrients and can be measured either by direct or indirect calorimetric methods. Discussion of these methods are beyond the scope of this paper, however, I once again refer the reader to Kleiber's, The Fire of Life (1961).

Early research focused on finding a relationship across species in order to make comparisons of metabolic rates within and among species. An initial relationship theorized that metabolic rate is proportional to the surface area of an animal. Max Rubner (Kleiber, 1961) measured the fasting metabolism of dogs varying in weight from 3 to 31 kg in 1883 and found that fasting metabolism per kg BW decreased with increasing size of animal, but expressed per unit of surface area all dogs produced similar amounts of heat (Blaxter, 1961). Other researchers (Kleiber, 1947; Brody, 1945) realized the difficulty of standardization of measurement of surface area and suggested that metabolism should be referred to as a function of BW. Brody (1945) found that "in species from mice to elephants" metabolism was a function of BW raised to the 0.73 power and that all species produced an average of 70.5 Kcal with a range of 61 to 81 Kcal. Simplicity of calculations favors BW raised to the 0.75 power and this has become the generally accepted relationship.

It should be noted that variations in fasting metabolism occur due to a number of factors. Fasting metabolism is affected by previous plane of nutrition. A low previous plane of nutrition will result in a lower fasting metabolic rate (NRC, 1996). This may be explained by changes in size of metabolically active organs. In addition, fasting metabolism is affected by age. In cattle, fasting metabolic rate decreased from greater

than 140 Kcal at birth to approximately 80 Kcal for adult cattle (Blaxter, 1962). Trained animals have lower metabolic rates than untrained animals, suggesting that environmental stimuli may have some effect. Finally, breed differences may exist, as indicated with cattle (NRC, 1996). Dairy breeds seem to have higher maintenance requirements, therefore, higher fasting metabolic rates, than beef breeds.

Energy partitioning. Food energy can be partitioned into major components associated with animal energetics (Ferrell, 1988). The energy provided to an animal in feedstuffs may first be partitioned into digestible and indigestible fractions. Digestion trials are often employed to determine apparent digestible energy (DE) of feedstuffs. These data are termed apparent because a portion of fecal energy represents body energy loss. Fecal energy losses are the greatest loss of feed energy representing 20 to 30% of feed energy in cattle and sheep fed concentrate diets (Maynard et al., 1979).

Metabolizable energy (ME) represents DE less urinary and gaseous energy losses. Urinary losses are generally measured by a quantitative collection of urine during a feeding period and determination of gross energy by bomb calorimeter (Church, 1988). Primarily, these losses consist of incompletely oxidized nitrogenous compounds such as urea, and endogenous nitrogen molecules such as creatinine (Galyean, 1997). Gaseous products, measured by respiratory exchange in a respiratory calorimeter, actually represent a digestive loss as they are products of fermentation of feedstuffs in the digestive tract; however, these losses are normally grouped with metabolic losses. Urinary losses account for 4 to 5% of energy losses in cattle while gaseous losses make up approximately 7 to 10% of feed energy losses (Maynard et al., 1979).

Net energy (NE) accounts for heat of fermentation and heat of nutrient metabolism by deduction of heat increment from ME. Heat of fermentation arises from chemical inefficiencies of fermentive processes within the animal (Galyean, 1997). Inefficiencies of nutrient metabolism give rise to the remaining portion of heat increment. The heat lost from nutrient metabolism varies with level of feeding, type of diet, and body function being supported thus, NE values should be discussed (see below) in context of the purpose of the diet.

Net Energy. Kleiber (1961) recognized that the partial efficiency of energy utilization for maintenance is greater than the partial efficiency of energy utilized for production. Thus, as feed intake exceeds that which is necessary to maintain body functions, partial efficiency is reduced. It is therefore necessary to express energy requirements for maintenance and energy associated with production separately. In the mid-1960's, Lofgreen and Garrett (1964) introduced a net energy system designed for use with growing and finishing phases of beef cattle production, the *California Net Energy System*.

In fed animals, heat production (HP) is comprised of heat produced by basal metabolism, activity, and heat increment, which is simply the heat of fermentation and the heat of nutrient metabolism. However, in fasting animals, heat increment is zero and HP consists of heat produced by basal metabolism and heat produced by activity. In developing an equation to estimate maintenance energy requirements for beef cattle, the researchers indirectly measured heat production by extrapolating data from five comparative slaughter trials that measured retained energy (RE). The experiments used 208 feeder cattle weighing approximately 230 kg to 300 kg, and consisted of four individual feeding trials and one group-feeding trial. Cattle were fed at either two or

three intake levels from maintenance to *ad libitum* and the dietary roughage level varied from 2% to 100% with trial. Heat production (HP) was calculated for each trial by deducting RE from metabolizable energy intake (ME). The relationship between daily HP expressed as Kcal per unit of metabolic body weight and daily ME intake expressed as Kcal per unit of metabolic body weight were characterized, and the mean HP value for fasting beef cattle was determined to be 77 Kcal per unit of metabolic body weight. This information can be used to determine the energy available to sustain function of body systems (NE_m) of a feedstuff. Simply, if one knows that at fasting an animal produces 77 Kcal/BW^{0.75}, NE_m of feed can be determined by feeding at any intake level, preferably several levels, above maintenance and calculating heat production and ME of that feed. A regression equation of heat produced over daily ME can be established and ME intake at fasting heat production determined.

Energy required for gain is simply the energy deposited in gain (Lofgreen and Garret, 1964). Two hundred and sixty-four steers and an equal number of heifers fed the same ration at different rates of gain were harvested to determine if the rate of energy deposited in gain (RE) was similar for steers and heifers and if energy concentration in weight gain changes with rate of gain (Lofgreen and Garret, 1964). These researchers concluded that in both sexes, energy concentration of weight gain increased as the rate of gain increased and that the increase was greater with heifers. The value of a feedstuff in providing energy for gain (NE_g) is typically determined by a "difference trial." In these experiments a feed is fed to cattle at any intake level above maintenance and RE is measured at harvest. NE_g is then determined by dividing the amount of energy retained by the amount of feed consumed above that required for maintenance.

Intake Regulation. Voluntary dry matter intake (VDMI) of forages by ruminants is considered to be limited by reticulorumenal distention resulting from low passage rates of forage. Since the NDF portion of forages typically ferments and passes from the rumen more slowly than other dietary components, it has a more pronounced filling effect than non-fibrous feed constituents (Allen, 1996). Other factors affecting fill include: particle size, chewing frequency and effectiveness, particle susceptibility to degradation, rate of fermentation of potentially digestible NDF, and reticulorumenal motility. Physiologically, distension of the reticulorumen activates tension receptors located in the cranial sac of the rumen, and the reticulo-omasal orifice signals the ventromedial hypothalamus (satiety center) of the brain to reduce intake.

In cattle consuming high-energy concentrate diets, models characterizing VDMI are somewhat varied. This is because intake control involves a neural integration of physiological phenomena such as learning and perceptual constraints (Povenza, 1995). The thermostatic theory states that feed consumption is a response to a decrease in heat production and that the termination of eating is in response to a rise in heat production (Blaxter, 1961). This theory is suspect, however, because ruminants grazing low-quality forages produce less heat than ruminants fed high quality rations. The lipostatic theory states that the level of feed intake and bodily activity is modified by the hypothalamus in response to changes in body lipid levels. Secondly, this theory states that animals attempt to maintain a steady state of fat. This seems unlikely in ruminants however, considering the ease with which cattle fatten. A third theory is that of chemostatic control. Mayor (1955) proved that simple-stomached animals responded to the arterio-venous difference in glucose concentration. In ruminants, however, glucose concentration is typically very

low and large intravenous glucose infusions have no effect on daily intake (Blaxter, 1961). In ruminants, the principle energy substrates utilized are volatile fatty acids (VFA). Mechanistically, concentration of these fatty acids (primarily acetate and propionate) seems to have an effect on the feeding centers in the hypothalamus by signaling chemoreceptors (Baile and Forbes, 1974). Infusion of propionate has been shown to have the greatest effect when infused into the right ruminal vein. Acetate mediated the greatest effect when infused into the dorsal sac of the rumen.

Gut hormones also may play a role in controlling VDMI either directly by signaling the hypothalamus or indirectly by controlling motility and nutrient absorption. Enkephalins, a family of opioid peptides, have been shown to stimulate intake while an array of peptide hormones signal cessation of intake. These include cholecystokinin, bombesin, pancreatic peptide, and somatostatin.

A theory proposed by Ketelaars and Tolkamp (1996) is based on the premise that animals optimize intake to meet requirements for gathering, eating, and digesting food, and absorption and utilization of end products of digestion. According to the hypothesis, animals strive to achieve a level of intake that maximizes the NE consumed per unit of oxygen consumed.

Eating Patterns

Unstressed calves generally consume feed in quantities sufficient to maintain adequate energy intake (Galyean et al., 1999). Thus, it would be expected that replacing high-energy feedstuffs with low-energy feedstuffs would increase consumption until bulk fill limits intake. Eating patterns are altered however, in stressed beef calves (Lofgreen, 1983). Voluntary feed intake was higher when stressed calves were offered a 75%

concentrate milled diet as compared to intake of calves offered all roughage. In addition, when calves were allowed to select from diets containing 20, 55, and 90% concentrate, calves exhibited a preference for the high-energy diet.

Energy Source and Concentration

California researchers conducted a series of experiments with market-stressed calves to determine optimal dietary energy concentrations of receiving diets (Lofgreen et al., 1975). In Experiment 1, diets with concentrations of .84, 1.01, and 1.10 Mcal/kg of NE_g were fed for 29 d followed by all treatment groups being fed the intermediate diet an additional 34 d. Calves on the intermediate- and high-energy dietary treatments consumed more feed and gained more weight during the 29 d receiving period, with the high-energy treatment group gaining more than the intermediate-energy treatment group. Calves on the high-energy and low-energy diets had lower morbidity rates than calves on the intermediate energy treatment; however, calves receiving the high-energy diet had higher medical treatment costs due to extra days morbid. All treatment groups had the highest morbidity during the first week of the receiving period. Given the outcome of this study, the researchers replaced the .84 Mcal/kg of NE_g diet with a 1.19 Mcal/kg NE_g diet to determine if gain would increase further with increased dietary energy concentration. Intake actually decreased when the higher energy diet was added and daily gain was not increased. Unlike the previous study, calves on the 1.10 Mcal/kg of NE_g diet consumed more feed than calves on the 1.01 Mcal/kg of NE_g diet. Morbidity tended to increase with increasing energy concentration.

More recently, others have examined the effects of dietary energy concentration on performance and health of receiving calves. Fluharty and Loerch (1996) fed corn silage-based diets with 1.15, 1.21, 1.25, or 1.30 Mcal/kg of NE_g to individually housed steers in a 28-d receiving trial. There was a linear increase in DMI with increasing dietary energy concentration, but there was no difference in daily gain, feed efficiency, or health status for the 28-d period. The cattle in this study, however, were individually fed and housed. This design does not accurately simulate the situation in a commercial setting in which calves are commingled and exposed to a wide variety of pathogens. Similarly, DMI was improved and daily gain was not different between high energy (1.17 Mcal/kg NE_g) and low energy (1.01 Mcal/kg NE_g) calves in a 28-d preconditioning study conducted by South Dakota researchers (Pritchard and Mendez, 1990). Unlike other studies, however, cattle on the low energy diet had a superior feed/gain.

Protein Source and Concentration

Dietary protein requirements for beef cattle can be calculated using the NRC (1984) factorial equations or the NRC (1996) metabolizable protein system. These systems are both integrated with body weight (BW) and energy intake. Energy intake seems to be the first-limiting factor associated with weight gain, thus protein deposited in gain is largely dependent on energy intake (Galyean et al., 1996). Since newly received stressed calves often have very low feed intake during the first few days, protein requirements might be low. Requirements would then increase as energy intake increases.

Effects of various protein levels and sources for newly received calves have been characterized. Galyean et al. (1996) fed three levels (12, 14, or 16%) of supplemental CP from soybean meal to 120 calves (185 kg) in a 42-d receiving trial. Daily gain increased and DMI tended to increase linearly with increasing CP concentration. Morbidity was higher for calves fed the high-protein diet compared with the 14% CP diet.

Ohio researchers (Fluharty and Loerch, 1995) conducted a series of experiments to assess protein requirements of newly arrived cattle. In Exp. 1, newly weaned Simmental x Angus crossbred (243 kg) steers were fed increasing CP (12, 14, 16, or 18%) from two sources; spray-dried blood meal or soybean meal. Gain:feed increased linearly with increasing CP levels for the first 7 d and for the entire 42-d feeding period. Daily gain increased linearly with increasing CP levels during the first week after arrival. For the entire receiving period, calves fed the blood meal diets had a 7.4% increase in gain compared with calves fed the soybean meal diets. Similar to data reported by Galyean et al. (1999), morbidity also increased linearly with increasing CP concentration. A second experiment was conducted in which 246-kg Simmental x Angus steers were fed 11, 14, 17, 20, 23, or 26% CP diets with protein supplied by spray-dried blood meal or soybean meal. In this trial DMI was not affected by CP concentration. Daily gain and feed efficiency were both affected quadratically, with the 20% CP diet yielding superior performance. There were no differences in health status between treatment groups. In a third experiment, researchers fed either 12.5% CP diets or phase-fed 23% CP in wk 1, 17% CP in wk 2, and 12.5% CP diets during wk 3 and 4, with dietary CP supplied by corn gluten meal, ring-dried blood meal, spray-dried blood meal, fish meal, or soybean meal to newly received Simmental x Angus (238 kg) calves. There were no performance

or health differences due to dietary protein source or concentration. From these data, the authors advocate higher CP concentrations in diets for newly received calves than are currently being used in feedlots.

Eck et al. (1988) conducted a series of 28-d trials to evaluate effects of protein level and source of incoming feeder calves. In Exp. 1 and 2, researchers fed 230 crossbred heifers (255 kg) and 360 crossbred steers (200 kg) steam-flaked sorghum based diets consisting of either 10.5 or 12.5% dietary CP. Crude protein was supplied in the diets from urea, cottonseed meal (CSM), or a 50:50 combination of blood meal (BM) and corn gluten meal (CGM). Calves on the 12.5% CP diets gained more rapidly and were more efficient compared with cattle on the 10.5% CP diets. Calves fed the BM:CGM diets had superior gains and were more efficient than calves fed the urea or CSM. An interaction was reported for DMI between protein source and level. At the higher CP level calves had greater DMI when fed the CSM supplemented diet compared with the urea or CGM supplemented diets, however, at the lower CP level, calves consumed more DM when offered the urea or CGM supplemented diets. At both levels, calves consuming the urea and CGM supplemented diets had similar intakes. Morbidity in the experiments was less than 10% and 30%, respectively. In the third trial, 370 crossbred steers (260 kg) were fed either steam-flaked or dry-rolled corn based diets supplemented at the 12.5% CP level with urea alone, 67% BM-CGM and 33% urea, 33% BM-CGM and 67% urea, or BM-CGM alone. Daily gain and gain:feed increased linearly as BM-CGM proportion increased in the supplement. Morbidity was less than 30% in this trial as well. These data suggest that optimal performance of receiving calves may be achieved by increasing undegradable intake protein (UIP).

In a similar receiving study, Nebraska researchers (McCoy et al., 1998) supplemented 398 steer calves (257 kg) with either urea or 80% feather meal (FTH) and 20% ring-dried blood meal to supply 9.2% degradable intake protein (DIP). Feed efficiency was improved by addition of supplemental escape protein (EP). Protein supplementation had no effect on DMI or daily gain. Metabolizable protein (MP) was greater in calves fed supplemental UIP compared with calves fed urea. The researchers noted a significant negative correlation ($r = -.61$) between MP supply and morbidity. Although further studies should be conducted, this may suggest that an increase in MP may improve health status. Van Koeveering et al. (1991) fed 466 stressed steer, bull, and heifer calves (223 kg) isocaloric (1.10 Mcal/kg) and isonitrogenous (14.5% CP) diets supplemented with SBM, SBM + bloodmeal (BM), milo distillers dried grains plus solubles (DDGS), or DDGS + BM to evaluate effects of UIP on receiving calves. If calves were never sick, daily gains were superior when cattle were fed diets containing SBM. However, for cattle classified as sick and for all cattle combined, gains were not different between dietary treatment groups. Morbidity rate was similar among supplementation groups, however, the percentage of calves recovering following the first treatment was improved when supplemented with DDGS. In contrast with previous research, these data suggest that supplemented DIP may be useful in improving health of receiving calves.

Zinn and Shen (1998) evaluated DIP and metabolizable indispensable amino acid (MIAA) requirements of feedlot calves during the early receiving period. Four Holstein steer calves (249 kg), with a cannulated rumen and proximal duodenum were used in Exp. 1 to study effects on OM and nitrogen (N) digestion and amino acid supply to the

small intestine. Treatment diets were steam-flaked corn based diets supplemented with urea, SBM, or fish meal (FM) at 1.5, 3.0, or 4.5%. Digestibility of feed N, OM in the rumen, and microbial N flow (MNF) to the small intestine decreased in diets without urea. However, postruminal OM digestibility was greater in calves fed FM compared with calves fed urea. As a result there were no total tract treatment effects on OM digestibility. Ruminal MN efficiency (MNEFF, g MN/kg OM fermented) increased linearly with increasing level of FM. Supply of MIAA to the small intestine increased linearly as level of FM in the diet increased. Daily gain, DMI, and feed efficiency increased linearly as additional FM was added to the diet.

Conclusions

Proper nutritional management of newly received stressed calves is key to health and productivity. However, due to low initial feed intake, energy generally limits the recovery process. Although more nutrient-dense diets can be formulated to aid calves in recovery, these diets still generally take several days to return stressed calves to positive energy and protein balances. The effect of high-energy receiving diets on health parameters of newly received calves is controversial. However, it appears that increased protein concentrations in the form of UIP may prove beneficial in reducing morbidity. Currently, mineral requirements have not been established for highly stressed calves. Research to determine the requirements of calves facing immune challenges could prove beneficial for formulating improved nutritional management plans for stressed calves.

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Chapter III

EFFECTS OF DIETARY ENERGY AND STARCH CONCENTRATIONS
ON GROWTH PERFORMANCE AND HEALTH OF
NEWLY RECEIVED FEEDLOT CALVES

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Abstract

Five hundred and seventy-two crossbred calves purchased from northern Texas, Arkansas, and Oklahoma auction markets were delivered to the Willard Sparks Beef Research Center in Stillwater, Oklahoma, and used to study the effects of dietary net energy for production (NE_g) and starch concentrations on performance and health of stressed calves during a 42-d receiving period. Upon arrival, calves were randomly assigned to one of two dietary energy levels (0.85 or 1.07 Mcal NE_g /kg feed, DM basis) and one of two dietary starch levels (34 or 48% of dietary metabolizable energy (ME) from starch). Diets consisted of dry-rolled corn, dried corn distillers grains, wheat middlings, soybean hulls, cottonseed hulls, alfalfa hay, and a monensin-containing supplement. Cattle were weighed and serum samples for antibody determination were collected on d 0, 7, 14, 28, and 42. Detailed individual animal records of morbidity were kept for all cases of respiratory and other disease. Nasal swabs were collected from each morbid animal and cultured for upper-respiratory pathogens. Daily gain (1.2 kg/d) and feed efficiency ($ADG:DMI = 0.18$) were not improved ($P > 0.10$) by increased dietary NE_g or starch concentrations. Calves offered low-energy diets tended ($P = 0.06$) to consume more DM. No differences ($P > 0.10$) were detected in sickness rate or retreatment rate for calves fed high-energy compared to low-energy diets. Calves fed

high-starch diets had higher ($P < .05$) morbidity rates compared to calves fed low-starch diets. There were no ($P > 0.10$) energy or starch effects on *M. haemolytica* or *P. multocida* antibody titers, however day effects ($P < 0.05$) occurred. By d 14, the high-energy, high-starch (HEHS) and low-energy, low-starch (LELS) treatment groups developed antibody titers for *P. multocida* that were significantly greater than titers on d 0, while the remaining treatment groups never varied from d 0. In addition, the HEHS treatment group had greater antibody titers to *M. haemolytica* by d 14 than d 0. Calves fed the low-energy, high-starch diet had a higher ($P < 0.05$) percentage of morbid calves with *H. somnus* isolates during the second pull and a tendency ($P < 0.10$) to have higher *H. somnus* isolates during the first pull and *M. haemolytica* during the third pull than calves on the remaining treatments.

Key Words: Net Energy, Starch, Stress, Calves

Introduction

Formulating a nutritional management plan for newly received stressed calves is often a considerable challenge for producers. Calves often arrive at the feedlot after being marketed through multiple auction markets and traveling great distances, making it impossible to determine the background or physiological condition of the calves. In addition, the stress of marketing is further compounded by such things as new feeds, noises, commingling, and processing upon arrival at the feedlot.

The nutritionist's challenge includes supplying adequate nutrients so that the animal does not rely on body stores for nutrients to survive and to mount an immune response to vaccines and pathogens. This often seems impossible because of the

frequently low feed intake of lightweight stressed calves. High-energy receiving diets, at least initially, would logically supply the calves with necessary nutrients and return the animal to a positive energy balance in the shortest time possible. However, high-energy diets are often comprised of large amounts of readily fermentable carbohydrate (i.e., starch) that may further stress an already unstable calf, making it even more susceptible to health problems.

The existing data is inconsistent with regard to the relationship of dietary energy concentration and respiratory disease. In the mid-1970's California researchers (Lofgreen et al., 1975) conducted a series of experiments feeding rolled barley-based diets at different concentrate levels to test the effects of concentrate level on health and performance of stressed calves. The energy concentrations of the different concentrate levels fed were 0.84, 1.01, 1.10, and 1.19 Mcal NE_g/kg of DM. These researchers reported in two of the three trials that the number of medical treatments required per calf increased as energy density of the ration increased, resulting in higher medical costs per animal. The increased cost vanished, however, when calculated on a per-unit-of-gain basis. More recently, Fluharty and Loerch (1996) fed calves four dietary energy concentrations (1.15, 1.21, 1.25, and 1.30 Mcal NE_g/kg of DM). They found that intake increased linearly with increasing energy concentration, but found no overall differences in daily gain or feed efficiency. Morbidity rate was similar (30%) for all concentrate levels fed.

No data could be found in the refereed literature relating starch content of feed to the health status of receiving calves. Low-starch by-products may offer alternatives to corn for formulating high-energy receiving diets. Therefore, the purpose of this

experiment was to determine the relationship of the energy concentration in diets with two levels of starch with performance and health of newly received calves.

Materials and Methods

Five hundred and seventy-two auction origin bull and steer calves (BW = 186 ± 27 kg) were received at the Willard Sparks Beef Research Center, Stillwater, OK from mid-November 2000 to late November 2001 (Table 3.1). Calves were purchased from numerous auction markets in Oklahoma, Texas, or Arkansas, assembled in Purcell, OK (205 km) or Texarkana, TX (514 km), and shipped to the research center. On arrival, calves were unloaded and allowed to rest for approximately one hour prior to pre-trial processing. Pre-trial processing included assessment of overall health, determination of sex, individual weighing of calves, and individually identifying each calf with a sequentially numbered ear tag. Calves were then distributed to holding pens where they were given 1.0 kg of prairie hay/calf and *ad libitum* access to water. Approximately 16 h later, calves were processed. Processing included individual weight, vaccination against viral respiratory pathogens (Frontier 4 Plus [Intervet, Millsboro, DE], 2 mL via subcutaneous injection [SubQ]); a clostridial bacterin-toxoid (Covexin 8 [Schering-Plough, Omaha, NE, 5 mL subcutaneous injection), and treatment with an injectible avermectin (Ivomec-Plus [Merial, Duluth, GA], 1.0 mL/45.4 kg of BW SubQ) for control of internal and external parasites. The viral respiratory vaccine was boosted on d 14. Calves' identification numbers (ID) were first sorted by corresponding sex (bull or steer) to provide each pen with an approximately equal number of bulls. Within each sex,

Table 3.1 Background information regarding calves received at Willard Sparks Beef Research Center for this study.

Group #	Origin	Date Received	# Calves ^a	# Bulls/Steers	Avg. Initial Weight, kg
1	Texas – Oklahoma -Arkansas	November 14, 2000	231	143/88	189 ± 14
2	Texas – Oklahoma - Arkansas	January 21, 2001	167	93/74	226 ± 16
3	Texas – Oklahoma - Arkansas	September 8, 2001	96	56/40	173 ± 14
4	Texas – Oklahoma - Arkansas	September 15, 2001	78	48/30	159 ± 11

^aTotal calves received = 572. Twenty-four calves were removed from the study due to mortality or animal welfare issues.

ID was again sorted according to weight. Calf ID was randomly assigned to home pen within each sex to create approximately equal average pen weights using a random number generator (Steel and Torrie, 1980). Each pen was randomly assigned to one of four dietary treatments. Truckloads one and two were commingled and assigned to eight pens (2 replicates; 29 hd/pen). Truckloads three and four were commingled and assigned to twelve pens (3 replicates; 14 hd/pen). Truckloads five and six were each assigned to twelve pens (3 replicates/load; 8 or 10 calves/pen). Calves/pen were reduced following truckloads one and two due to inclement weather resulting in poor pen conditions. Dietary treatments (Table 3.2) included two dietary NE_g concentrations (0.85 or 1.07 Mcal NE_g /kg feed, DM basis) and two dietary starch (34 or 48% of dietary metabolizable energy from starch) levels calculated using NRC (1996).

Following processing, calves were moved to assigned pens and supplied 1.4 kg of their appropriate experimental diet (Table 3.2). Pen sizes were uniform across treatments (12.2 m x 30.5 m). Feed was delivered into concrete fence line feed-bunks (12.2 m of linear bunk space per pen). Prairie hay was supplied for the initial 2 to 5 days of the receiving period and reduced as intake of experimental rations increased. Free-choice water was supplied via automatic water basins positioned to supply water to two adjacent pens/basin. Feed bunks were evaluated at approximately 0630 and 1830 for remaining feed and feed delivery was adjusted daily in order to produce a slick bunk immediately prior to the morning feeding. Feed was weighed into individual containers on a platform scale and delivered twice daily at 0700 and 1300.

Table 3.2. Experimental diets (DM basis).

Ingredient, % of Diet	High Energy		Low Energy	
	High Starch	Low Starch	High Starch	Low Starch
Alfalfa hay - fair	17.5	17.5	10.0	10.0
Cottonseed hulls	17.5	17.5	35.0	35.0
Cracked corn	37.5	22.1	24.5	13.0
Corn distillers grain ^a	9.9	19.5	6.5	12.8
Soybean hulls	5.6	14.4	1.0	10.0
Wheat midds	.8	.9	12.9	10.5
Cottonseed meal	6.5	1.0	2.0	1.5
Soybean meal 47.7	3.4	6.1	7.0	6.3
Limestone 38%	1.0	.9	.8	.8
Salt	.2	.2	.2	.3
Other ^b	.04	.04	.04	.04

^aDried corn distillers grains without solubles

^bVitamin A 30 KIU/lb to provide 2200 IU/kg Vitamin A, RumensinTM 80 to provide 25 g/ton monensin, Vit. E 50% to provide 60 IU Vitamin E/kg and Selenium 600 to provide .1 mg Selenium/kg

Calves were weighed on days 0, 7, 14, 28, and 42 of the study. All weights with the exception of d -1 and d 42 were shrunk by 4% to calculate daily gain and feed efficiency. On d 41, calves received morning feed only, and the water was turned off at approximately 1700 hours until after processing the following morning. Due to cold weather ($< -6^{\circ}\text{C}$), calves from truckloads three and four were not shrunk on d 41 and therefore, received a 4% calculated shrink. On days 0, 7, 14, and 28 blood samples were collected via jugular venipuncture from a random subset of calves from each treatment (10 mL Vacutainer tube with no additive) and allowed to equilibrate to ambient temperature prior to overnight storage at 4°C . Serum was separated the following day and stored at -10°C until laboratory analysis could be conducted.

Prior to feeding each morning, calves were evaluated in their pen by experienced personnel for signs of respiratory and other diseases. Each calf was given a severity score: 0) normal, 1) slight, 2) moderate, 3) severe, or 4) mortally ill. An animal was designated as sick and transferred to the processing facility if it was scored as "severe" or if it was scored as "mild" and exhibited two or more clinical signs of disease including depression, lack of fill compared with pen-mates, cough, altered gait, ocular or nasal discharge, or general physical weakness. Upon entering the treatment facility, sick calves were restrained in a hydraulic squeeze-chute and BW, rectal temperature, and a severity score were recorded. If the rectal temperature was 40°C or greater, a predetermined therapeutic antimicrobial regimen was initiated (Table 3.3). In addition, one blood sample (10 mL Vacutainer[®] tube with no additive) was collected for antibody determination and one nasal swab was collected to determine the prevalence of upper-

Table 3.3. Antimicrobial treatment protocol^a.

Pull	Severity Score ^b	Rectal Temperature	Antimicrobial Treatment ^c
First Returned to home pen for at least 48 h before eligible for next treatment	Mild or greater	40° C or higher	Micotil™
Second Returned to home pen for at least 72 h before eligible for next treatment	Mild or greater	40° C or higher	Nuflor™
Third Repeat therapy in 48 h; may be given a third dose if remains morbid	Mild or greater	40° C or higher	Excenel™

^aAntimicrobial pharmaceuticals administered under supervision of a licensed veterinarian

^bSubjective severity score: 0 = healthy; 1 = slightly morbid; 2 = moderately morbid; 3 = severely morbid; 4 = fatally morbid

^cAntimicrobials were administered at labeled dosages

respiratory pathogens. Blood was processed as discussed above and nasal swabs were stored overnight at 4°C until they could be transported to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) the next morning for culture. Regardless of antimicrobial treatment, all information was recorded on individual sick cards and filed for future reference.

If a calf was scored as slight or moderate and the rectal temperature was less than 40° C, all information was recorded, and the calf was returned to its home pen without antimicrobial treatment.

Serum antibody concentrations were determined by ELISA. Enzyme-linked immunosorbent assay wells were coated with 100 µL of antigen at a concentration of 1 ng/µL of coating buffer for outer-membrane proteins (OMPs) and 1.850 optical density (OD) @ 650 nm in 0.4% formalized PBS for whole cells. Plates were covered and rocked overnight in an incubator at 37° C. The following day, plates were washed (3x) and dried prior to addition of sample. Standard and test samples were diluted in PBS-Twn20-1%BSA (OMPs, 1:400; WC, 1:800), 100 µL/well was added and the plates were covered and incubated on a rocker for 1.5 h at 37° C. Plates were again washed (3x) with PBS-Twn20 and dried. A dilute (1:400 in PBS-Twn20-1%BSA anti-bovine IgG) conjugated secondary antibody was added (100 µL/well) and plates were incubated as before. Following a final wash (6x), 100 µL/well of a color substrate consisting of OPD, dH₂O, and H₂O₂ was added to each well. Plates were allowed to color for approximately five minutes prior to a stop reagent being added. Optical density (OD₄₉₀) was determined for each plate in an automated plate reader (V Max Kinetic Microplate Reader, Molecular Devices, Inc.).

Table 3.4. Nutrient composition of experimental rations (DMB).

Nutrient	High Energy		Low Energy	
	High Starch	Low Starch	High Starch	Low Starch
ME ^a , Mcal/kg	2.61	2.60	2.36	2.34
NE _m ^a , Mcal/kg	1.69	1.68	1.47	1.45
NE _g ^a , Mcal/kg	1.07	1.07	0.86	0.85
DM ^b , %	89.2	89.5	89.4	89.6
OM ^b , %	89.0	88.3	89.3	89.3
CP ^a , %	15.5	16.3	14.4	14.8
CP ^b , %	16.5	17.4	15.2	16.2
DIP ^a , g/kg	88.5	89.8	85.0	84.1
DIP ^b , g/kg	94.7	92.7	91.8	95.6
Starch ^a , %	32.3	23.6	26.9	18.8
ME from starch ^a , %	50	36	46	32
NDF ^b , %	64.7	62.9	67.0	66.5
ADF ^b , %	29.8	32.9	35.1	39.2

^aCalculated using NRC, 1996 formulas and tabular ingredient values

^bAnalyzed

Feed samples were collected weekly and combined by receiving period subsequent to the study. Analysis (Table 3.4) included % DM and OM (AOAC, 1996), CP concentration analysis by combustion (Leco, NS2000, St. Joseph, MI), partitioning of nitrogen into degradable (DIP) and undegradable (UIP) protein utilizing the method of Roe et al. (1991), evaluation of starch concentration (Galyean, 1997), and separation of fiber into cell wall and cell content portions by neutral detergent fiber (NDF) analysis and further separating hemicellulose from cellulose and lignin by acid detergent fiber (ADF) analysis (Van Soest et al., 1991). A survey of consulting nutritionist revealed that these levels represent diets currently being fed in receiving feedlots (Hansen; Armbruster, Personal Communication). Protein content of experimental diets was balanced to provide adequate DIP for microbial growth, whereas by-products used to create low-starch diets did not allow for balanced adequate DIP for microbial growth, whereas by-products used to create low-starch diets did not allow for balanced CP. Overall, mixed ration CP values were unexpectedly high compared with calculated values from reference feeds used to formulate the experimental diets (NRC, 1996).

Statistical Analysis. Data were analyzed using the Mixed procedure of SAS (SAS, 1996). Pen was used as the experimental unit for performance data variables such as daily gain, intake, and feed conversion. The experiment was designed as a randomized complete block, blocked by feeding group, with a 2 x 2 factorial treatment structure. Main effects were two levels of NE_g/kg feed and two levels ME contribution from starch. Two-way interactions between main effects and block were tested. The three-way interaction between main effects and block (group) was used as the overall error term (σ_e^2) and used to test main effects. Calves were assigned to pens such that each pen

would have approximately the same number of bulls in order to minimize sex effects. An alpha error term of 0.05 was chosen as significant for all data. For variables related to health such as first and second pull rates, recovery rates, antibody titers, and upper respiratory pathogen data, individual calves were used as the experimental unit. Covariate analysis was conducted to determine sex (bull or steer) effects. Individual animal nested within block by energy by starch combination was used as the error term to test main effects. Antibody titers were analyzed as repeated measures over time utilizing the individual animal by group by starch by energy within day as the error term.

Results and Discussion

Animal Performance. Feedlot performance data are shown in Table 3.5. Feeding group (blocking factor) x starch and group x energy interaction terms were not significant ($P > 0.10$) and were removed from the model. Twenty-four calves were removed from the study due to mortality or animal welfare issues (Table 3.6). Daily gain (ADG) and feed efficiency (ADG:DMI) were not affected ($P > 0.10$) by dietary energy or starch levels. Overall, calves gained similarly averaging $1.17 \pm .44$ kg calf¹/d. Similarly, Ohio researchers (Fluharty and Loerch, 1996) fed four dietary NE_g levels (1.15, 1.21, 1.25, and 1.30 Mcal/kg of DM) to stressed calves and found no energy effects on daily gain or feed efficiency. In contrast, Lofgreen et al. (1975) reported a linear increase in daily gain of stressed calves fed .84, 1.01, or 1.10 Mcal NE_g/kg of DM as dietary energy levels increased. In our experiment, dietary starch level had no effect ($P > 0.29$) on either overall or interval period DMI. Calves fed low-energy diets tended ($P < 0.06$) to consume more DM (3.8 %) than calves fed high-energy diets during the overall feeding

TABLE 3.5. Receiving period performance by dietary treatment of calves fed 42 days.

	High Energy		Low Energy		SEM ^a	Probability > F		
	High Starch	Low Starch	High Starch	Low Starch		Starch	Energy	Starch x Energy
Pens, n	10	10	10	10	---	---	---	---
Calves	84	86	85	86	---	---	---	---
<u>Weight, kg</u>								
d 0	186.6	186.5	186.2	186.5	14.19	0.91	0.90	0.87
d 42	236.7	238.2	235.6	238.9	18.38	0.41	0.93	0.67
<u>Daily gain, kg</u>								
d 0 - 7	-.21	-.28	-.43	-.19	0.33	0.46	0.59	0.28
d 8 - 14 ^b	.81	.70	.73	.75	0.24	0.60	0.91	0.43
d 15 - 28	1.56	1.56	1.62	1.74	0.23	0.37	0.16	0.42
d 29 - 42	1.32	1.48	1.27	1.39	0.25	0.25	0.46	0.73
d 0 - 42	1.14	1.16	1.11	1.19	0.11	0.47	0.98	0.49
<u>Intake, kg/d</u>								
d 0 - 7	2.8	2.7	2.8	2.8	0.48	0.56	0.47	0.64
d 8 - 14	4.3	4.3	4.5	4.6	0.53	0.70	0.09	0.71
d 15 - 28	5.6	5.6	5.8	5.8	0.42	0.74	0.09	0.86
d 29 - 42	6.9	7.2	7.4	7.5	1.11	0.28	0.08	0.50
d 0 - 42	5.4	5.4	5.6	5.6	0.63	0.65	0.06	0.94
<u>Gain/feed</u>								
d 0 - 7	-.20	-.33	-.04	-.23	0.15	0.30	0.39	0.83
d 8 - 14 ^b	.24	.08	.27	.17	0.14	0.52	0.60	0.79
d 15 - 28	.23	.25	.25	.25	0.04	0.65	0.69	0.56
d 29 - 42	.21	.21	.18	.20	0.05	0.38	0.16	0.41
d 0 - 42	.18	.16	.19	.18	0.03	0.64	0.36	0.83

^aStandard error of the mean

^bData from load 4 was omitted from analyses due to scale malfunction

Table 3.6. Calves removed from experiment.

ID #	Date		Group	Diagnosis	Outcome
	Received	Removed			
1724	11/14/00	11/15/00	1	Crippled	Off trial
1749	11/14/00	11/15/00	1	Crippled	Off trial
1851	11/14/00	11/17/00	1	Crippled	Off trial
1773	11/14/00	11/18/00	1	Crippled	Off trial
1939	11/14/00	11/18/00	1	Crippled	Off trial
1763	11/14/00	11/22/00	1	Crippled	Off trial
1868	11/14/00	11/22/00	1	Crippled	Off trial
1906	11/14/00	11/27/00	1	Crippled	Off trial
1923	11/14/00	11/29/00	1	Crippled	Off trial
1793	11/14/00	12/10/00	1	Crippled	Off trial
1855	11/14/00	12/10/00	1	Pneumonia	Dead
1806	11/14/00	12/29/00	1	Crippled	Off trial
1866	11/14/00	12/30/00	1	Pneumonia	Dead
859	01/21/01	02/01/01	2	Crippled	Off trial
920	01/21/01	02/01/01	2	Crippled	Off trial
935	01/21/01	02/01/01	2	Crippled	Off trial
955	01/21/01	02/01/01	2	Crippled	Off trial
1574	09/08/01	09/18/01	3	Crippled	Off trial
1554	09/08/01	09/22/01	3	Polioencephalomalacia	Off trial
1591	09/08/01	10/28/01	3	Respiratory chronic	Off trial
1716	09/15/01	9/20/01	4	Pneumonia	Dead
1700	09/15/01	9/26/01	4	Not eating	Off trial
1715	09/15/01	9/26/01	4	Not eating	Off trial
1676	09/15/01	10/11/01	4	Respiratory chronic	Off trial

period. However, this difference did not affect feed efficiency. These differences are in contrast to previously reported experiments (Fluharty and Loerch, 1996; Lofgreen et al., 1975) that reported increasing DMI as dietary NE_g levels increased. Similar to previous studies (Fluharty and Loerch, 1996; Lofgreen et al., 1975), during the critical initial week of the receiving period, when intake is typically very low, intake was similar for all diets. Mean DMI for all groups was approximately 2.8 ± 0.5 kg during the first week and 5.5 ± 0.6 kg for the entire 42-d receiving period. Calves on low-energy diets gained unexpectedly well. Table 3.7 shows the differences between expected gains estimated on the basis of NRC (1996) published energy values and actual gains. Estimated and actual gains were similar for calves consuming high-energy diets. Calves fed diets estimated to provide only 0.85 Mcal/kg of DM gained 137% of estimated for the high-starch diet and 151% of estimated for the low-starch diet. These deviations from expected performance may be explained in part by the increased intake of calves consuming the low-energy diets. The increased intake is largely unexplainable, however, one may speculate that calves fed the low energy gained an intake advantage over calves fed high-energy rations due to less concentrated rations. The increased roughage may have aided calves in adjustment to the mixed ration. The increased roughage may have also created a positive associative effect, slowing the passage rate of fermentable carbohydrates and raising digestible energy and thus, raising net energy values of the diets. It is also plausible that, at least in part, the deviance of observed gain from expected gain was due to increased gut-fill of calves fed the less concentrated rations.

In contrast to the present study, Lofgreen et al. (1975) reported that stressed, newly received calves generally consume greater quantities of higher-concentrate diets.

Table 3.7. Evaluation of actual performance as a percentage of expected performance by dietary treatment and calculation of actual net energy for gain.

	High Energy		Low Energy	
	High Starch	Low Starch	High Starch	Low Starch
Results				
Mean BW, kg	211.7	212.4	210.9	212.7
Daily feed consumed, kg	5.4	5.4	5.6	5.6
Daily gain, kg	1.14	1.16	1.11	1.19
Tabular NE values of ration				
NE _m , Mcal/kg	1.69	1.68	1.47	1.45
NE _g , Mcal/kg	1.07	1.07	.86	.85
Calculations				
NE _m required per day, Mcal	4.27	4.28	4.26	4.29
Feed required for maintenance, kg	2.53	2.55	2.90	2.96
Feed available for gain, kg	2.87	2.85	2.70	2.64
NE _g available for gain, Mcal	3.07	3.05	2.32	2.24
NE _g required per kg gain, Mcal ^a	2.84	2.85	2.84	2.85
Expected gain, kg/d	1.08	1.07	0.81	0.79
Observed gain as % of expected gain	106	108	137	151
NE_g calculation				
Empty body wt ^b , kg	188.6	189.2	187.9	189.5
Empty body gain ^c , kg	1.09	1.11	1.06	1.14
Retained Energy ^d , Mcal/d	3.34	3.42	3.23	3.52
NE _g of ration ^e , Mcal/kg	1.16	1.20	1.19	1.33

^aDetermined from Table 10 of NRC, 1984

^bEmpty body weight (EBW) = 0.891 x shrunk BW

^cEmpty body gain (EBG) = .956 x shrunk weight gain

^dRetained energy (RE) = (52.72 EBG + 6.84 EBG²)(EBW^{.75}) (Lofgreen and Garrett, 1964)

^eNE_g = RE / (DMI - Intake_{maintenance})

The NE_g of diets was calculated using equations established by Lofgreen and Garrett (1964) (Table 3.7). These calculations illustrate the reliance of NE_g on intake. Calves fed low energy rations consumed more DM and gained similarly to calves fed high-energy diets. Thus, when the NE_g of individual diets were calculated from retained energy and compared, all rations were similar with the exception of the low-starch, low energy diet. This ration had a higher NE_g concentration as compared with all other rations.

Although the metabolizable protein (MP) concentration differed between treatment groups, calves were not limited by protein in any of the experimental diets. Table 3.8 shows metabolizable protein (MP) requirements for maintenance and growth based on NRC (1996), MP equations and MP supplied based on intake data. Regardless of diet, calves appeared to be limited by energy intake rather than protein intake. Effects of varying protein levels in receiving diets have been characterized. Galyean et al. (1993) reported increased daily gain with increasing supplemental soybean meal fed to 185 kg calves in a 42 d receiving study. Similarly, Ohio researchers (Fluharty and Loerch, 1995) assessed protein requirements of newly received calves by feeding 12, 14, 16 or 18% CP diets and reported a linear increase in daily gain for the first week after arrival. In a second experiment, these researchers fed 11, 14, 17, 20, 23, or 26% CP diets. Daily gain and feed efficiency were both affected quadratically with the 20% CP diet yielding superior performance. Eck et al., (1988) conducted a series of 28-d trials to evaluate protein concentration in incoming feeder calves. In Exp. 1 and 2, calves were fed steam-flaked sorghum grain based diets consisting of either 10.5 or 12.5% dietary CP.

Table 3.8. Metabolizable protein requirements and intake during 42-d receiving period.

	High Energy		Low Energy	
	High Starch	Low Starch	High Starch	Low Starch
MCP intake^a, g/d				
d 0 - 7	169	162	154	152
d 8 - 14	259	258	247	250
d 15 - 28	337	336	318	315
d 29 - 42	416	432	406	407
d 0 - 42	325	324	307	304
MP intake^b, g/d				
d 0 - 7	326	338	288	301
d 8 - 14	501	538	463	494
d 15 - 28	652	700	597	623
d 29 - 42	804	900	762	806
d 0 - 42	629	675	577	602
MP available for gain^c, g/d				
d 0 - 7	134	146	97	109
d 8 - 14	304	342	268	297
d 15 - 28	438	488	385	410
d 29 - 42	583	680	542	584
d 0 - 42	418	445	348	371
MP requirement for gain^d, g/d				
d 0 - 7	-47	-40	-26	-49
d 8 - 14	237	206	215	220
d 15 - 28	230	230	229	228
d 29 - 42	231	230	231	230
d 0 - 42	233	233	234	233

^aMicrobial crude protein yield calculated using $MCP = 0.13 \times TDN$ (NRC, 1996).

^bMetabolizable protein supply calculated using $MP_{tot} = MP_{bact} + MP_{feed}$, where $MP_{bact} = MCP \times 80\%$ and $MP_{feed} = UIP_{intake} \times 80\%$ (NRC, 1996).

^cMetabolizable protein available for gain calculated using $MP_{intake} - MP_{maint}$; where $MP_{maint} = 3.8 \times Shrunk\ BW^{0.75}$ (NRC, 1996).

^dMetabolizable protein required for actual gain (Table 3.5) calculated using $MP_g = NP_g / (0.83 - (EQEBW \times 0.00114))$; where $NP_g = SWG (268 - (29.4 (RE/SWG)))$. NRC (1996); EQEBW and RE relationships shown in Table 3.6.

Calves fed the 12.5% CP diet gained more rapidly and were more efficiently fed than calves fed the 10.5% CP diet.

Health performance. Health performance for calves from groups 1 and 2 was omitted from analysis because of an electronic thermometer malfunction, which was not detected until late in the receiving period. Fifty-seven percent of calves in all treatments received at least one antimicrobial treatment with 16% of calves treated receiving requiring a second treatment and 2% requiring a third treatment (Table 3.9). A significant ($P < .05$) starch effect existed for first pull rate. This difference existed, however, because of a relatively low number of calves pulled from the high-energy, low-starch treatment group. No energy effects were present ($P > 0.10$) for first, second, or third pull rates. These data are consistent with Ohio research (Fluharty and Loerch, 1996) that reported similar incidences of respiratory disease for calves fed diets varying in NE_g from 1.15 to 1.30 Mcal. In contrast, Lofgreen et al. (1975) reported that morbidity tended to increase with increasing dietary NE_g . However, results were inconsistent throughout their trials. The starch effect may have only occurred in the high-energy rations due to a stress effect of the readily fermentable carbohydrate in the HSHE diet.

Antibody titers. No starch or energy effects ($P > 0.10$) were detected for either *M. haemolytica* or *P. multocida* antibody titers (Table 3.10). However, significant ($P < 0.05$) day effects were present for cattle fed high-energy for *M. haemolytica* and for cattle fed HEHS and LELS rations for *P. multocida*. Calves fed HEHS diets developed antibody titers greater ($P < 0.05$) than initial levels for both pathogens by d 14. No previous data could be found relating energy concentration or source to antibody titers for BRDC pathogens.

Table 3.9. Health performance of calves during the 42-d receiving period.

	High Energy		Low Energy		SEM ^a	Probability > F	
	High Starch	Low Starch	High Starch	Low Starch		Starch	Energy
<u>First Pulls^{bc}, #</u>	55 (65%)	39 (45%)	50 (59%)	50 (58%)	9.927	0.05	0.55
d 1 – 7, %	59.4	50.8	61.1	61.8	--	--	--
d 8 – 42, %	40.6	49.2	38.9	38.2	--	--	--
Day treated ^e	5.9	5.0	4.9	4.6	2.298	0.14	0.22
<u>Second Pulls^{bc}, #</u>	8 (15%)	6 (15%)	6 (12%)	10(20%)	3.182	0.57	0.56
Day treated ^e	15.5	10.2	12.9	11.3	2.367	0.66	0.04
<u>Third Pulls^{bc}, #</u>	0 (0%)	2(5%)	1(2%)	3(6%)	0.988	0.10	0.41
Day treated ^e	18.9	21.1	20.0	19.4	5.485	0.94	0.82

^aStandard error of the mean

^bNumber of animals in dietary treatment group receiving antimicrobial treatment

^cAnalyzed using Chi-square P value

^dNumber of animals pulled prior to recovery from first respiratory disease event

^eAnalyzed using Proc Mixed procedure of SAS (1996)

Table 3.10. Antibody titers to *Mannheimia haemolytica* and *Pasteurella multocida* by treatment and day^a.

	High Energy		Low Energy		SEM ^b	Probability > F		
	High Starch	Low Starch	High Starch	Low Starch		Starch	Energy	Starch x Energy
<u>Outer membrane protein^c</u>					0.148	0.2083	0.4046	0.8269
d 0	.80 ^e	1.07	1.05	1.00 ^e				
d 7	1.07 ^{ef}	1.29	1.16	1.29 ^{eg}				
d 14	1.31 ^f	1.33	1.34	1.47 ^{fg}				
d 28	1.36 ^f	1.27	1.28	1.39 ^{fg}				
<u>Whole cell protein^d</u>					0.050	0.7166	0.5003	0.6889
d 0	.129 ^e	.116	.117	.171				
d 7	.202 ^{ef}	.261	.170	.190				
d 14	.276 ^f	.226	.216	.169				
d 28	.224 ^{ef}	.151	.220	.197				

^aMeasured as nanograms of secondary antibody that bound to sample

^bStandard error of the mean

^c*P. multocida*

^d*M. haemolytica*

^{e,f,g}Means in same column with different superscript differ significantly (P < .05)

Upper respiratory pathogens. *M. haemolytica* was the most prevalent pathogen isolated from the upper respiratory tract of morbid calves, and was detected in 74% of morbid calves at the time of first treatment, 37% at second treatment, and 29% at third treatment (Table 3.11). Although no significant main effects ($P > 0.10$) were detected on *M. haemolytica* presence, in pairwise least squares means comparisons calves fed the HEHS diet had lower ($P < 0.05$) incidence rates during the third pull. Prevalence of *P. multocida* in the upper respiratory tract of morbid calves measured at the first pull was lowest for calves fed the HEHS diet, but only differed ($P < 0.05$) significantly from calves fed the LEHS diet. Prevalence of *P. multocida* was less ($P < 0.05$) at the second pull for calves on the HEHS ration than calves on the HELS diet, and no difference could be detected from isolates obtained at the third pull. Presence of *H. somnus* in the nasal swabs of cattle treated for respiratory disease was low; 5.4% during the first pull, 8.7% in calves pulled a second time, and 22% in calves pulled a third time. High-energy treatment groups tended ($P = 0.08$) to have a lower percentage of cattle with *H. somnus* isolates at the initial pull, however, these groups were similar ($P > .05$) to the LELS group. During the second pull, calves on high-energy diets had a lower ($P < 0.05$) percentage of isolates compared with calves fed low-energy diets. No relationship between energy ($P > 0.05$) and *H. somnus* was found during the third pull. Calves on high-starch treatments tended ($P < .08$) to have a higher percentage *H. somnus* isolates during the first pull compared to calves fed low-starch diets. Both this effect and the energy effect were largely due to the high percentage of calves fed the LEHS diet with *H. somnus* in the upper respiratory tract. No starch effect was detected for isolates during

Table 3.11. Prevalence of pathogens in nasal cavity of calves treated as sick by dietary treatment.

	High Energy		Low Energy		SEM ^a	Probability > F		
	High Starch	Low Starch	High Starch	Low Starch		Starch	Energy	Starch x Energy
First Pull^b								
<i>M. haemolytica</i> , %	64.4	77.1	75.9	80.0	0.80	0.12	0.19	0.43
<i>P. multocida</i> , %	17.7 ^f	26.2 ^{ef}	35.3 ^e	26.1 ^{ef}	0.07	0.96	0.17	0.17
<i>H. somnus</i> , %	3.3 ^f	2.3 ^f	12.7 ^e	3.3 ^f	0.06	0.07	0.07	0.15
Second Pull^c								
<i>M. haemolytica</i> , %	45.2	21.0	38.0	42.5	0.13	0.38	0.53	0.20
<i>P. multocida</i> , %	7.3 ^f	32.6 ^e	18.5 ^{ef}	17.8 ^{ef}	0.14	0.14	0.82	0.11
<i>H. somnus</i> , %	1.4 ^f	6.6 ^{ef}	12.2 ^{ef}	14.6 ^e	0.09	0.44	0.05	0.76
Third Pull^d								
<i>M. haemolytica</i> , %	0.0 ^f	28.6 ^{ef}	62.5 ^e	25.0 ^{ef}	0.18	0.79	0.09	0.06
<i>P. multocida</i> , %	2.5	10.6	13.7	35.0	0.18	0.31	0.21	0.64
<i>H. somnus</i> , %	20.0	24.7	13.4	28.0	0.18	0.08	0.75	0.32

^aStandard error of the mean

^bPercentage of calves having positive cultures for pathogen during first pull

^cPercentage of calves pulled twice having positive cultures for pathogen

^dPercentage of calves pulled three times having positive cultures for pathogen

^{e,f}Means in same row with different superscript differ (P < .05) significantly

the second pull, however, in pairwise least squares means comparison, the HEHS treatment group had significantly ($P < 0.05$) less *H. somnus* isolates than calves fed the LELS diet. Starch had no effect ($P > 0.05$) on isolates present at the third pull. Frank et al., (1983) reported similar percentages of *M. haemolytica* in feedlot calves treated for respiratory disease in a 1976 study with percentages reaching almost 100% in a 1977 study. No data correlating *P. multocita* or *H. somnus* found in the upper-respiratory tract to incidence of respiratory disease could be found in the refereed literature. In addition, data relating upper-respiratory tract pathogens to re-pulls could not be found.

Conclusions

Daily gain and feed efficiency were not affected by either dietary energy concentration or starch level in this study. Dry matter intake was increased ($P < .08$) in cattle offered the low-energy receiving diets on d 7, 14, 28, 42, and the overall receiving period. The lack of differences in feed efficiency in this study was a function of a small difference in intake relative to the number of replications (10). Fewer calves fed the high-energy, low-starch ration were treated for BRD as compared to other treatment groups, however, no starch or energy effects were detected. No starch or energy effects on antibody titers to either *M. haemolytica* or *P. multocida* were noted, however, cattle in the high-energy, high starch treatment group developed antibody titers to both pathogens that were significantly different from arrival titers by d 14. In addition, cattle in the low-energy, low starch treatment group developed significant antibody titers to *P. multocida* by d 14.

Implications

No adjustment to energy requirements for stressed calves has been established to date. Although growth performance was unaffected by energy level in this study, calves fed low-energy rations tended to have higher feed intake. This effect was possibly due to superior energy provided to meet the calves maintenance requirements by low energy diets. In addition, although high starch levels in diets did not affect growth performance by metabolically restricting calves, energy possibly did. Because highly concentrated diets did not negatively affect the health of the calves in this study, economics should dictate the feeding strategy. Further research into the effect of respiratory disease on energy requirements would provide useful information allowing producers to formulate rations to meet the calf's needs. In addition, this study was of a very limited scope. A wider range of energy and starch concentrations should be examined to determine upper limits of energy concentrations that may be fed to stressed calves.

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CHAPTER IV

MEASURING CHANGES IN ACUTE PHASE
PROTEIN CONCENTRATION TO PREDICT
BOVINE RESPIRATORY DISEASE

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Abstract

Five hundred and seventy-two market stressed male calves (186 ± 27 kg) were received at the Willard Sparks Beef Research Center near Stillwater, Oklahoma in truckload lots in November, 2000, January, 2001, and September 2001 in order to study the effects of dietary energy concentration and source on growth performance, health performance, and immune response. Plasma and serum samples were collected from a subset of animals and used to characterize the acute phase response.

The purpose of this study was to determine if one or more of the hepatic proteins associated with the acute phase response could be used as a diagnostic or prognostic tool for calves affected by bovine respiratory disease. Three acute phase proteins (APP) were measured: fibrinogen (Fb), haptoglobin (Hp), and serum amyloid-A (SaA). Blood samples were collected on days 0, 7, 14, and 28 in order to determine the variation and normal levels. In addition samples were collected upon medical treatment and seven days following treatment to determine if the APP could be used as a diagnostic tool.

Serum concentrations of Fb, Hp, and SaA were similar between dietary treatment groups on d 0. Fb concentration remained constant over time, while Hp and SaA concentrations decreased by d 28. No differences were detected between dietary

treatment groups throughout the receiving period. The ratio of Fb/total blood proteins differed significantly ($P < .05$) on d 0, 7, and 28 between calves never treated for respiratory disease and those treated multiple times. Hp concentrations differed ($P < .05$) between calves never treated, calves treated once, and calves treated multiple times on d 0 and d 7, and differed ($P < .05$) between calves never treated and calves treated multiple times on d 14 and d 28. Hp concentration was significantly ($P < .01$) elevated in calves on medical treatment days as compared with recovery days. These results suggest that Hp may be used as a diagnostic tool to make management decisions as to treatment protocols of calves with BRD.

Introduction

Although producers continue to utilize a number of vaccines and antimicrobials to control the major pathogenic contributors to bovine respiratory disease (BRD), it continues to be the most economically significant disease condition in feedlots. In 1999 BRD affected 14.4% of placements (U.S.D.A., 1999) and at last report, led to 28% of the total cattle deaths (U.S.D.A., 1996) valued at \$478 million. Approximately two-thirds of the cattle that died were calves, rather than yearlings. Loneragan et al. (2001) measure trends in mortality among cattle in feedlots. These researchers calculated yearly and monthly mortality ratios of approximately 21.8 million cattle entering 121 feedlots from 1994 to 1999. Averaged over time, the mortality ratio was 1.26 deaths/100 cattle entering the feedlots. Compared to cattle entering the feedlot in 1994, cattle received in 1999 had a significantly increased risk (relative risk, 1.46) of dying of respiratory disease. In addition, respiratory tract disorders accounted for 57.1% of all deaths. BRD is a

multifactorial event involving the host animal, pathogen, and the environment (Perino, 1992). In addition, data exist that show the relationship between viral and bacterial pathogens and their contribution to the prevalence of pneumonia in calves (Roth and Perino, 1998). These factors make BRD difficult to diagnose making effective treatment difficult.

Diagnosis of respiratory disease in cattle is subjective and is often conducted by personnel with limited training. A diagnostic tool to identify infection would be useful to producers and veterinarians by supplying objectivity to diagnosis of sick animals. The measurement of acute phase proteins (APP) has potential as a diagnostic and prognostic tool in cattle. An increase in APP concentrations in plasma following inflammatory stimulus results from increased rates of synthesis and secretion of APP by liver parenchymal cells (Kushner and Mackiewicz, 1987). These APP function to minimize damage caused by pathogens and promote tissue repair (Faulkner et al., 1992). Acute phase proteins are released into the plasma within hours of the insult and increase from 25% to 1000% above normal concentrations (Kushner and Mackiewicz, 1987).

In humans, ceruloplasmin, complement components C3 and C4, α_1 acid glycoprotein, α_1 proteinase inhibitor, α_1 anti-chymotrypsin, haptoglobin, fibrinogen, C-reactive protein, and serum amyloid-A have all been proven to increase during inflammation (Kushner and Mackiewicz, 1987). Although APP release is species dependent, haptoglobin, ceruloplasmin, α_1 proteinase inhibitor, fibrinogen, and α_1 acid glycoprotein have all been shown to increase during the acute phase response in cattle (Conner et al., 1988). Also of some interest as diagnostic tools are the pro-inflammatory

cytokines (interleukin-1, interleukin-6, and tumor-necrosis factors) that mediate the acute phase response.

Serum haptoglobin (Hp) concentrations have been described in sick and healthy cattle. Wittum et al., (1996) reported a mean initial Hp concentration in feedlot calves with clinical respiratory disease as 67 ± 108 mg/dL with a range of 508 mg/dL. Calves receiving antimicrobial treatment had Hp concentrations at or near zero at final examination, whereas calves not receiving antimicrobial treatment had only slightly lower concentrations at final examination as compared with initial examination. Young et al., (1996) measured serum Hp concentrations of calves at three time periods (d 0, d 40, d 60) during a feeding trial. These researchers reported that 58% of the calves sampled had detectable serum Hp concentrations in at least one sample. Calves with subsequent clinical respiratory tract disease had a higher ($P < .05$) proportion of calves with elevated Hp concentrations.

Severity of illness, as compared with Hp and serum amyloid-A (SaA) concentrations, were characterized in healthy cows, cows with spontaneous acute, subacute, or chronic inflammatory diseases (Alsemgeest, 1994). Plasma concentrations of both APP differed ($P < .001$) between healthy animals and animals with inflammatory disease. APP from cows with acute inflammatory disease were significantly different ($P < .01$) as compared with cows with chronic disease. Some evidence exists, however, questioning the efficacy of SaA as a diagnostic tool as it may be elevated under stress situations (Alsemgeest, 1995). To test this hypothesis, two stress levels were created by housing groups of five cows on different types of floors, videotaping the animals, and assessing stress. Plasma SaA concentrations were elevated ($P < .001$) in cattle housed on

floors associated with the highest levels of physical stress whereas Hp concentrations were not elevated. Carter (2000) tested the value of using SaA, Fb, α_1 - acid glycoprotein, or Hp blood concentrations to predict morbidity in stressed calves and concluded that Hp concentrations were the only stand-alone test with any predictive value, although Hp/SaA ratios may be an important predictor.

Fibrinogen (Fb) has been described as the most recognized and most frequently employed APP assay in cattle (Eckersall and Conner, 1988). However, Hirvonen et al., (1996) suggested that Fb has limitations as a prognostic tool. In addition, further research (Carter et al., 2002) concluded that Fb concentration lead to inconsistent results and was not useful as a diagnostic or prognostic tool.

The purpose of this study was to evaluate the serum concentrations of Fb/total protein, Hp, and SAA in a large group of feedlot cattle receiving various starch-supplemented rations, and to correlate changes in acute phase proteins with development of respiratory disease and responses to antimicrobial therapy.

Materials and Methods

Experimental Design. A study was conducted at the Willard Sparks Beef Research Center (WSBRC) near Oklahoma State University, Stillwater, Oklahoma beginning in November, 2000 to determine the effects of varying energy levels and starch concentrations in receiving diets on growth performance, health parameters, and immune function of newly received stressed calves. Health records were kept on each individual animal treated for respiratory disease, and blood samples were collected from a subsample of calves. Since measurement of health parameters was one of the primary

objectives of the study, a measurement of the intensity of the acute phase response was a valuable concurrent trial.

Five hundred and seventy-two auction origin male calves (186 ± 27 kg) were received in truckload lots in November 2000, January 2001, and September 2001 (Table 3.1). On arrival calves were allowed to commingle for approximately one hour prior to pre-processing. Pre-processing included application of sequentially numbered identification tags in the left ear, determination of arrival weight, determination of sex, and an overall health assessment (healthy or sick). Calves were distributed to holding pens, allowed free-choice water and approximately 1.0 kg of prairie hay. The following morning (d 0) calves were processed between 0600 and 0700 hours prior to feeding. Processing consisted of vaccination against viral respiratory pathogens (Frontier 4 Plus [Intervet, Millsboro, DE], 2 mL via subcutaneous injection [SubQ]); a clostridial bacterin-toxoid (Covexin 8 [Schering-Plough, Omaha, NE], 5 mL subcutaneous injection), and administered an injectible avermectin (Ivomec-Plus [Merial, Duluth, GA], 1.0 mL/45.4 kg of BW SubQ) for control of internal and external parasites. Calves were boosted with the viral respiratory vaccine on d 14. Bulls were left intact until after d 42 of the receiving period because of the potential elevation of APP due to tissue damage.

Calves' identification numbers were separated according to calf sex and according to descending weight. Identification numbers were randomly assigned to home pens to produce equal average pen weights utilizing a random number generator. Pens were randomly assigned to dietary treatment by a random number generator. Truckloads one and two were commingled and assigned to eight pens (2 replicates; 29 hd/pen). Truckloads three and four were commingled and assigned to twelve pens (3 replicates; 14

hd/pen). Truckloads five and six were each assigned to twelve pens (3 replicates/load; 8 or 10 calves/pen). Calves/pen were reduced following truckloads one and two due to inclement weather causing poor pen conditions. Each group received at the WSBRC was fed for a 42 d period. Home pens were uniform in size across all treatments (12.5 m x 30.5 m) with water basins being shared by alternating adjacent pens. Feed bunks were evaluated at approximately 0630 and 1200 hours and managed to provide a “slick-bunk” immediately prior to the morning feeding. Feed was weighed in individual containers for each pen and delivered twice daily at 0700 and 1300 hours. Calves were weighed on d – 1, 0, 7, 14, 28, and 42 of the receiving period. Blood samples for APP determination were collected on d 0, 7, 14, and 28.

Experimental rations are shown in Table 3.2. Rations were formulated to provide dietary net energy for gain (NE_g) concentrations of either 1.07 or .85 Mcal NE_g /kg feed. These concentrations are consistent with diets typically fed in commercial receiving lots (Hansen, 2000, Armbruster, 2000). Within each energy level, rations were also formulated to provide approximately either 34 or 48% of dietary metabolizable energy (ME) from starch. Prairie hay was offered to each pen for the first 2 to 5 d after arrival and was gradually removed as intake increased.

Prior to processing, a sub-sample of six calves per pen (30 calves/dietary treatment) from groups 2 and 6 was randomly selected using a random number generator for whole blood sample (1 plasma and 1 serum sample) collection. Blood samples were allowed to equilibrate to ambient temperature. One plasma sample was collected for each animal (10 mL Vacutainer[®] with sodium heparin) and separated within 12 h of collection, stored overnight at 4° C and fibrinogen (Fb) concentration determined the following day.

The serum sample (10 mL Vacutainer[®] with no additive) was stored overnight at 4° C, separated, and stored at -10° C until laboratory analysis could be performed. Analysis included determination of the plasma APP, haptoglobin, fibrinogen, and serum amyloid-A concentrations.

Prior to feeding each morning calves were evaluated for signs of respiratory and other diseases by experienced personnel. Calves were given a subjective clinical severity score (0 thru 4 scale) based on severity of illness. Clinical scores were: 0 = healthy; 1 = slightly ill; 2 = moderately ill; 3 = severely ill; and 4 = mortally ill. Two or more signs of clinical sickness or a subjective severity score of “severe” was required for the calf to be removed from its home pen and taken to the treatment area. Signs of clinical illness included depression, nasal or ocular discharge, cough, weakness, lack of fill compared to pen-mates, and lack of appetite. Once in the processing area, the calf was restrained in a hydraulic-squeeze-chute, weighed, and its rectal temperature was measured. If the rectal temperature was 40° C or greater, or if the rectal temperature was less than 40° C but the severity score was “severe”, the calf was treated with tilmicosin (10 mg/kg injected subcutaneously). If a second antimicrobial treatment was necessary, florfenicol was administered (40 mg/kg injected subcutaneously). Ceftiofur HCl was administered (2.2 mg/kg injected subcutaneously) if a third treatment was required.

Upon diagnosis of respiratory disease, blood samples were collected from calves from groups 2, 5, and 6 for APP (Hp and SaA) determination. Seven days following the initial antimicrobial treatment, calves were evaluated for signs of continuing respiratory disease. If the calf showed no signs of respiratory disease an additional blood sample

was collected for APP determination. In the case of animals that remained morbid, blood samples were collected upon clinical recovery.

Haptoglobin (Hp). Hp concentrations were determined using Bovine Serum Haptoglobin radial immunodiffusion kits (Code No. P0105-1, Cardiotech Svcs., Inc., Louisville, Kentucky). Bovine serum samples (100 μ L) were treated with an equal amount of 40 mM solution of L-Cysteine (24 mg L-Cysteine dissolved in 5 mL L-Cysteine solvent (1-e)), and both were added to a single mixing well. Two standards were loaded (5 μ L) into specified wells. Similarly, one treated sample was loaded into each additional well of the plate. The plate was covered and incubated at 37° C for at least 24 h. Following incubation, results were determined by measuring the external diameter of each precipitin ring to the nearest .01 mm with a supplied plastic scale. Absence of a precipitin ring indicated Hp concentration of less than 10 μ L/mL, a normal range for healthy cattle. The results were plotted on the vertical axis of a semi-logarithmic graph and Hp concentration was determined from the horizontal axis. A 2x dilution factor was used to develop the reference curve by plotting the ring diameters against two known dilutions. The coefficient of variation for the kit was stated as less than four percent for repeated, identical measurements of the same specimen. Procedures used for this experiment were derived from or validated by Makimura et al., 1982; Van Rijn et al., 1987; Conner and Eckersall, 1988; Conner et al., 1989; Makimura et al., 1990; Skinner et al., 1991.

Fibrinogen (Fb). Plasma samples were delivered to the Boren Veterinary Teaching Hospital Clinical Pathology Laboratory located on the campus of Oklahoma State University in Stillwater, OK for fibrinogen concentration analyses. Samples were

vortexed prior to depositing 100 μ L of sample plasma into a microhematocrit tube. Fb concentrations were then determined using heat precipitation (60° C for three minutes) and refractometry as described by Duncan et al. (1994).

Serum Amyloid-A (SaA). SaA concentrations were determined using commercial ELISA kits (The Tridelta PhaseTM Range kit, Tridelta Development, Ltd., Wicklow, Ireland). Serum samples were vortexed and added (50 μ L diluted 1:500; one sample per well) to each well of a 12 x 8 microtiter strip coated with a monoclonal antibody (Ab) specific for SaA along with biotinylated anti-SaA monoclonal Ab (50 μ L; diluted 1:100 in 1x diluent buffer). Plates were covered and incubated at 37° C for at least one hour, followed by a complete wash (6x) to remove unbound material. Streptavidin-horseradish peroxidase diluted 1:4000 (100 μ L) was added to each well and the plate was incubated at room temperature in darkness for 30 minutes. The plates were washed again (6x) and tapped dry. TMB substrate (100 μ L) was added, and the plates were incubated at room temperature in darkness for an additional 30 minutes. Stop solution was added and the plates were read in an automated plate reader (V Max Kinetic Microplate Reader, Molecular Devices, Inc.) at OD₄₉₀. SAA concentrations were measured in ng/mL.

Statistics. Data were analyzed using the Mixed procedures of SAS (1996). The experimental design was a completely randomized block design with Group as a blocking factor. Residual experimental error (σ_e^2) for the model was individual animal nested within each block by energy by starch combination. Individual animal was used as the experimental unit because health data was recorded on each animal. Starch and energy main effects were analyzed as fixed effects, whereas block was analyzed as a random effect. The treatment structure was a repeated measure over time with time being d 0, 7,

14, or 28. Time and main effects were tested utilizing individual animal by energy by starch within time as the designated error term. Samples from animals that became sick were analyzed as day sick and day well. Repeated measures were also constructed to determine if differences in APP concentrations could be detected at d 0 or d 7 in calves that would never become sick, calves that would become sick only once, and calves that would require multiple treatments. In addition, regression analysis was conducted using the REG procedures of SAS (1996) with number of times treated (0, 1, >1) as the independent variable and APP concentration as a dependent variable.

Results and Discussion

Fibrinogen (Fb). Means of serum Fb concentrations by dietary treatment are shown in Table 4.1. There were no dietary effects on Fb concentration (mg/dL) for calves on any of the dietary treatments. In addition, Fb concentration (mg/dL) was not affected by day (d 0, 7, 14, 28). However, when expressed as a percentage of total blood proteins (mg/mg), Fb concentration decreased ($P < .05$) in all dietary treatment groups by d 28. Fb as a percentage of total blood protein remained constant for d 0 thru d 14. Results of Fb concentrations for calves receiving no (Med0), one (Med1), or more than one (Med>1) antimicrobial treatment are shown in Table 4.2. Significant ($P < .05$) day effects existed in all groups. Calves receiving 0 or 1 treatment had lower ($P < .05$) concentrations by d 28 while calves receiving multiple treatments remained constant over time. In addition, calves receiving multiple treatments had elevated Fb concentrations ($P < .05$) on d 7. Calves in group Med>1 had elevated ($P < .05$) Fb/total protein concentrations on d 0, 7, and 28 as compared with calves in Med0 and Med1. These data

Table 4.1. Results of laboratory analysis for plasma and serum acute phase protein concentrations by dietary treatment.

Factor Measured	D0	D7	D14	D28
Fibrinogen (Fb), mg/dl				
HSHE ^a	574.6 ± 41.0	605.5 ± 42.4	517.8 ± 43.4	420.0 ± 43.0
LSHE ^b	579.9 ± 41.0	568.8 ± 41.0	507.3 ± 41.0	423.6 ± 41.0
HSLE ^c	573.6 ± 41.6	528.5 ± 42.5	525.5 ± 43.4	408.5 ± 42.5
LSLE ^d	562.7 ± 40.2	555.6 ± 40.2	485.7 ± 40.7	468.8 ± 41.0
Fb/Total Protein Ratio				
HSHE ^a	80.4 ^A ± 5.3	84.1 ^A ± 5.5	74.0 ^A ± 5.6	57.9 ^B ± 5.6
LSHE ^b	77.4 ^A ± 5.3	79.8 ^A ± 5.3	71.3 ^{AB} ± 5.3	58.4 ^B ± 5.4
HSLE ^c	75.2 ^A ± 5.4	72.7 ^A ± 5.5	71.8 ^A ± 5.6	55.1 ^B ± 5.5
LSLE ^d	76.8 ^A ± 5.2	75.4 ^A ± 5.2	65.2 ^{AB} ± 5.3	62.9 ^B ± 5.3
Haptoglobin (Hp), µg/ml				
HSHE ^a	757.1 ^A ± 148.8	1037.5 ^A ± 148.8	235.0 ^B ± 152.6	388.2 ^B ± 149.9
LSHE ^b	435.8 ^A ± 140.3	801.2 ^B ± 144.7	303.4 ^A ± 141.3	221.8 ^A ± 140.3
HSLE ^c	552.4 ± 144.7	569.2 ± 144.7	254.1 ± 148.4	304.8 ± 144.7
LSLE ^d	818.1 ^A ± 137.2	867.2 ^A ± 141.3	340.0 ^B ± 139.0	323.6 ^B ± 140.3
Amyloid-A (SaA) ^e , ng/ml				
HSHE ^a	189.9 ^{AB} ± 44.3	217.2 ^A ± 44.3	122.5 ^{AB} ± 46.1	99.8 ^B ± 45.2
LSHE ^b	164.5 ^A ± 42.1	119.1 ^{AB} ± 42.1	132.6 ^{AB} ± 43.6	42.4 ^B ± 42.8
HSLE ^c	164.2 ± 42.9	196.5 ± 42.9	119.6 ± 44.5	130.8 ± 43.8
LSLE ^d	146.1 ± 40.7	185.7 ± 40.7	132.9 ± 41.4	97.3 ± 42.1

^{A,B}Day effect. Means in same row with different superscript differ significantly (P < .05)

^aHSHE = high starch, high energy dietary treatment

^bLSHE = low starch, high energy dietary treatment

^cHSLE = high starch, low energy dietary treatment

^dLSLE = low starch, low energy dietary treatment

^eSerum Amyloid-A means for Group 2 only.

Table 4.2. Results of laboratory analysis of serum and plasma acute phase protein concentration by number of medical treatments.

Factor Measured	D0	D7	D14	D28
Fibrinogen (Fb), mg/dl				
Med0	547.5 ^A ± 31.2	485.8 ^{AB} ± 32.2	503.9 ^A ± 33.4	406.3 ^B ± 32.9
Med1	592.8 ^A ± 35.0	549.9 ^{AB} ± 35.0	499.6 ^B ± 35.0	396.3 ^C ± 35.3
Med>1	642.0 ^A ± 39.4	745.3 ^B ± 39.4	578.6 ^A ± 39.4	555.3 ^A ± 39.4
Fb/Total Protein Ratio				
Med0	73.3 ^{aA} ± 4.0	67.8 ^{aA} ± 4.2	69.3 ^A ± 4.3	56.5 ^{aB} ± 4.2
Med1	80.8 ^{acA} ± 4.5	76.1 ^{acA} ± 4.5	69.6 ^A ± 4.5	54.7 ^{aB} ± 4.6
Med>1	85.7 ^{bcA} ± 5.0	100.1 ^{bcB} ± 5.0	78.7 ^{AC} ± 5.0	71.5 ^{bc} ± 5.0
Haptoglobin (Hp), µg/ml				
Med0	153.9 ^{aA} ± 86.3	446.6 ^{aB} ± 88.5	10.0 ^{aA} ± 90.9	152.6 ^{aA} ± 88.5
Med1	751.1 ^{ba} ± 93.4	921.9 ^{ba} ± 96.2	264.0 ^{aB} ± 93.4	158.3 ^{aB} ± 93.4
Med>1	1188.7 ^{ca} ± 100.9	1201.0 ^{ca} ± 100.9	661.4 ^{bb} ± 102.6	688.3 ^{bb} ± 100.9
Amyloid-A (SaA), ng/ml				
Med0	140.2 ^{AB} ± 29.3	179.6 ^A ± 29.3	148.0 ^{AB} ± 30.5	97.9 ^B ± 30.0
Med1	202.9 ^A ± 40.5	163.2 ^A ± 40.5	116.4 ^{AB} ± 41.0	75.6 ^B ± 41.0
Med>1	203.8 ± 56.7	216.2 ± 56.7	79.8 ± 56.7	107.8 ± 56.7

^{a,b,c}Medical treatment effect. Means in same column within APP measured with different superscripts differ significantly (P < .05).

^{A,B,C}Day effect. Means in same row with different superscript differ significantly (P < .05).

suggest that Fb/total protein concentration may be useful in predicting calves that will require multiple antimicrobial treatments. Serum Fb and Fb/total protein concentrations are commonly used clinically to assess inflammatory diseases in domestic animals (Duncan et al., 1994). Carter et al. (2002) recently found no significant value for serum Fb concentrations for predicting illness or responses to antimicrobial treatment in cattle with naturally acquired respiratory disease. However, Fb/total protein concentrations were not examined in that study and are known to be more accurate at assessing the acute phase response than are serum Fb concentrations alone (Duncan et al., 1994).

Haptoglobin (Hp). Serum Hp was not different ($P > .05$) between dietary treatments for any of the sampling periods (Table 4.1). Significant ($P < .05$) day effects did occur in both high-energy groups and the low starch, low energy group. The HSHE and LSLE groups both had lower ($P < .05$) Hp concentrations by d 14 while the LSHE treatment group had elevated Hp concentrations on d 7. Haptoglobin concentration increased linearly ($P < .01$) a number of antimicrobial treatments increased for d 0 and 7 (Table 4.2). This data agrees with Carter, (2000) who reported elevated Hp concentrations in calves requiring multiple treatments. In addition, Hp concentrations of calves never treated until d 7 remained lower than HP concentrations of calves requiring antimicrobial treatment. This suggests that correlations may be developed to determine if calves should receive therapeutic treatment at processing in order to maximize the effects of respiratory disease treatment and minimize the medical costs. Figure 4.1 and 4.2 show the regression relationships of Hp concentration on d 0 and d 7 to number of antimicrobial treatments. Although the correlation coefficients of these models were only .33 and .21, respectively, these models are promising. Due to extremely high variation in

Figure 4.1. Regression analysis of Hp concentration ($\mu\text{l/ml}$) of day 0 blood samples on number of medical treatments required.

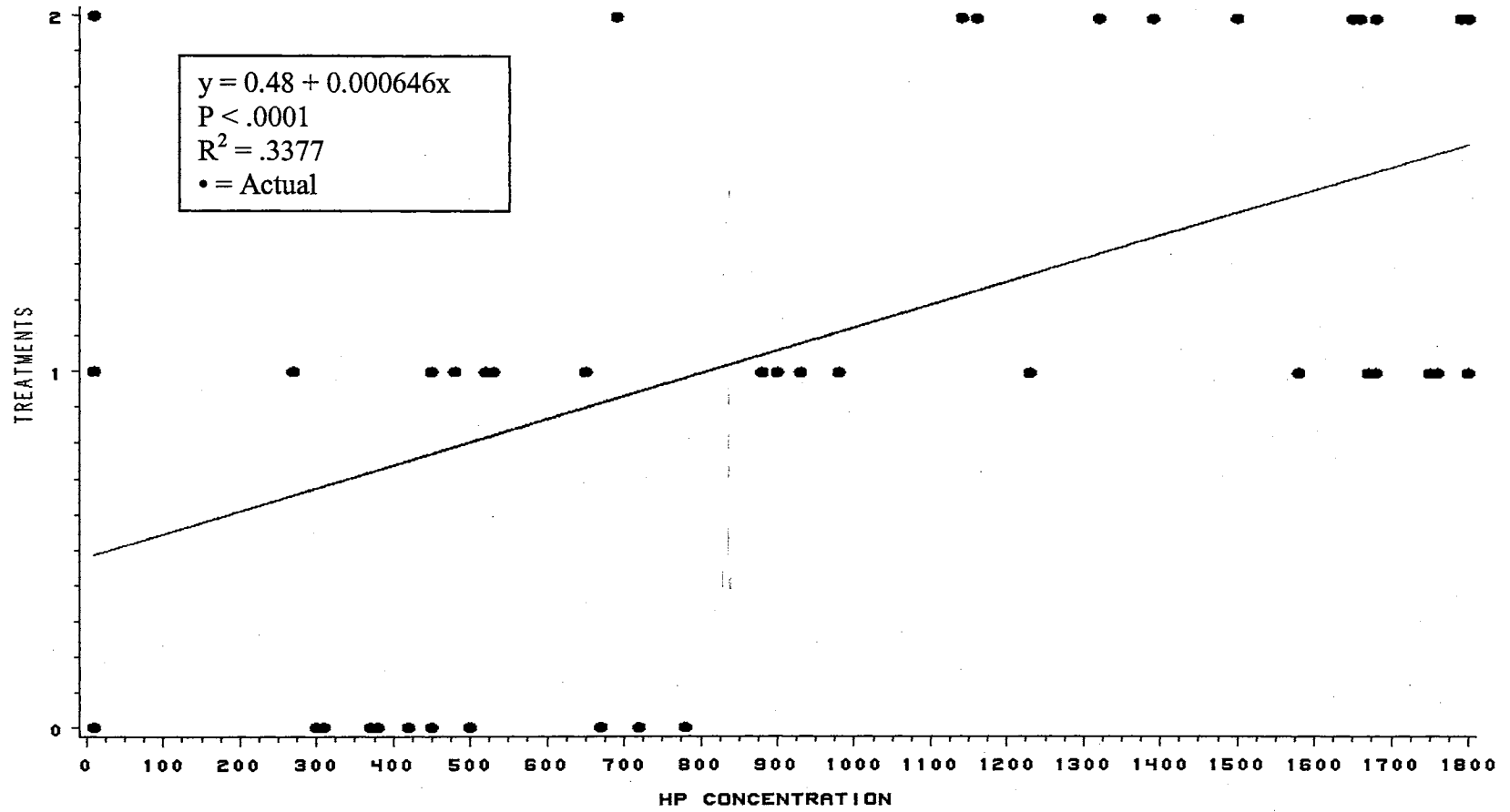
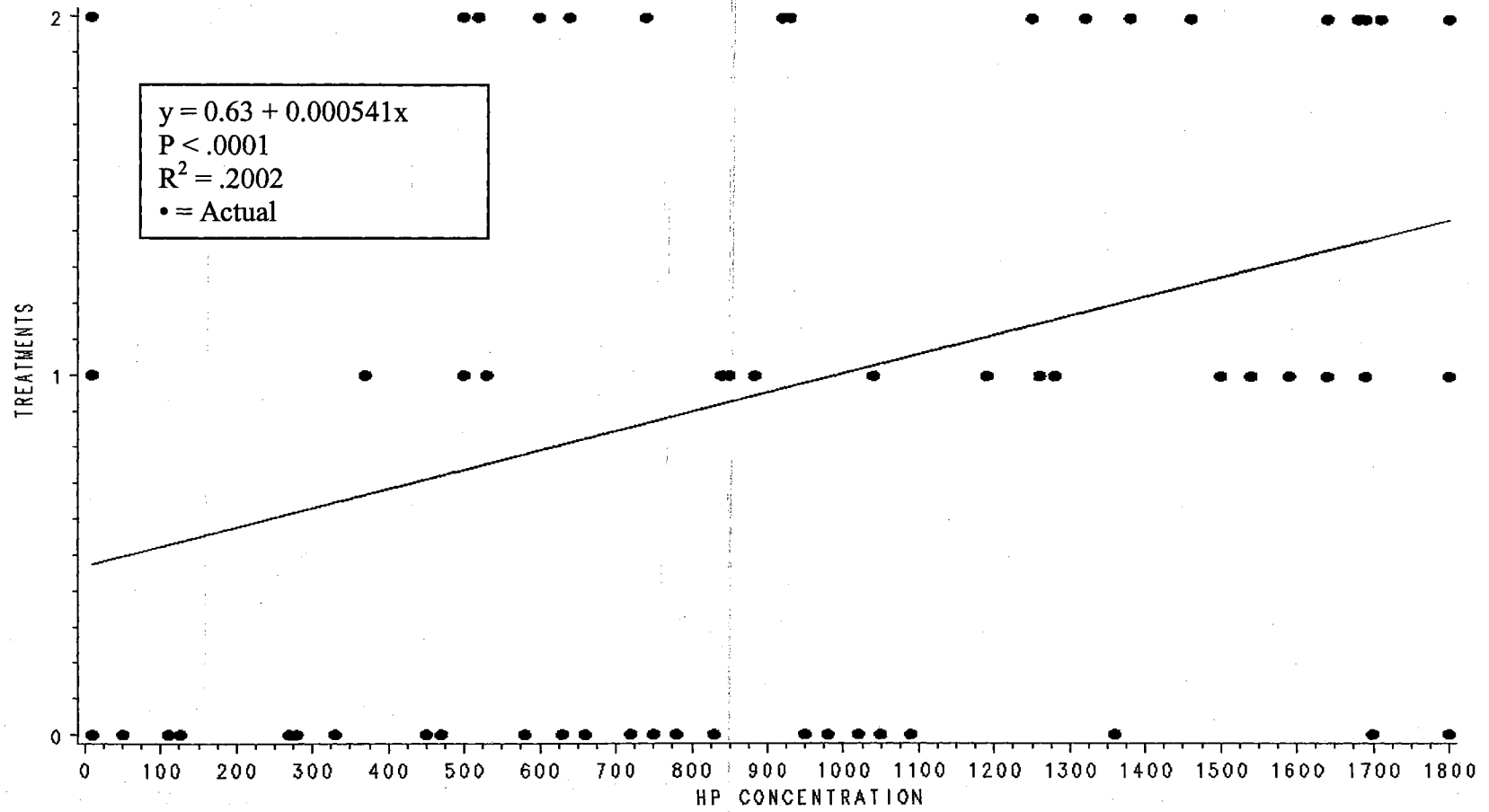


Figure 4.2. Regression analysis of Hp concentration ($\mu\text{l/ml}$) of day 7 blood samples on number of medical treatments required.



Hp concentration, a more expansive number of samples should provide for revisions to the model. In addition, rises in APP concentration are not specific to respiratory disease. Any inflammation will cause an acute-phase response causing further variation. Serum Hp concentrations were elevated ($P < .05$) on d 7 for calves never treated and returned to d 0 levels by d 14 while calves treated for respiratory disease had constant Hp concentrations until d 14. Table 4.3 shows the Hp concentration of calves collected upon medical treatment and upon recovery. The high starch, low energy treatment group had lower ($P < .0001$) Hp concentrations as compared with all other groups upon medical treatment. All four groups had elevated ($P < .05$) Hp concentrations as compared to recovery levels suggesting Hp concentration may have some use as a diagnostic tool.

Serum Amyloid-A (SaA). SaA concentrations did not differ ($P > .05$) among dietary treatment groups (Table 4.1). In addition, no day effects were detected in low energy groups. SaA concentrations remained constant from d 0 to 14 for high-energy groups with d 28 concentrations being decreased ($P < .05$). SaA concentrations were not different ($P < .05$) for calves requiring 0, 1, or more than 1 antimicrobial treatment. However, calves requiring one or no treatments had decreased ($P < .05$) SaA concentrations by d 28, while SaA concentrations of calves requiring more than one treatment remained constant throughout the study. SaA concentrations were also constant in the comparison of blood collections from sick calves and recovered calves. These data suggest that SaA may not be useful as a diagnostic or prognostic tool, however, only one load of cattle are included in this study. Due to the high variability SaA more calves may prove the assay useful.

Table 4.3. Results of laboratory analysis for serum acute phase proteins when cattle were diagnosed as sick and when cattle were determined to be recovered

Factor Measured	Medical Treatment ^a	Recovery ^b
Haptoglobin (Hp), $\mu\text{g/ml}$		
HSHE ^{cA}	1401.7 ^{gi} \pm 69.6	557.1 \pm 70.2
LSHE ^{dA}	1540.7 ^g \pm 78.6	686.9 \pm 79.3
HSLE ^{eA}	1254.4 ^{hi} \pm 70.7	510.3 \pm 71.2
LSLE ^{fA}	1549.3 ^g \pm 66.4	688.2 \pm 66.8
Amyloid-A (SaA), ng/ml		
HSHE	178.8 \pm 28.3	106.4 \pm 28.3
LSHE	215.7 \pm 49.0	186.9 \pm 46.2
HSLE	155.1 \pm 29.6	128.6 \pm 29.6
LSLE ^A	216.0 \pm 29.6	91.2 \pm 31.0

^aBlood sample collected prior to antimicrobial treatment

^bBlood sample collected 7 d following antimicrobial treatment

^cHSHE = high starch, high energy dietary treatment

^dLSHE = low starch, high energy dietary treatment

^eHSLE = high starch, low energy dietary treatment

^fLSLE = low starch, low energy dietary treatment

^{g,h,i} Means in same column with different superscript differ significantly ($P < .05$)

^AMedical treatment effect ($P < .05$)

Numerous reports on the use of acute phase protein responses to predict severity or chronicity of cattle diseases have been published (Alsemgeest et al, 1994; Carter et al., 2002; Hirvonen et al, 1996; Horadagoda et al., 1999; Wittum et al., 1996; Young et al., 1996). Alsemgeest et al. (1994) indicated that serum Hp and SAA concentrations as well as Hp/SAA ratios were elevated in cattle with inflammatory diseases and that serum Hp concentrations and Hp/SAA values were significantly elevated in cases of chronic rather than acute inflammation. In contrast, Horadagoda et al. (1999) found that Hp, SAA, and alpha -1-acid glyoprotein (AGP) were significantly greater in cases of acute compared with chronic inflammation. Because those studies examined a large variety of inflammatory diseases, their results may not be comparable to those found in the current study or those found by Carter et al. (2002) wherein only naturally acquired respiratory disease was studied. More appropriate to the current study, Godson et al. (1996) found that serum Hp concentrations corresponded to the severity of respiratory disease in calves experimentally inoculated with bovine herpesvirus-1 and *Mannheimia haemolytica*. Heegaard et al. (2000) found that the magnitude and duration of serum Hp concentration correlated well with the severity of experimental bovine respiratory syncytial virus infection, whereas serum SAA concentrations increased most rapidly following infection. In the present study, serum Hp concentrations correlated well with the number of antimicrobial treatments required to successfully treat respiratory disease.

Conclusions

Acute phase protein concentrations did not differ between dietary treatments; however, obviously the hepatic proteins did vary in response to morbidity in calves. Haptoglobin

proved to be a strong indicator of sickness and a good predictor as to the number of treatments a calf will require. Serum amyloid-A concentrations provided little information, however, only a small data set was collected for this assay. The most commonly used APP, fibrinogen, also provided some guidance as to the prognosis of morbid animals as concentrations were elevated in calves that would require multiple treatments. Both fibrinogen and haptoglobin concentrations or haptoglobin concentrations alone may be useful for producers to make health management decisions based on objective evaluations.

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VITA 2

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