NUTRIENT MEDIATION OF THE IMMUNE RESPONSE IN SHIPPING STRESSED FEEDLOT CATTLE

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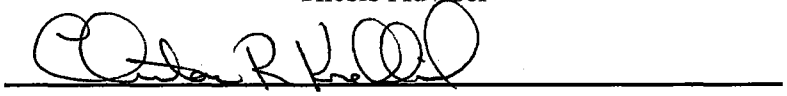

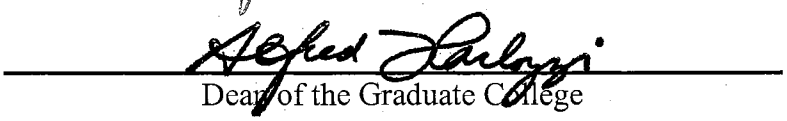

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NOMENCLATURE

ADG	average daily gain
AMT	antimicrobial treatments
APP	acute phase protein
BRD	bovine respiratory disease
E	vitamin E
F/G	feed to gain, or feed conversion ratio
HCW	hot carcass weight
kg	kilograms
lb	pounds
Med0	number of cattle receiving zero AMT
Med1	number of cattle receiving exactly one AMT
Med>1	number of cattle receiving more than one AMT
mg/dL	milligrams per deciliter
mg/mL	milligrams per milliliter
QG	carcass quality grade
YG	carcass yield grade

CHAPTER I

INTRODUCTION

Vitamins are generally characterized as organic, low molecular weight substances that are key (even essential) to animal nutrition and as such function as metabolic catalysts or regulators. Contrary to popular belief, vitamins are not used to provide energy or to provide amino acids for protein deposition. Vitamins are segregated into families of similar or related substances known as vitamers, and are further categorized by their respective solubility, i.e., whether they are fat-soluble or water-soluble. Fat-soluble vitamins are soluble in non-polar solvents and are unique in structure—they all have an isoprenoid unit derived from acetyl-CoA. Vitamins A, D, E, and K are fat-soluble vitamins. Water-soluble vitamins are soluble in polar solvents and are rather diverse in their chemical structures. B-vitamins make up the majority of water-soluble vitamins.

Vitamin E is the general descriptor for tocol and tocotrienol derivatives with biological activity for α-tocopherol; tocopherols are insoluble in water and relatively soluble in non-polar solvents. Another of the fat-soluble vitamins, E can accommodate unpaired electrons (free radicals), but is destroyed in doing so. It is transformed (reduced) into a quinone. Although tocopherols are resistant to heat, they can be easily oxidized; consequently, the rate of oxidation is increased with heat, level of rancid fat (which is already highly oxidized), moisture, and mineral concentrations. Selenium, a trace mineral, is important for the biological function of vitamin E; it can preserve the integrity of the pancreas for fat digestion and absorption and can decrease the

concentration of vitamin E required for glutathione peroxidase synthesis. The free radical destruction of vitamin E is accomplished by peroxides that react with cellular organelles and cell membranes to destroy the vitamin. Peroxides (destroyed by glutathione peroxidase) are recognized as highly reactive compounds. Vitamin E functions as an antioxidant preventing oxidation of unsaturated lipid materials and protecting other structures (like vitamin A and carotenoids) against hydroperoxide damage. The international standard for measuring vitamin E is in international units; one mg of α-tocopherol acetate is equal to 1 I.U.

An important function of vitamin E that is not well recognized is its impact on blood coagulation by acting as an inhibitor of platelet aggregation, and its function on prostaglandin E synthesis. In the latter role, vitamin E may protect against arachadonic acid degradation. It appears that all vitamins, especially A, D, and E, have some direct impact on immune system function. Careful study of metabolic pathways would indicate their importance in numerous reactions such that absence of any could effect metabolism greatly. Their roles range from a greater incidence of disease when these vitamins are deficient, to a reduction in lymphocytes, and degeneration of organs (thymus and spleen in rats). This increased atrophy of these organs is very important to immune function. These organs are sites of maturation of immune cells (mostly T-cells) which can fight off infections and disease.

Serum antibody production is also affected by vitamin status. Parasitic infections can be reduced if vitamin status is adequate. Further, differentiation of some immune cells has been improved by retinoids. Vitamins have also been shown to improve

phagocytic action of macrophages, and cytokine-like product release, which improves the action of T-helper cells.

The first study was designed to determine the effects of supplemental vitamin E fed at one level over four time-periods. These four periods were within the frame of time in which most cases of respiratory disease are commonly observed in newly arrived feedlot cattle. Therefore, our goal was to determine if vitamin E supplementation that coincided with typical disease pathogenesis would provide a boost to the immune system, and simultaneously improve animal performance. Textured and completely mixed diets were fed shipping stressed calves during a 42-d receiving period. Dietary effects were determined based on growth, feed conversion, percent morbidity, frequency and duration of sick days, drug treatment costs, and measurement of serum total cholesterol, vitamin E, and antibody concentrations.

The second study tested an experimental supplement, which had recently been patented by another research institution, to determine its effects on growth, immune response, and carcass characteristics, including carcass value. Response variables similar to those in the first study were tested, with the exception of physiological parameters obtained from serum.

Performed in conjunction with the first study, acute phase protein concentrations over time and in response to stress and disease were studied. Plasma and serum samples were collected at regular intervals and upon an animal's entry into and departure from the hospital where disease status was assessed and drug treatment was employed. Characterization of the change of the circulating levels of these hepatic proteins might assist in the prognosis and diagnosis of respiratory disease in cattle. Improved

knowledge regarding these proteins might also aid in the development of new drugs by providing a tool for use in drug efficacy trials.

CHAPTER II

REVIEW OF LITERATURE

Homeostasis

Maintenance of the Steady State

A resounding feature of the mechanism of stress is its participation in the regulation, or successful interruption of homeostasis (Breazile, 1988). Disturbances to, or the attempted interruption of homeostasis is the method by which invading organisms cause damage to the host and cause infection, tissue injury, or immunological disorders (Hochepied et al., 2000). Homeostasis can be defined as a tendency toward stability in the normal body state (the internal environment) of the animal (National Library of Medicine, 2000). Coffee (1998) defines homeostasis as the maintenance of a constant cellular environment. Homeostasis is achieved by a system of regulatory or control mechanisms activated by negative feedback. For example, a high level of carbon dioxide in extracellular fluid triggers increased pulmonary ventilation, which, in turn, causes a decrease in carbon dioxide concentration in the extracellular matrix.

Ultimately, living organisms are not at equilibrium. They must continually be supplied with energy from external sources. There is a price to pay, however, for maintaining an orderly system and that price is in the form of energy. A supply of free energy into the body is required, which comes in the form of food or feed (Voet and Voet, 1995). Many metabolic reactions occur and do so on a continual basis; these reactions couple favorable and unfavorable, or exergonic (nutrient oxidation) and

endergonic (mechanical work, active transport of molecules against concentration gradients, biosynthesis of other, more complex molecules) reactions. Most animals obtain their energy ultimately from plant sources, in one way or another. Plants have derived their energy from the sun, the ultimate source of energy, through photosynthesis. The coupled reactions previously mentioned are usually facilitated by the intermediate synthesis of adenosine triphosphate (ATP) (Voet and Voet, 1995).

Nutrients are degraded through a series of reactions to common intermediate substances that are used as precursors in the biosynthesis of other biological molecules (Voet and Voet, 1995). Although the internal processes of animals are extremely complex, they somehow maintain a steady state. One example of this steady state existence is the normal adult human. Over a 40-year period, thousands of pounds of nutrients and more than 20,000 liters of water can be consumed without a significant change in total body weight (Franklin Leach, personal communication). It seems logical that some system of closely regulated, metabolic controls must exist.

The metabolic pathways that we all recognize when displayed on pathways maps comprise many compounds and display a series of very specific enzymatic reactions (using more than 2000 enzymes) to convert one chemical to another (Nicholson, 1997). Rate limiting metabolic pathways are characteristic in at least four ways: they are irreversible; every pathway has a first committed step; all pathways are in some way regulated; and they are localized or compartmentalized (Lehninger, 1993; Voet and Voet, 1995; Coffee, 1998). Metabolism can be generally described as the degradation (catabolism) of many diverse compounds to common intermediates (the most common is acetyl-CoA) and the nearly simultaneous biosynthesis (anabolism) of common

intermediates (acetyl-CoA, pyruvate, and TCA-cycle intermediates) into useful products, such as glucose (Coffee, 1998). A perpetual adjustment of metabolic pathway rates helps to ensure that an adequate supply of substrate, as well as energy, is available to carry out normal cellular, and thus bodily, functions (Coffee, 1998).

Since animals are open systems, they can never be at equilibrium (Voet and Voet, 2000). They must continually ingest nutrients having characteristics of high-enthalpy and low-entropy, which are ultimately converted to waste products with the opposite energetic characteristics. The difference in the energy in this process of metabolism allows for the high degree of cellular organization and the ability to produce some type of product (lean muscle, milk, wool, etc.), all characteristic of life. If for any reason, the balance or process is interrupted, the organism or animal attains a state of equilibrium. In open systems, equilibrium is the ultimate resting state, death.

Maintenance of the state of non-equilibrium must be maintained for several reasons. Work can only be derived from processes in non-equilibrium. Once an organism reaches equilibrium, it can not be directed; non-equilibrium is required by the precise regulatory functions that are characteristic of life. A constant influx of free energy is required by open systems in order to maintain the simultaneous degradation and regeneration of the biochemical compounds of which and from which they exist (Voet and Voet, 1995). If an interruption or suspension of metabolism occurs for a long enough period of time to allow for complete exhaustion of the ATP supply, the switch for metabolism of glucose can not be turned back on. It ultimately remains, then, that in non-equilibrium processes, some "thing" must flow, whether it is matter (food), electrical charge, or heat, and change the spatial distribution of the "thing".

Vitalism

It seems appropriate now, and necessary to discuss the theory known as Vitalism. The Vitalism theory states that inorganic materials or compounds do not contain the "vital force" of life (National Library of Medicine, 2000). As this theory relates to thermodynamics, classical or equilibrium thermodynamics function in regard primarily to processes in closed systems (inanimate) that can be reversed. For example, inorganic material can be melted, but can always be recovered by taking away the heat source. In contrast, organic compounds (open systems) change form upon heating and can not be recovered intact after removal of the heat source. Open systems (animals) can remain in a state of non-equilibrium, provided they can obtain free energy (in the form of food, heat, work, etc.) from their surroundings (Voet and Voet, 1995; National Library of Medicine, 2000). Converse to the classical state of equilibrium, time is considered.

Although it seems ironic, and even in contrast to the above description, living systems can remain in a steady state over time as long as the "flow" stays in motion. As referenced in the water and food consumption in humans example, all flows in living systems remain constant; over time the system does not change. Ilya Prigogine, one of the first to study and describe irreversible thermodynamics, summarized that the steady state of an open, living system is the system's own state of maximal thermodynamic efficiency (Voet and Voet, 1995). A steady state open system is therefore undifferentiated from closed systems in a steady state and both are considered stable.

Stress and Disease in Cattle

Stress and Homeostasis

Stress has become a common descriptor for a variety of reactions to external and internal stimuli in all species of animals, including man. Classical literature indicates clearly that the transport of cattle, especially calves, will produce stress (Shaw and Nichols, 1964). The term typically implies a negative reaction, when, in fact, stress can produce beneficial responses. In fact, stress can motivate an animal to search for food, water, or even shelter. Adaptation to novel stimuli, often recognized as stress reactions, can be considered as a modification of continuing physiological mechanisms allowing the animal to maintain homeostasis with minimal interruption of the steady state (Breazile, 1988). Organizing mechanisms that allow animals to deliver a homeostatic response to stressors imposed on them by man or their environment are primarily dependent on closely regulated and precise relationships of both the nervous and endocrine systems. Measurable physiological responses to stressors can be determined due to the activation of the sympathetic portion of the autonomic nervous system, which will unleash adrenaline and noradrenaline from the adrenal medulla (Stephens, 1980).

Physiological Responses to Stress

Attempting to define, categorize, or measure stress in animals has eluded many scientific investigators. The challenge exists mostly because a majority of the definitions have dealt with outcomes, resulting in a classification of either behavioral or physiological responses (Levine, 1985). Conversely, stress should be considered as a syndrome without a specific etiology, biological response, or even a singular effect on the

animal. Rather, it typically manifests as a multitude of effects (Moberg, 1985). Stephens (1980) rather clearly defines stress as a stimulus at some rate significantly deviating from what is normal (as perceived by the animal), or when that stimulus is abnormally prolonged, or intense. Disease does not occur in cattle of its own volition. A traditionally accepted belief links exposure to stressful stimuli and viral or bacterial challenges with the pathology of disease (Blecha, 1988).

To recite traditional dogma regarding stress, there are three primary forms that can be identified and discussed: eustress, neutral stress, and distress (Breazile, 1988). Eustress can be categorized as good stress. In this, the animal can identify certain stimuli that are not harmful, but will initiate some benefit for the animal. As mentioned previously, these are the stressors that can initiate feeding and drinking, as well as other responses necessary in the maintenance of homeostasis, such as respiration, changes in metabolism, cardiovascular activity variation, temperature regulation, and fight or flight reactions (Stephens, 1980). Neutral stress, although not inherently harmful, may result in responses by the animal that are neither detrimental, nor helpful (Breazile, 1988). Usually mild in intensity, these stressors typically involve central neural activity. Any response is unnoticed by the animal because it results either in a stimulus to which the animal has already adapted, or in a biochemical change that lies within a "normal" range of variation. Distress is characterized by stimuli that are not inherently harmful, but will result in a harmful response, which can ultimately interfere with the homeostatic self-regulation by the animal (Breazile, 1988). Overt pathological changes can be observed in organs and tissues because of distress, and the overall pathogenesis of disease can be accelerated.

Infectious agents are frequently a component of distress, and therefore, merit our attention and understanding, particularly when we attempt to provide relief to the animal and assistance in maintaining homeostasis through therapeutic methods (Breazile, 1988). In addition, when one carefully studies the mechanisms involved in responses to stressors, observations can usually be found on the nervous and endocrine system interaction (Stephens, 1980).

Jones and Gillham (1988) indicated a correlation between distress responses and the increased production of adrenocorticotropic hormone (ACTH) resulting in an increased secretion of glucocorticoids by the adrenal cortex. Similarly, Wood et al. (1982), Keller-Wood et al. (1983), and Keller-Wood et al. (1984), suggested that the quantities of ACTH and glucocorticoids produced were in direct proportion to the stress stimulus received. Metabolically speaking, glucocorticoids can significantly impact carbohydrate metabolism by diverting glucose metabolism from muscle to brain and other tissues, thus helping to prevent the incidence of hypoglycemia (Breazile, 1988). Tight regulation of these hormones is essential. If the distress stimulus remains persistent, hyperglucocorticoidism can result, leading to further states of disease, such as ketosis, or acidosis, and eventually delayed wound healing or immune deficiencies can result (Breazile, 1988).

Management Induced Stress

Physiological stress can be induced in cattle in a variety of ways. It can result from typical management practices such as weaning, co-mingling with unrecognized cattle in the same herd, or co-mingling with "new" cattle (both imply establishment of social order), transit, changes in feeding patterns, and even space allowance (Hays, 1987b).

Cole et al. (1982) investigated the effects of marketing and transit on calves that just arrived at the feedyard by measuring blood packed cell volumes (PCV) on arrival. In this study, PCV was lower in calves that had been weaned in advance of delivery to an auction facility rather than the day of, suggesting that dehydration was less frequent, and thus, stress was reduced. However, pre-weaned calves did not perform better than their day-of-sale weaned counterparts, further suggesting that this stress was a recoverable event, and not a life-threatening one.

In several feedlot studies in Canada (Wieringa et al., 1976; Martin et al., 1982), researchers determined that weaning and co-mingling are two of the greatest stressors that we impose on calves. In the latter of the two, Martin et al. (1982) observed significantly higher morbidity and mortality rates among groups of calves that were mixed upon arrival at the feedlot versus calves that remained with their own herd group. Staples and Haugse (1974) concur; their data also indicate a significant increase in mortality when calves are transported for long periods or for long distances. Under adverse transport conditions, Shaw and Nichols (1964) observed an immediate and persistent change (for up to four weeks) in the level of plasma 17-hydroxycorticosteroids in long-haul calves, compared to calves neither co-mingled nor hauled.

Transit and exposure to unrecognized other cattle has even been found to be a more significant stressor in terms of the adrenal response (i.e., plasma corticosteroid levels) than withdrawal from water for up to 48 hours, or invasive surgery such as castration or dehorning (Johnston and Buckland, 1976). Stress, it seems, takes its toll due to this adrenal response which can ultimately limit a calf's ability to mount a response to life-threatening environmental stressors, such as viruses or bacteria (Stephens, 1980).

Husband et al. (1973) found that calves injected with corticosteroids within hours of birth had a reduced absorption efficiency of necessary immunoglobulins. Additionally, these same calves were slower to produce some types of immunoglobulins when exposed to environmental viruses and bacteria. Subclinical and clinical disease states and increased distress levels are easily related to intensified production and higher densities of animals in confined areas (Cunha, 1985).

Stress Effects Vitamin E Concentrations

Nockels et al. (1996) measured the effects of stress on tissue α-tocopherol concentration in a 28 d study using 16 crossbred heifers maintained on a corn and corn silage-based diet. The diet was supplemented with either 0 or 1000 I.U. of dl-α-tocopherol acetate for 28 d. Samples of plasma, red blood cells (RBC), as well as liver, trapezius and longissimus muscles, and subcutaneous fat tissue were collected. Stress induced by various levels and duration of feed and water restrictions affected average heifer weight negatively, while serum cortisol, creatine kinase, and urea were increased. Once cattle were returned to a normal or stress-free regimen of feed and water, vitamin E heifers showed a greater positive response in the previously described parameters. Specifically, vitamin E reduced the magnitude of the increase observed in creatine kinase, and serum selenium increased in vitamin E supplemented heifers compared to non-supplemented control-fed heifers. Other parameters, such as RBC, liver, and subcutaneous fat concentrations of α-tocopherol, were similarly increased when heifers were fed supplemental vitamin E. Cattle experiencing stress may be found to have decreased levels of α-tocopherol in some tissues. Therefore, supplemental vitamin E

before, during, or after an imposed stress may assist in the restoration of adequate levels of α-tocopherol in many tissues.

The Bovine Respiratory Disease Complex

Monetary Effects

The bovine respiratory disease complex commonly recognized as BRD, after many years of investigative research remains even today as a constant nemesis to beef cattle production. This is in spite of the cadre of vaccines and antimicrobial drugs developed specifically to aid in its control. Understanding of the pathogenesis and etiology of the disease complex also remains incomplete (Roth and Perino, 1998). Symptoms of BRD can be caused by many factors acting congruently that allow microbial colonization within the lungs, resulting in severe respiratory distress (Merck, 1991). In extremely severe cases, death is the ultimate fate. Ultimately, BRD is an economically significant disease (Godson et al., 1996; Young et al., 1996). Respiratory diseases accounted for losses of nearly $500 million in 1995 to the U.S. beef industry (NASS, 1996; Loerch and Fluharty, 1999). Comparatively, digestive disorders and calving problems combined accounted for approximately thirty-five percent of mortality in cattle ($586.5 million). Predators (coyotes, dogs, mountain lions, bobcats, bears and wolves) accounted for only about three percent of all cattle deaths ($40 million) (NASS, 1996).

Viral and Bacterial Interactions

Epidemiological data strongly points to evidence that viral pathogens can, and do predispose animals to bacterial pneumonia (Roth, 1984; Roth and Perino, 1998). Often, a

synergism is seen between viral and bacterial pathogens making accurate diagnosis and treatment difficult (Godson et al., 1996; Merck, 1991). All prominent members of the disease complex, bovine herpes virus (BHV, a.k.a. IBR), parainfluenza-3 (PI3), bovine viral diarrhea (BVD), and bovine respiratory syncitial virus (BRSV) can predispose, or pre-infect the animal. Thus, in this manner any member of the disease complex can clear a path for the development of severe bacterial pneumonia invariably due to *Mannheimia* (formerly *Pasteurella*) *haemolytica, Pasteurella multocida,* or *Haemophilus somnus* (Roth and Perino, 1998). Other pathogens can also play significant roles in the severity of the disease: chlamydiae, mycoplasmas, helminths, and fungi (Hays, 1987; Merck, 1991).

Bacteria are typically unable to migrate to lower regions of the respiratory tract in normal, healthy cattle (Roth and Perino, 1998). However, under certain conditions of distress imposed by altering the animal's environment (weaning, transportation, changes in or deprivation of diet, mingling with other animals that may be shedding a variety of pathogens, crowding, exposure to inadequate ventilation), the likelihood of disease transmission is enhanced (Merck, 1991). The interaction between environmental stressors, viral and bacterial pathogens, and even other unrecognized immunosuppressive factors lead to the notion that the BRD complex has a multifactorial etiology (Hays, 1987; Roth and Perino, 1998). These factors acting separately and (or) sequentially, or in concert effectively suppress native defense mechanisms in the respiratory tract allowing consolidation of bacterial pathogens.

Shipping Fever

Commonly referred to as "shipping fever," pneumonic pasteurellosis (clinical reference) is not age specific in beef animals; however, these bacterial species are more commonly observed in younger animals that have just been transported for some time and/or distance (Merck, 1991). Typical etiology of shipping fever includes alveolar edema, serofibrinous exudation into the alveoli with hemorrhage, microvascular thrombosis and endothelial cell swelling, as well as numerous virulence factors (formation of a polysaccharide capsule, and production of endotoxins, and leukotoxins) (Cheryk et al., 1998). Adding to the strain of transport and handling, concurrent exposure to a variety of infectious pathogens may lead to the development of pneumonia.

Nutrition and Animal Health

Metabolic Systems of Ruminants

Mammals are comprised of a network of closely regulated and interrelated cycles, pathways, and systems. Without these systems and their intricate functions, animals would quickly reach the ultimate state of homeostasis described earlier. Nutrition, and more specifically adequate nutrition, provides the animal with a continuous influx of the macro and micronutrients required for sustaining life. Further, adequate nutrition is essential for proper metabolism, which is the ultimate means by which living systems obtain and make use of the free energy they need in order to carry out their productive and reproductive functions (Voet and Voet, 1995). Although mammalian systems are similar, if not identical across species, ruminants possess another environment with a separate system making these animals unique among their counterparts (Nagaraja, et al.,

1998); ruminants contain a microbial metabolism system within the reticulo-rumen. The microbial system's requirements are somewhat different from that of mammalian tissues. Thus, balanced nutrition focused on optimizing both metabolic systems in order to maximize the animal's performance is essential.

The Acute Phase Response

The appropriate balance of nutrients, macro and micro, depends on many factors including level of production, environment, and even genetics. As described previously (homeostasis), an influx of energy is always necessary and becomes critical under conditions of stress and disease. During the acute-phase of disease pathogenesis, nutrient intake can be compromised. Acute-phase reactions include increased slow-wave sleep, anorexia, accelerated degeneration of skeletal muscle proteins, hypotension, hepatic synthesis of acute-phase proteins (including complement) and an alteration of the circulating pool of white blood cells (Kumar, et al., 1997). Energy is primarily provided by macronutrients and is usually classified as carbohydrates, protein, or fat. Nearly forty percent of the energy found in these energy-containing nutrients is used to synthesize the fire of life, adenosine triphosphate (ATP) (Coffee, 1998).

Adequate nutrition is important in disease prevention. In human populations especially, disease pathogenesis occurs as a result of a breakdown in some disease mechanism; malnutrition is often a leading cause, while other factors such as pathogenic organisms play a similarly important role (Simon et al., 1998). When disease or infection occurs, anorexia can cause animals to rely on body stores of energy for sustenance. Disease can effect energy metabolism by affecting the basal metabolic rate (BMR). Basal metabolic rate can be defined as the amount of energy required for maintenance of

basic physiologic functions while the body is at rest (Coffee, 1998). For instance, hyperthyroidism can increase BMR from 120 percent to 150 percent more than normal, and hypothyroidism can decrease BMR 20 to 40 percent from normal (Coffee, 1998). Generally, injury or sepsis can increase BMR by 30 to 60 percent over normal levels (Coffee, 1998).

Mammalian Immune Systems and Nutrition

Three primary components make up the immune system of mammals: mucosal barrier immunity, humoral immunity, and cell-mediated immunity (Cole, 1996). Roth and Perino (1998) describe an additional component of the immune system known as native defense mechanisms (NDM). These include salivary enzymes, enzymes in tears, gastric acids, fatty acids found on the skin, and normal flora found on mucosal surfaces. The NDM also include the complement system, as well as phagocytic white blood cells (phagocytes) fully capable of killing certain bacteria and viruses. From a learning standpoint, these systems are typically first considered separately; however, in reality each of these systems is closely linked to the other. Cole (1996) describes ways in which nutrition can affect any or all of these component systems: anatomic development of lymphoid tissue; production of mucus; synthesis of immunologically active compounds; cellular proliferation, activation, and movement; intracellular killing; and modulation and regulation of immune processes. Although malnutrition can most obviously affect immune responses, less severe nutrient deficiencies or nutrient imbalances can also impair the animal's natural ability to defend against pathological invaders.

Distress and disease can create a state of hypermetabolism in animals, thus decreasing nutrient balance (Cole et al., 1986). Observation of this phenomenon is not

necessarily accompanied by a decrease in feed or nutrient intake. Nagaraja et al. (1998) asserted that special nutrient management must be given to stressed cattle, otherwise, inadequate nutrition can result in exacerbate the effects of stress. Lofgreen (1988) observed that stressed calves have a distinctly different pattern of consumption when compared to herd mates that were not similarly stressed. Light weight cattle are especially prone to a more frequent occurrence of BRD when transported to commercial feedyards (Lofgreen, 1988). In part, low pre- and post-transport feed intakes resulting in nutritional deficiencies combined with the stress of weaning and transport compromised immune system function (Cole, 1996). Therefore, nutritionists should be more mindful of the fact that stressed animals are at greater risk of infection due to impaired immune "response-ability" and must constantly reevaluate the level of vitamins necessary to maximize responsiveness of the immune system (Nockels, 1988).

Nutrition After Arrival in the Feedyard

Further compounding the effects of stress, and thus animal health, is the reduction of feed and water intake observed after arrival. Hutcheson (1988) compiled data from seven years of receiving experiments in Texas. Healthy cattle consistently consumed more feed during the first seven, 28, and 56 days after arrival than their morbid counterparts. The author indicated that it is nearly impossible to create a diet that will meet animal nutrient requirements for gain when intakes are at or below one percent of body weight. Shaw and Nichols (1964) showed a continued effect of persistent corticoid levels, and thus the effects of stress remained for at least three weeks after the arrival of feedlot cattle and calves. Lofgreen (1988) confirmed the importance of adequate nutrition during the first 21-28 days after arrival in a feedlot. Further, eating patterns of stressed cattle were

described as follows: eating behavior of stressed calves is opposite that of non-stressed calves, they will eat greater quantities of high-energy diets; transport stressed calves will also select diets (when given a choice) higher in concentrates than their unstressed mates; and calves hauled for long distances or periods of time will reach a normal level of feed intake sometime during the third week after arrival, thus indicating at least a 21-d receiving period as optimal. Lofgreen (1988) also reported that receiving diets containing 50 to 75 percent concentrate combined with native grass hay (fed for only the first week) have produced excellent results in terms of animal performance as measured by average daily gain and animal health represented by percent morbidity and mortality.

Regardless of how well we may manage animals in native conditions, invariably Mother Nature delivers conditions that make optimal nutrient management difficult, at best. Be it cold stress or heat stress combined with diminished forage availability, often the result can be a 10-20 percent reduction in body weight (Sheffy and Williams, 1982). In this negative nutrient balance, one could not expect these environmental factors to contribute positively to the animal's optimal immune system function. Reddy and Frey (1990) agree that the nutritional status of the animal can be significant with respect to its ability to resist disease-causing agents. Successful therapeutic treatment may also be influenced by this same balance.

It is clear that disease in cattle, specifically disease related to the BRD complex, negatively effects the performance and economics of feeding beef cattle (Nagaraja et al., 1998). It may also effect beef carcass quality (Stovall et al., 2000). Perino (1992) described the BRD complex as unique in its ability to manifest itself throughout the production phase of beef cattle. BRD significantly impacts profits by raising therapeutic

treatment costs, increasing overall production costs due to lost or diminished performance, decreasing salvage values, and increasing mortality and associated costs.

Acute Phase Proteins

Characteristics and Usefulness

By definition, acute phase proteins (APP) are proteins, mainly glycoproteins, that are synthesized and secreted as part of the acute (meaning quick, within a day or two; G.A. Campbell, personal communication) phase response (Kent, 1992; Wright et al., 1995). They are produced by the liver in reference to some type of systemic infection, toxic insult, or other immune system injury and work to restrict cellular damage and promote repair of already damaged tissue (Faulkner et al., 1992). They can be alternately described as coagulation proteins, and also may include complement, another soluble mediator whose primary function is the generation of a membrane attack complex (MAC) that effectively creates perforations in invading microbes (Kumar et al., 1997). Characteristics that make a good APP include very low, or nearly negligible basal values, a narrow reference range unaffected by age, sex, or genetics, and quick response times with great magnitude changes (>100x) to infectious or inflammatory conditions (Kent, 1992). Further, APP are very stable compounds allowing samples to be frozen and analyzed later (Horadagoda, 1999). Development of one or more reliable measures of APP as a screening test that could indicate the general health status of loads or pens of cattle, predict when and if antimicrobial treatment is necessary, identify sick animals that do not exhibit clinical signs, monitor antimicrobial treatment response, and (or) evaluate the severity of disease would be an invaluable tool for feedlot veterinarians and the beef

industry (Young et al., 1996). This tool would be particularly valuable in the case of transient and self-limiting infections, which often go unrecognized by even expertly trained personnel (Hirvonen et al., 1996).

Initiation and Production

The acute phase reaction can also include specific cytokines, which are polypeptide products produced by a variety of cell types (primarily derived from lymphocytes and macrophages) to modulate the function of other cell types (Werling, 1996). The most dominant cytokines that mediate inflammation increasing nonspecific immunity (or resistance), and thus acute phase reactions, are interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor-necrosis factors (TNF) (Hochepied, et al., 2000). These cytokines can be released independently, but most frequently are released as part of a cytokine cascade. For example, TNF can stimulate production of IL-1, which can subsequently induce IL-6 production. With respect to hepatic production of APP, IL-6 is commonly recognized as a primary induction factor (Kumar et al., 1997). These compounds can effectively regulate the hepatic synthesis of at least two important acute phase proteins (Werling, 1996). Once an infection is recognized by the system, the serum concentration of APP increases rapidly (Roitt et al., 1998).

Form and Function

A few examples of APP include haptoglobin (Hp), fibrinogen (Fb), serum amyloid-A (SAA), α-1-acid glycoprotein (AGP), ceruloplasmin (Cp), and C-reactive protein (CRP) (Kent, 1992; Hirvonen et al., 1996; Kumar et al, 1997; Roitt et al, 1998). Each APP is named somewhat in relation to its function. For example, CRP is named for its ability to

bind to the C-protein of pneumococci, which will subsequently promote the uptake of this complex by phagocytes. C-reactive protein is always detectable in the bovine serum, even in healthy animals (Conner and Eckersall, 1988; Eckersall and Conner, 1988). This detection and adherence process is known as opsonization (the bacterial surface is coated, which allows for better recognition and enhanced "sticking" by phagocytes) (Roitt et al, 1998). Fibrinogen is another hepatic protein characterized by polymerized long, branched, fibrin threads important in blood coagulation (Guyton, 1987). Plasma or serum concentrations of Hp can be effectively used to quantify a particular host's response to disease, injury, or other stressors (Faulkner et al., 1992; Hirvonen et al., 1996). Hepatic synthesis of plasma proteins can be as great as 50 g per day, in extreme cases (Guyton, 1987). These plasma proteins can be a source of amino acids for protein-depleted and even damaged tissues. Therefore, not only do APP function as soluble mediators of the immune response, they also function as a rapidly available source of amino acids on demand, as well as a labile protein storage medium for tissues (Guyton, 1987). Interestingly, as the serum concentration of APP rises, the serum concentration of other hepatic-produced proteins, like albumin, decreases (Eckersall, and Conner, 1988).

Response to Infection

Although initially limited by suitable laboratory methods for detection, Hp (part of a macromolecular protein complex) and Fb have recently gained new attention for their ability to detect disease in various animal species, including deer (Cross et al., 1991) and sheep (Pfeffers and Rogers, 1989). Haptoglobin was named for the ability of one of its components, α_2-globulin, to bind to hemoglobin (Eckersall and Conner, 1989). Evidence

exists that points to Hp as the predominant APP in bovine (Kent, 1992; Horadagoda, et al., 1999). Its concentration has clearly been shown to increase in response to infection clinically observed in adult cattle and stressed feeder calves (Wright et al, 1995), and in calves challenged with *M. haemolytica* (Conner et al., 1989). Godson et al. (1996) even suggested that measurement of Hp concentrations might detect sick animals before clinical signs of disease become evident. Although Hp is normally detected in the sera of healthy humans, it is absent, or at negligible levels, in the sera of healthy bovine and is detectable during an acute phase response (Eckersall and Conner, 1988). Thus, Hp meets one of the criteria for a good APP previously discussed. Especially in the case of bacterial challenge or infection (*M. haemolytica*), as well as endotoxin injection, and even clinical administration of turpentine, Hp production is adequately stimulated to be recognized as an acute phase response (Conner and Eckersall, 1988; Eckersall, and Conner, 1988; Conner et al., 1989; Cheryk et al., 1998).

Consequently, however, if the acute phase response is concurrent with a hemolytic crisis (as in babesis), all Hp will be bound to the hemoglobin and the complex is removed by the liver (Bremner, 1964). This phenomenon would obviously then restrict the use of Hp as an indicator of disease in certain disease states. Another useful aspect of Hp concentrations has been identified by Wittum et al. (1996). In their observations using feedlot cattle, those with clinically diagnosed respiratory disease given antimicrobial therapy had lower Hp levels upon final examination than did their non-treated counterparts. Ultimately, they suggest as do Young et al. (1996), that Hp may be effective in monitoring antimicrobial drug efficacy, or it may also be effective in the preliminary stages of BRD.

Work by Murata and Miyamoto (1993) revealed a negative aspect of the increase in plasma/serum Hp. Their study showed that Hp suppressed lymphocyte activity (blastogenesis) indicating that Hp may be an immunomodulator, further indicating a potential role for Hp as a diagnostic aid. In this case, particularly, Hp displayed potential as an aid to evaluate the vulnerability of the immune response of these transported and stressed calves.

As previously mentioned, Fb is a circulating precursor to fibrin, and is easily the most recognized and most commonly used assay with regard to the acute phase response, especially in bovine (Eckersall and Conner, 1988). Similar to the response of Hp, the serum concentration of Fb is observed to increase in response to bacterial challenge (Hirvonen et al., 1996), endotoxin administration, and even injection with turpentine. In an experiment evaluating the response of APP to clinically induced mastitis in dairy heifers due to either *A. pyogenes, F. necrophorum,* or *Pept. Indolicus*, Hirvonen et al. (1996) measured Fb levels prior to bacterial challenge, within the first two days after challenge, and periodically thereafter for two weeks. Levels of Fb began rising on day two, reached a maximum level by day five (Fb concentration was doubled from pre-challenge measurements), and returned to initial levels after two weeks. The rate, however, at which Fb concentrations increased was not as rapid as that of other APP. The authors reported that this response pattern has been observed in other experiments (Conner et al., 1986). Although the study by Hirvonen et al. (1996) revealed that Fb is a reliable indicator of bacterial infection, it was not necessarily a good prognostic tool, especially for mastitis. Thus, perhaps other APP can provide results that are more specific.

A 14-kD protein, SaA, likely the precursor to amyloid protein A (a major component of the amyloid protein), is an APP similar in nature and function to CRP, especially in humans (Eckersall and Conner, 1988; Husebekk et al., 1988). Alsemgeest et al. (1994) considered the value of SaA and Hp measurements in order to distinguish between healthy animals and those with some level of inflammatory disease, either, acute, subacute, or chronic. Their results clearly indicated that circulating concentrations of SaA and Hp could be clinically useful. Not only can these chemical variables determine healthy vs sick animals; the ratio of these proteins' concentrations (Hp/SaA) may also be beneficial in the determination of disease. Although these results provided an extremely beneficial break-through for clinicians, the mechanism of these APP was still unclear.

Response to Physical Stress

Alsemgeest et al. (1995) further studied SaA and Hp in an attempt to determine a mechanism for stress related proteins. In this experiment, physical stress was compared to the stress of disease. Calves whose mobility was impaired by being housed on plastic flooring had significantly ($P<.001$) higher SaA concentrations than calves housed on wooden flooring with a rubberized top layer. The concentration of Hp was low in all animals and not different. These authors suggested that SaA might be more an indicator of physical stress, whereas Hp is not effected by physical stress and may be more specific for disease. Conversely, in a study comparing the results of SaA and Hp concentrations in 81 clinically ill cattle, Horadagoda et al. (1999) reported that SaA increased in 100 percent of the animals, while Hp increased in only 68 percent. These results suggested that SaA is likely more sensitive to physical stimulation, but that both SaA and Hp can give clinicians adequate evidence toward the extent of infection. Horadagoda et al.

(1999) suggested that the non-favored status of SaA, particularly in bovine studies as opposed to human medicine, primarily was due to the difficulty in purification and quantification caused by SaA's chemical structure. Serum amyloid-A is a complexed apo-lipoprotein within the high-density lipoprotein (HDL) fraction in serum (Husebekk et al., 1988; Horadagoda et al., 1999); however, an efficient enzyme-linked immunosorbant assay (ELISA) has been developed. The value of results from these quantifications of bovine SaA is yet to be determined.

Vitamin E

History

Recognized initially as a fat-soluble vitamin important primarily for reproduction in rats (Church and Pond, 1988), vitamin E was first isolated in 1922 by researchers at the University of California, Berkeley (Evans and Bishop, 1923). They described this compound as an unidentified factor found in vegetable oils essential for reproduction in female rats. Their experiments monitored all phases of estrus, mating and early pregnancy, and found that fetuses died within the first trimester and were reabsorbed unless diets were supplemented with either wheat germ, dried alfalfa leaves, or fresh lettuce in small amounts, all of which naturally contain vitamin E.

Originally recognized as factor X, vitamin E was renamed as such by Sure (1925) and Evans (1925). This designation was a logical choice as it was simply the next serial alphabetical designation available (McDowell, 1989a). Pappenheimer and Goettsch (1931) conducted classical experiments, which revealed vitamin E's efficacy in the prevention of numerous diseases in animals, including encephalomalacia in chicks and

nutritional muscular dystrophy in rabbits and guinea pigs. These same researchers later found that more than one vitamin E deficiency disease could be concurrent in the chick: exudative diathesis, encephalomalacia, and muscular dystrophy. Adamstone et al. (1949) were among the first researchers to perform a controlled experiment studying vitamin E deficiencies in swine. Their findings revealed the positive effects supplemental dietary vitamin E can have on reproductive efficiency, as well as improving muscular function (locomotor coordination) and diminishing muscular necrosis. Other diseases such as hepatic necrosis, and fibrinoid degeneration of vascular walls were observed in animals consuming vitamin E deficient diets, both naturally and experimentally (Obel, 1953).

In the years following these discoveries, selenium was found to have a critical role in these vitamin E deficient induced disease states (McDowell, 1989a). Specifically, selenium was isolated as the active ingredient in brewer's yeast, which could functionally replace vitamin E. It could effectively diminish the incidence of exudative diathesis observed previously in chicks, as well as reducing the severity of tissue degeneration in swine, and muscular degeneration in cattle and other young ruminants.

Chemical Forms

Vitamin E, or tocopherols are a family of lipid soluble compounds which contain a substituted aromatic ring and a long hydrocarbon side chain (Lehninger et al., 1993). They are light yellow oils at room temperature, insoluble in water, and relatively soluble in non-polar solvents, or fats (McDowell, 1989a). Tocopherols are resistant to heat, but can be readily oxidized (oxidation is accelerated by heat, so heat can cause damage indirectly).

The most potent form of vitamin E is α-tocopherol (5, 7, 8-trimethyltocol); β-, γ-, and δ-tocopherols are only 50, 20, and 10% as effective compared to the α form (Huber, 1988). These tocopherols and their various forms are found naturally in the oils of plants (NRC, 1996). One compound with the highest biochemically active level of vitamin E is known as RRR-α-tocopherol (previously known as D-α-tocopherol) (U.S. Pharmacopeia, 1985). The biopotency of RRR-α-tocopherol is equal to 1.36 moles of all-*rac*-α-tocopherol, which is a synthetically produced mixture of eight stereoisomers. Although most commercially available forms of vitamin E are offered as dl-α tocopheryl acetate, the d-form, or isomer, is more biochemically active than the l-form. Tocopheryl acetate (an ester), as found in commercial compounds does not occur naturally in plants, and thus in many animal feedstuffs. The ester form effectively replaces the formation of a hydroxyl group on C-6 of the aromatic ring making the molecule much more stable and able to avoid degradation in feed milling procedures, such as pelleting. Functionally, this form is superior to natural forms because the natural forms are highly unstable (Church and Pond, 1988). However, the natural forms are likely more bioavailable.

An alcohol group linked to the acetate restricts the degradation of the tocopherol in blended feeds. In ruminants, once the vitamin E containing feed is consumed and eventually passed from the rumino-reticulum into the small intestine, the ester is hydrolyzed making the tocopherol absorbable and available to the animal (NRC, 1996). Oxidation of the natural form is increased in the presence of either polyunsaturated fatty-acids (PUFA), or certain minerals (Church and Pond, 1988). The detrimental affects that PUFA have may be limited, or at least reduced somewhat in ruminant animals. Ruminal microorganisms can effectively saturate fatty acids, but not all PUFA will be effectively,

or completely hydrogenated by these bacteria leaving behind a residual concentration to perform their oxidative dismantling of vitamin E (NRC, 1996).

Molecules that exhibit the biological activity of α-tocopherol are composed of isoprenoid side-chain derivatives of 6-chromanol (McDowell, 1989a). These compounds have the ability to accept unpaired electrons within the resonance structure of the ring. However, in the process the structure is reduced to a quinone. Glutathione reductase (a sulfur amino acid [SAA] enzyme) can regenerate the hydroxyl group (C-6) of α-tocopherol. This enzyme may also help to detoxify cells by assisting in the conversion of hydrogen peroxide (H_2O_2) to water (H_20).

Primary Functions

Vitamin E, or more specifically α-tocopherol, protects cattle at the tissue level and helps to prevent diseases such as muscular dystrophy (McDowell, 1989a). It can also help to boost the animal's immune system (Nockels, 1988) in order to defend itself against a more common series of pathogens, the bovine respiratory disease (BRD) complex of viral and bacterial diseases.

Metabolically, vitamins are important as they enable animals to efficiently make use of other nutrients (NRC, 1996). Vitamins are also recognized as precursors to important coenzymes, which are typically involved in metabolically important pathways and are either a complex organic molecule, or a metallo-organic molecule (Lehninger et al., 1993).

Little question or controversy surrounds vitamin E with respect to its primary function as an antioxidant. It is universally considered as an excellent inter- and

intracellular antioxidant (NRC, 1996). Although other vitamins have antioxidant capability, namely vitamins A and C, vitamin E is the primary fat-soluble antioxidant found in the body (Roche, 1999). In this role it can prevent the oxidative destruction of DNA and RNA, as well as the peroxidative degradation of lipids (mostly with PUFAs) in animal cell membranes, which if allowed to occur, can initiate a chain reaction formation of free-radical peroxides (Roche, 1999). Consequently, this lipid peroxidation can also effectively diminish cell membrane fluidity. Bendich (1993) has shown that this reaction is directly related to a reduction in lymphocyte responsiveness when the animal is faced with an immune system challenge. When unsaturated fatty acids react with oxygen, rancidity ensues; when similar reactions occur within an animal cell, membrane damage can result, leading ultimately to irreversible cell injury or death (Lehninger et al., 1993; Kumar et al, 1997).

Some lipid-soluble antioxidant compounds (ubiquinol-10, α-tocopherol, or α-tocopheryl hydroquinone) can facilitate electron transfer across the cellular membrane from intracellular ascorbate to extracellular ascorbate-free radicals (May, 1999). This is unique in that traditional understanding places a restriction on the location of long-chain quinones (α-tocopheryl hydroquinone) and limits its functional geography to the center of the lipid bilayer with no flexibility or movement to either membrane surface (Ulrich et al., 1985). Conversely, the chromanol ring of α-tocopherol typically is found next to the polar head-groups of the bilayer (Buettner, 1993) creating a link to the membrane surface. However, electron transfer across membranes by α-tocopherol can be somewhat restricted by its low frequency of movement across the bilayer and by the low rate of

redox reactions occurring on either side of the bilayer at the lipid-water interface (May, 1999).

The maintenance of structural and functional integrity of muscle, including skeletal, cardiac, and smooth muscle, as well as the peripheral vascular system is aided by the presence and function of tocopherols (Hutchenson, 1991). Vitamin E may provide a sparing effect for other nutrients by its antioxidant capability, which can protect the structural integrity of cells.

Free Radicals

Vitamin E functions not only to prevent biological oxidation within cells, but also scavenges electrons known as free-radicals that can cause the most severe cellular damage (NRC, 1996). These reactive oxygen compounds can cause severe damage to cellular membranes (Hill and Williams, 1995), and even inhibit the biochemical action of certain enzymes (Huber, 1988). Free radicals, fortunately, are highly unstable and will decay rather spontaneously (Kumar et al., 1997). Originating from oxygen molecules, free radicals are molecules that contain at least one unpaired electron that seek other molecules from which they may acquire or donate an electron to balance their charge and improve their stability (Roche, 1999).

Vitamin E's activity as a scavenger of free radicals and an aid to the immune system in the resistance of disease challenge has been clearly documented (Tappel, 1972; Hoekstra, 1975, Secrist et al., 1977; McCay and King, 1980; Cipriano et al., 1982; Gill et al., 1986; Nockels, 1988; Droke and Loerch, 1989; Coelho, 1991; Eicher-Pruiett et al., 1992; Galyean et al., 1999).

Oxidation-reduction type reactions always affect vitamin E on its aromatic ring structure (Lehninger et al., 1993). In the case of free radical's reaction with a PUFA molecule, an oxygen molecule is inserted in place of a previously abstracted hydrogen atom transforming the PUFA into a peroxy radical (Roche, 1999). This conversion sets up a chain reaction that will continue to abstract hydrogen atoms from nonradical PUFA molecules until all substrate is exhausted and the cell is depleted. Vitamin E can interrupt this chain-reaction, but in the process becomes oxidized and deactivated (Roche, 1999).

A normal by-product of oxidative metabolism, free radicals can also be produced in the body by immune cells (neutrophils and macrophages) as a normal and typically beneficial action (Roche, 1999). These free radicals are derived from the NADPH oxidase pathway and are released from the immune cells after stimulation by chemotactic agents, immune complexes, or by phagocytic activity (Kumar et al., 1997). Various tissue injuries have been noted due to the insult of free radicals: endothelial damage; protease activation with degradation of the extracellular matrix; and direct injury to adjacent healthy cell types, including erythrocytes and parenchymal cells (Kumar et al., 1997). Any free excess concentration of α-tocopherol is converted into 2, 5, 7, 8-tetramethyl-2 (2'-carboxyethyl) -6-hydroxychroman (α-CEHC) and excreted via urine (Brigelius-Flohe and Traber, 1999).

Determining the extent to which free radicals actually contribute to cellular damage and disease pathogenesis is extremely difficult due to the short life span of these molecular species and the lack of sufficiently sensitive equipment to recognize them (Pryor, 1986; Cheeseman and Slater, 1993; Brigelius-Flohe and Traber, 1999). Furthermore, in the pathogenesis of many disease states, it remains unclear whether free

radicals are the indisputable cause of the injury, or conversely are formed as a result of the disease (deZwart et al., 1999).

Simply put, oxidative stress is a gross abundance of oxygen within the cell, itself (James E. Breazile, personal communication). Cell death occurs when ROM species disrupt cellular membrane integrity, increase eicosanoid production, impede protein function, alter DNA structure, or interrupt energy production. Membranous concentrations of vitamin E helps to prevent oxidative damage to lipid bilayers from ROM whose concentrations are increased during distress. During distress, a cascade of hormones (corticotrophin-releasing factor stimulates the pituitary to release adrenocorticotropic hormone [ACTH] resulting in an increased synthesis of glucocorticoids via the mitochondrial P-450 monooxygenase system [Mayes, 1988]) including epinephrine and norepinephrine, mobilize energetic substrates (fatty acids and glucose) from tissues thus increasing cellular energy production with the simultaneous production of ROM. Consequently, macrophages can also produce free radicals during respiratory burst reactions (Tizard, 1988), making the immune function a contributor to the demise of the phagocyte, adjacent cells, or even serious tissue damage if antioxidants are insufficient.

Synergism with Vitamin C

Fortunately, vitamin E can be recycled by a reduction process with vitamin C or glutathione, returning vitamin E to its active state and antioxidant capabilities (May, 1999). Eicher-Pruiett et al. (1992) in a review of Bendich (1987) clearly suggests that synergistic effects exist between vitamins C and E. The relationship proceeds as follows. Vitamin E is primarily responsible for protection of cellular membranes against

oxidation. When vitamin E reacts with free radicals and other ROM, it is reduced in form. Vitamin C in high concentrations at the membrane surface works to regenerate vitamin E previously reduced by oxido-reduction reactions. The mechanism by which this synergism and vitamin E-regenerating activity occurs is not clear, however.

As with many other molecules, vitamin C is multi-functional. Ascorbate, or vitamin C, is a potent water-soluble antioxidant that can scavenge reactive oxygen and nitrogen species, and can functionally regenerate other antioxidants (α-tocopherol, glutathione, urate, and β-carotene) from their free radical-degenerated state (Carr and Frei, 1999). Some debate continues whether vitamin C actually recycles, or simply spares α-tocopherol *in vivo* (Burton et al., 1990; Jacob et al, 1996). In fact, in a review of more than 40 *in vivo* studies with vitamins C and E, Carr and Frei (1999) concluded that vitamin C does not "spare" or act as a pro-oxidant under physiological conditions. Regardless, the interaction of ascorbate and α-tocopherol radical (α-tocopheroxyl) does indeed prevent further tocopherol-mediated peroxidation (Neuzil et al., 1997) lending more power to the notion of providing a balance of nutrients in appropriate combinations, rather than mega-doses of any one nutrient (Roche, 1999).

Synergism with Selenium

Vitamin E and selenium can cooperatively impact disease resistance by protecting leukocytes and macrophages during phagocytosis of bacteria. Vitamin E is the first-line defender against cellular oxidative damage; however, some peroxides can still be formed. Glutathione peroxidase, a SAA-requiring enzyme, spares vitamin E by destroying peroxides. Selenium can preserve pancreatic integrity for fat digestion and absorption; it

will also spare vitamin E needed for the maintenance of glutathione peroxidase (McDowell, 1989a). Vitamin E can be stored in all tissues of the body including adipose tissue, but is found in highest concentrations in the liver. Assessment of vitamin E status in animals is best performed by collecting whole blood samples and separating the plasma or serum; either can be used as a reliable means of quantifying the nutrient status of an animal (Adams, 1982). As reported by Droke and Loerch (1989), serum immunoglobulin G (IgG) titers to *M. haemolytica* were increased at 14 days after a 25 mg injection of selenium and a 340 I.U. injection of vitamin E.

In a five-trial experiment with beef steers new to the feedlot environment, Droke and Loerch (1989) examined the effects of parenteral selenium and vitamin E on animal performance, morbidity, mortality, and humoral immune response. Steers in this experiment that were given at least one injection of selenium and vitamin E had significantly greater immunoglobulin-G (IgG) titers in response to a vaccination with *P. haemolytica* than did control steers, thus indicating immunocompetence. Unfortunately, these results were not consistent within each trial group. The variations could be attributed to morbidity caused by other pathogens of the BRD complex, rather than only *M. haemolytica.* Additionally, control steers in this study produced very low seroconversion values, possibly indicating immunosuppression, leading to a conclusion that Se and E can easily improve the chance of resisting disease simply by improving immune system function.

Protection of Other Antioxidant Compounds

Vitamin E also protects vitamin A and other carotenoids against peroxidative damage, inhibits platelet aggregation (improves blood clotting or coagulation time), and

can effect prostaglandin-E (PGE) synthesis by protecting against arachadonic acid degradation. Beta-carotene (a precursor to vitamin A) is another important antioxidant, especially in extracellular fluids (Roche, 1999). Vitamin A is clearly the most powerful and efficient scavenger of singlet oxygen molecules; estimates of its scope indicate that one β-carotene molecule is capable of dissipating the energy contained in up to 1,000 molecules of singlet oxygen (Roche, 1999). Due to its chemical structure, β-carotene/vitamin A absorbs and dissipates the energy contained in the singlet oxygen molecule and releases the energy as heat (Roche, 1999). Although both vitamin E and vitamin A function as powerful antioxidants, their effects are not redundant. Rather, these vitamins seem to have a complementary effect due to the "geography" of their function. Beta-carotene/vitamin A functions in tissues where the partial pressure of oxygen is low, while vitamin E function favors environments where oxygen pressure is much greater (Roche, 1999).

Role in the Immune System

Vitamin E has a dual function in animal systems—it meets certain basic physiologic requirements, as well as stimulates the immune system, which helps to increase the animal's resistance to disease (Coelho, 1991). This boost to the immune system is primarily in the form of the vitamin's antioxidant abilities. It scavenges free radicals. Important functions of vitamin E not yet discussed include the synthesis of coenzyme Q, maintenance of low cellular peroxide concentrations, aids cellular respiration, and helps maintain muscle structures particularly in the gastrointestinal and reproductive systems. Vitamin E improves immune system performance by enhancing mechanisms involved in

the defense against primary infections. Specifically, it stimulates glutathione peroxidase activity in circulating neutrophils, macrophages (peritoneal and pulmonary alveolar), as well as stimulating the activity of helper T-cells (T_H), chemotaxis, phagocytosis, and antibody production (Coelho, 1991).

Once engulfed by neutrophils, macrophages, or other phagocytes, killing of microbes is ultimately achieved by a phenomenon known as an oxidative burst (Kumar et al., 1997). The oxidative burst is characterized by a rapid consumption of oxygen, increased glycogenolysis, and glycolysis, resulting in the production of reactive oxygen metabolites. The oxidation of NADPH reduces oxygen to a superoxide ion ($O_2^{\bullet -}$), which is converted, almost spontaneously to hydrogen peroxide (H_2O_2). An adequate killing compound itself, the total volume of H_2O_2 actually produced is insufficient to completely kill most bacteria. Fortunately, *azurophilic granules* (lysosomes of neutrophils) contain myeloperoxidase (MPO), an enzyme effective in the conversion of H_2O_2 to a hypochlorous radical molecule ($HOCl^{\bullet}$) when adequate quantities of a halide such as chloride (Cl^-) are present. A very powerful oxidant and similar to the active ingredient in chlorine bleach ($NaOCl^{\bullet}$), $HOCl^{\bullet}$ effectively kills bacteria by halogenation, or by protein or lipid peroxidation (Kumar et al., 1997). Clinical deficiencies of vitamin E can lead to hydroperoxide formation from the oxidation of fat resulting in the decreased structural integrity of the cell and metabolic derangement (Kumar et al., 1997).

Average calf mortality due to respiratory disease in a large New York dairy averaged greater than thirty percent (Cipriano et al., 1982). These same authors revealed a direct correlation between low serum immunoglobulin concentrations and disease in young

calves; they also indicated that dietary vitamin E can effectively enhance the humoral immune response observed in both domestic and laboratory animals.

Vitamin E has been proven to have valuable effects on the immune system of young beef and dairy calves (Cipriano et al., 1982; Reddy et al., 1986; Reddy et al., 1987; Eicher-Pruiett et al., 1992). Thirty newborn Holstein bull calves were used in an experiment (Eicher-Pruiett et al., 1992) to examine the effects of dietary supplementation of vitamins C and E on neutrophil and lymphocyte response. Clinical signs of respiratory disease measured by severity of ocular and nasal discharge were lower in vitamin supplemented calves compared to non-supplemented controls. Although vitamin C alone tended to impede neutrophil-mediated phagocytosis and antibody-dependent cellular cytotoxicity (ADCC), calves supplemented with both vitamin C and E produced improved neutrophil and ADCC activity when compared to controls or calves supplemented with vitamin C only.

It seems clear that supplemental vitamin E in receiving diets provides many benefits, including improved performance and decreased morbidity; the majority of these benefits seem to be mediated by positive effects on the immune system itself (Galyean et al., 1999).

Protection of Cellular Membranes

Vitamin E protects cellular membranes from peroxidative destruction caused by highly reactive oxygen-containing molecules (ROM) (Adams and Zimmerman, 1984; Nockels, 1991). These ROM species include such molecules as the hydroxyl radical ($HO^{\bullet}$), the peroxy radical ($ROO^{\bullet}$), and super oxide ($O_2^{\bullet -}$). Normal products of metabolic

processes, ROM are produced by enzymes such as oxidases, cyclooxygenases, lipoxygenases, dehydrogenases, peroxidases, (Machlin and Bendich, 1987), as well as by metal ions and xenobiotics (Rice and Kennedy, 1988). These enzymes are found in the aqueous regions of the cell in numerous membranes including plasma, mitochondrial, endoplasmic reticulum, and nuclear, as well as in phagocytes (Weiss and Buglio, 1982

Several studies (Boyne and Arthur, 1979; Eskew et al., 1985) have shown that some components, and thus the responsiveness of immune systems, can be suppressed by deficiencies of selenium and vitamin E leading to an increase in susceptibility to disease during conditions of stress. Other researchers (Spallholz et al., 1975; Peplowski et al., 1980; Teige et al., 1982; Smith et al., 1985) have concluded that supplementation with selenium and vitamin E can improve humoral immune responses, as well as enhance an animal's natural ability to defend against an immune challenge.

Frequently, inaccuracies in vitamin E data can be a result of an inability to quantify the compound because a majority of the vitamin may be bound to cellular membranes and unavailable for mobilization (Nockels, 1996). Further, this impaired mobilization may be alternately explained by inadequate concentrations of α-tocopherol binding protein (EBP). Lipid soluble compounds are transported from aqueous portions of membranes to phospholipid regions via specific carrier proteins (Dutta-Ray et al., 1993; Nockels et al., 1996). Liver tissue deficient in EBP concentration may not be sufficient for the rapid mobilization of vitamin E and can result in vitamin E-deficient animals, including cattle (Nockels et al., 1996).

Requirements

Vitamins are required in animal diets in very small quantities relative to other nutrients. However, each vitamin has a very specific function, or array of functions, and the omission of any one vitamin from the diet of a particular species requiring that vitamin can lead to specific deficiency symptoms. In severe deficiencies, even death may be observed (Church and Pond, 1988). Laboratory animals fed diets deficient in vitamin E often exhibit predictable deficiency symptoms including rough and scaly skin, muscular weakness, cachexia, and even sterility (Lehninger et al., 1993; Kumar, et al., 1997). Ruminant animals have vitamin requirements at the tissue level similar to other species of animals; however, ruminal microorganisms are capable of producing B-vitamins and vitamin K, but do not produce the fat soluble vitamins A, D, and E (Huber, 1988). Therefore, these vitamins must be provided in the diets of ruminants.

Vitamin E is an essential nutrient for all animal species (Robert Teeter, personal communication). Several nutrients are involved metabolically with vitamin E such as selenium, sulfur-containing amino acids (SAA), and PUFA. In contrast to other vitamins, such as vitamin A, vitamin E is not stored in significant quantities in hepatic tissue to be mobilized for later use by the animal (Hill and Williams, 1995). Therefore, dietary supplementation with vitamin E is necessary, especially under conditions when animals are unusually stressed, or when the dietary level of vitamin E is insufficient. Whole body concentrations of vitamin E as measured by plasma or serum α-tocopherol levels can become depleted very rapidly (Hill, 1987; Hill et al., 1990; and Hill et al., 1993).

Gill et al. (1986) reported that sick cattle fed vitamin E (1600 I.U./d) had improved weight gains and fewer sick days (days of treatment with an antimicrobial drug) than did

their counterparts not fed vitamin E. These same researchers also observed that when the data was analyzed excluding cattle that were identified as sick on arrival (d 0), the vitamin E fed cattle gained weight faster (22.2%), and had a reduced incidence of morbidity (11.7%). Finally, they concluded that the vitamin E requirement for young, stressed cattle is higher than the 33-132 I.U./lb of dry diet recommended by NRC (1984).

Adams (1982) reported on data from more than 280 head of feedlot cattle sampled for quantification of vitamin E status. Sixty percent of the cattle were only marginally sufficient while forty percent were deficient in circulating vitamin E concentrations. Normal plasma tocopherol levels were set at .72 mg/100ml or greater as defined by Sheldon (1980). This study resulted in a recommendation that cattle receive 20 to 50 I.U./day of supplemental vitamin E during the finishing period, and not less than 50 I.U./day when daily feed intakes of mainly roughage diets are low.

Hutchenson (1991) defines the minimum amount of dietary vitamin E for receiving calves as 100-125 I.U./lb of dry diet for cattle with initial weights of approximately 182 kg and 50-75 I.U./lb of dry diet for cattle with initial weights of 318 kg or greater. According to NRC (1996) recommendations, which are based on calculation by its own subcommittee and on published data (Hutcheson, 1990), dietary nutrient concentrations for stressed calves lie in the range of 400-500 I.U./d for a 250-kg calf. These recommendations are based on variable rates of feed intake over the first 14-d; adequate intakes during this phase of the receiving period are critical. Target intake levels are 1.55% of body weight (BW) for days 0 through 7, and 1.90% BW for days 0 through 14 (Hutcheson, 1990).

Vitamin requirements as suggested by NRC are typically defined as some minimum level above which deficiency signs and adequate health and performance could be expected (McDowell, 1989b). Common sense should prevail when diets are being developed and NRC recommendations should be used only as a guide, and not an absolute optimum. Moreover, optimal allocations that will provide a maximal response may be significantly different from one animal to another, or even from day to day (Roche, 1979). Cunha (1985) suggested that nutrient levels adequate for intensified production factors (daily gain, feed efficiency, gestation and lactation) likely are not sufficient to maintain normal immunity, nor are these nutrient levels adequate to maximize the animal's resistance to disease. The author further suggested that the combination of induced distress and exposure to disease might increase the animal's requirement for certain vitamins, especially vitamins A, D, and E.

Supplemental Vitamin E Forms

Research has been done comparing the efficacy of injected versus dietary vitamin E supplementation (May et al., 1987). Heifers receiving injectable vitamin E performed no better than control heifers receiving no injection; performance was measured by daily gain for a twenty-eight day receiving period. However, among the two levels of injectable vitamin E tested, the lower level (1250 I.U.) allowed for faster daily gains among heifers in this experiment. In a subsequent experiment, steers received either no supplemental vitamin E (control), were injected with vitamin E (2500 I.U.), were fed 1000 I.U. vitamin E, or received both the fed and the injectable forms at the same levels as the previous two treatment groups. Vitamin E supplementation provided no benefit to daily gains, regardless of route of administration. However, dietary vitamin E

significantly improved plasma tocopherol concentrations when compared to controls and injectable treatments (.48 vs .34 vs .26 mg/100 mL, respectively).

Lee et al. (1985) compared a combination of dietary vitamin supplements and measured animal performance, morbidity, and mortality. Treatments included a control diet (contained supplemental vitamins A and D), a vitamin E diet (control diet plus 450 I.U. per head per day of vitamin E), and a B-complex plus E diet (vitamin E diet plus an array of B-complex vitamins). Animal performance as measured by average daily gain (ADG) and feed conversion (F/G) were improved for vitamin E and vitamin E plus B-complex diets compared to controls (ADG=1.25 kg/d vs 1.31 vs 1.18; F/G=5.90 vs 5.31 vs 6.33, respectively). No significant differences were observed in morbidity or mortality among the dietary treatments.

Little controversy remains with regard to the most efficacious and least harmful means of supplementing vitamin E. Due to the recurrence of injection-site reactions, commercially available injectable compounds are less favorable than other available forms, specifically dietary supplements or oral drenches (Galyean et al., 1991). Hays et al. (1987a) confirms some of the negative aspects of injectable vitamin E preparations. In their study, injection of 3000 I.U. vitamin E significantly increased the number of hospital days per sick or morbid calf. Conversely, dietary supplementation with 800 I.U. per day of vitamin E not only decreased total morbidity and the number of hospital days per sick calf, it also significantly improved average daily gains.

Motivation for Further Research

Ruminant animals can typically consume adequate quantities of vitamin E in green forages to satisfy their dietary requirement for this nutrient (Hill and Williams, 1995).

However, under the conditions of modern production systems grains make up a greater proportion of many calves' lifetime dietary intakes than do forages. Additionally, stress levels are frequently elevated due to transport and co-mingling, thus increasing the animal's requirement for vitamin E.

Griffin (1983) reported that not only do marketing and transport typically include periods of feed and water deprivation, the extent of weight loss caused by these factors can be directly related to subsequent morbidity and mortality levels in feedlot cattle. As a result of the general decrease of feed and water intake during these periods, abnormalities in metabolism and even appetite may occur for as long as three weeks after arrival in the feedlot (Dubeski, 1992). Hutcheson and Cole (1986) reported that likely the most critical period for disease resistance and the immune response in feedlot cattle was the 14 to 28 d period after arrival in the feedlot. Previous research has primarily focused on determining an optimal level of vitamin E to include in the diet for the entire receiving period (Gill et al., 1986; Hays, 1987) rather than feeding a similarly high level only during the critical 14 to 28 period previously described. Performance results determined by daily gain, feed intake, and feed conversion (G/F) were variable for vitamin E supplemented cattle; however, morbidity was also increased (Lee et al., 1985; Gill et al., 1986; Hays, 1987; May et al., 1987).

Although requirements for vitamin E supplementation are not precise (Secrist et al., 1977), data exists regarding the efficacy of vitamin E in immune system function (Cipriano et al., 1982; Nockels, 1988; Droke and Loerch, 1989; Nockels, 1991; Eicher-Pruiett et al., 1992). None of this research correlates subjective determination of disease

with physiological parameters such as APP determination that would define the pathogenesis of BRD.

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CHAPTER III

VITAMIN E SUPPLEMENTATION IN THE DIET OF NEWLY ARRIVED FEEDLOT CATTLE

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ABSTRACT

Seven hundred fifteen crossbred (mostly British) calves purchased in southern Oklahoma and northern Texas auction barns were received at the Willard Sparks Beef Research Center (WSBRC) and used to study the effects of dietary vitamin E during a 42-d receiving period on animal performance and health, and serum cholesterol and vitamin E concentrations. On arrival cattle were blocked by weight (light (L) and heavy (H)), and randomly assigned to one of four dietary treatments. The basal diet (CON; 14.8% CP) consisted of soybean hulls, corn, wheat middlings, a lasalocid-containing protein supplement, and cottonseed hulls. Experimental diets provided 2000 IU/hd/d of supplemental vitamin E for 7 (E7), 14 (E14), or 28 (E28) d. The vitamin E was delivered in a pelleted supplement and added at to the basal diet at decreasing rates as dry matter intake increased (2.0 kg of DMI=6%; 4.0 kg of DMI=4%; 6.0 kg of DMI=2%). Serum samples were collected on d 0, 14, 28, and 42 for determination of cholesterol, α-tocopherol, and antibody (IgG) concentrations. Detailed records of all cases of respiratory and other disease were maintained. Regardless of dietary vitamin E treatment, daily gain (0.9 kg/d) and feed conversion (F/G = 5.3) were not improved. Serum cholesterol concentrations decreased significantly among all treatment levels from d 0 (136.8 mg/dL) to d 14 (63.8 mg/dL); cholesterol concentrations were higher in H

cattle compared with L cattle in all four periods. Similarly, serum α-tocopherol decreased from d 0 (5.2 μg/mL) to d 28 (1.8μg/mL) and even further by d 42 (1.5 μg/mL). Significantly higher serum α-tocopherol was observed on d 28 (3.4 μg/mL) among cattle in E28 compared to those in CON (1.1 μg/mL), E7 (1.2 μg/mL), and E14 (1.5 μg/mL). Respiratory disease affected 64.6% of the cattle in this study. Medical costs were decreased among E28 ($4.88/hd) compared to CON ($6.29/hd). Medical costs per total kg gain (COSTGN) were lower among H cattle ($0.004/kg) than L ($0.04/kg). Serum antibody response of cattle vaccinated against keyhole limpet haemocyanin (KLH) was significantly improved from d 0 to d 14 and d 28, but was unaffected by dietary vitamin E level. Carcass characteristics were not affected by dietary treatments. Vitamin E decreased medical costs among treatment and weight class blocks and improved serum concentrations of α-tocopherol while as cholesterol concentrations deficient levels.

Key Words: Vitamin E, Stress, Disease, Cattle

Introduction

The nebulous mechanisms of stress in cattle caused by marketing, transit, weaning, and other management practices and its interaction with infectious disease has long been recognized, but not well understood (Breazile, 1988). Vitamin E (α-tocopherol) is a potent lipid-soluble antioxidant that functions in the prevention of chronic diseases associated with oxidative stress (Cipriano et al., 1982; Eicher-Pruiett et al., 1992; Galyean et al., 1999). It remains unclear whether the free radicals produced as part of normal metabolism are injurious by themselves, or if they are formed as a result of disease (deZwart et al., 1998).

Although many studies have attempted to determine the optimal level of vitamin E required in the diet of ruminants, a definitive value remains unclear. Minimally, growing cattle require between 15 and 60 IU/kg body weight of vitamin E (NRC, 1996). Previous studies (Gill et al., 1986; Hays et al., 1987) evaluating the effects of vitamin E supplemented cattle revealed improvements in animal performance and improved immune system function when cattle received 800 IU of supplemental dietary vitamin E. Han et al. (1999) observed a marked reduction in plasma α-tocopherol concentrations of supplemented cattle in response to transit stress. Similarly, Smith et al. (1999) observed a decrease in plasma vitamin E levels just prior to parturition in dairy cows.

Our objective in this study was to measure the influence of supplemental dietary vitamin E (2000 IU/hd/d) fed for either 0, 7, 14, or 28 d in a 42-d receiving period on animal performance (total gain, ADG, and F/G), and health (number of drug treatments and antibody response to KLH). Additionally, serum cholesterol and α-tocopherol concentrations were quantified to report physiological responses to our dietary treatments. This objective was in contrast to previous research that has attempted to quantify an optimal level of vitamin E fed continually for extended periods.

Materials and Methods

Seven truckloads of sale barn-origin calves (568 heifers, 197 kg initially; 126 bulls and steers, 151 kg initially) were received at the Willard Sparks Beef Cattle Research Center from July to mid-December 1999 (Table 1). Calves were purchased from numerous auction barns in south central Oklahoma and northern Texas, hauled to a facility in central Oklahoma and sorted into truckload lots. They were then hauled

Table 1. General information regarding cattle received at Willard Sparks Beef Research Center for this study.

Load #	Origin	Date Received	# Head in Load[1]	Average Weight, kg
1	Texas-Oklahoma	July 15, 1999	86	234 ± 33
2	Texas-Oklahoma	August 12, 1999	85	233 ± 33
3	Texas-Oklahoma	August 20, 1999	85	227 ± 33
4	Texas-Oklahoma	September 4, 1999	130[2]	151 ± 11
5	Texas-Oklahoma	October 4, 1999	111	168 ± 33
6	Texas-Oklahoma	December 8, 1999	106	172 ± 33
7	Texas-Oklahoma	December 16, 1999	112	174 ± 33

[1]Total cattle received=715. Not all cattle completed the 42-d receiving trial due to mortality or morbidity. In total, twenty-one head were removed due to morbidity and were chronic, or due to death loss (<1%).

[2]Bulls and steers; all other loads were heifers.

approximately 145 km to our facility. On arrival, calves were allowed to co-mingle and rest for at least one hour in a processing facility alley prior to a pre-processing procedure. This procedure included assessment of overall health, individual weight (INWT) of each calf and application of a sequentially numbered identification tag in the left ear. Calves were randomly distributed to six holding pens for no more than 36 hours before inception of the study. While in these holding pens, 0.9 kg of prairie hay and 1.4 kg of the control diet (Table 2) were fed per head. On d 0, calves were processed at approximately 0600 hours prior to feeding. Processing included: individual weight, vaccination for viral respiratory diseases (BRSV-Vac 4™, 2 mL via intramuscular injection [IM]; Vision-7™, 2 mL via subcutaneous injection [Sub-Q; heifers]; or Covexin 8™, 5 mL Sub-Q [bulls and steers]), and treatment with anthelmintics for internal and external parasites (Ivomec-Plus™, 1.0 ml/110 lb SubQ); viral respiratory was boostered at d 14.

Calves were blocked by weight using INWT into two weight blocks, light (L), and heavy (H), and randomly assigned to one of four dietary treatments; treatments were randomly assigned to eight pens. Dietary treatments are represented by the number of days that the control diet was supplemented with 2000 IU of vitamin E (Supp. B-171; Table 3): 0 days = Control (CON), 7 days = E7, 14 days = E14, or 28 days = E28. Supplement B-171 was included in the diet to provide 2000 IU of vitamin E on a constant basis as DMI increased (2.0 kg of DMI=6%; 4.0 kg of DMI=4%; 6.0 kg of DMI=2%).

After d-0 processing, calves were immediately taken to their assigned pens and 2.3 kg of the control and experimental (Table 4) rations were delivered into concrete feed-bunks (12.2 m of linear bunk space per pen). Prairie hay was fed for the first seven days only (.75 kg/d). As the amount of hay in the diet was reduced and as calves became

Table 2. Control diet composition.[1,2]

Ingredient	% of Diet (DM basis)
Soybean hulls	32.5
Whole corn	27.0
Wheat middlings	17.0
Sparks 99 supplement	13.5
Cottonseed hulls	10.0

[1]Fed ad-libitum.

[2]Control diet provided 127 IU vitamin E per 4.5 kg feed from added and natural sources.

Table 3. Supplement B-171 (vitamin E) composition[1].

Ingredient	% of Supplement
Wheat middlings	97.0
Vitamin E-50 Adsorbate™	3.0

[1]Fed at a constant rate of 0.14 kg/hd/d to provide 2000 IU of supplemental vitamin E.

Table 4. Experimental diet (Vitamin E-2000) composition.[1,2]

Ingredient	% of Diet (DM basis)
Soybean hulls	32.5
Whole corn	27.0
Wheat middlings[3]	15.0
Sparks 99 supplement	13.5
Cottonseed hulls	10.0
B-171 Supplement[3]	2.0

[1]Fed ad-libitum.

[2]Control diet provided 127 IU vitamin E per 4.5 kg feed from added and natural sources.

[3]A pelleted supplement (for composition of B-171, see Table 3) replaced wheat middlings in experimental diets and was added to provide 2000 IU per day of vitamin E. Inclusion in the diet was based on average daily feed intake: 2.3 kg = 6%; 4.5 kg = 4%; 6.8 kg = 2%).

acclimated to the new environment and diets, feed was increased on an ad libitum basis. The basal diet was a well-balanced, textured, and highly palatable feed ideal for receiving cattle and was supplemented with natural proteins, calcium, vitamins A and E, selenium, and lasalocid as a low-level coccidiostat (Table 5). It was developed to meet the nutritional requirements of incoming cattle that may be stressed due to a short term feed and water deficiency, or even a deprivation of both in the marketing system. The nutrient composition of the basal diet is shown in Table 6.

Our experimental diet was analyzed (Roche Laboratories, Nutley, NJ) to determine the actual level of vitamin E supplied by both the supplement pellet (B-171) and the total mixed ration (TMR). The results of these analyses are presented in Table 7. Ideally, these results would be at approximately 85 percent or greater of our calculated amount. Our results were consistently below that acceptable threshold, and in some cases appeared to be very inadequate. It was finally determined that a starch matrix was occluding the analytical procedure to the extent that the results were masked (J. Wilson, Roche Vitamins, Inc., personal communication). The problematic starch matrix was contributed by the wheat middlings, which comprised more than 95 percent of the B-171 pellet (Table 3). The final sample collected prior to the pelleting process was determined to be adequate. We relied on our supplement mixing and pelleting procedures, as well as our ration balancing and delivery procedures and remain confident that our experimental diet provided vitamin E at, or relatively near to our calculated amounts.

Pen size was uniform across all treatments (12.2 m x 30.5 m) and alternating pens shared automatic water basins. Feed was delivered once daily at approximately 0700

Table 5. Sparks 99 supplement composition[1].

Ingredient	% of Supplement (DM basis)
Cottonseed meal (43% CP)	55.16
Soybean meal (48% CP)	30.6
Limestone	5.4
Pellet partner	6.5
Salt	1.9
Vitamin A (30,000 IU/gm)	0.15
Vitamin E-50™ Adsorbate	0.02
Bovatec™ 68 [2]	0.09
Selenium (0.02%)	0.18

[1]Crude protein = 37.9%; fat = 1.9%; fiber = 7.3%; TDN = 66.8%; Ca = 2.5%; P = 0.7%; K = 1.6%; Na = 1.8%.

[2] Bovatec™ included at 221 g / ton or 110 mg / lb.

Table 6. Control diet nutrient composition.[1]

Nutrient	% AF	% DM
NEm, Mcal/cwt	74.3	82.8
NEg, Mcal/cwt	46.1	51.4
TDN, %	64.6	72.0
Fat, %	2.6	2.9
Crude fiber, %	10.7	11.9
Crude protein[2], %	13.8	15.4
Ca, %	0.9	1.1
P, %	0.7	0.8
K, %	0.4	0.5

[1]Calculated values.
[2] Crude protein analyzed value (DM%) = 14.8%.

Table 7. Results of chemical analysis of experimental diet for vitamin E concentrations.

Inclusion rate	n	Claim (IU)[1]	Result[2]	%
6%	5	140	95.7	68.4
4%	4	207	137.6	66.5
2%	9	407	226.6	55.7
4%	1	207	180.0	87.0
2%	1	407	270.0	66.3
Pelleted B-171	6	6,667	2610.7	39.2
Pelleted B-171	4	6,667	5168.8	77.5
Pelleted B-171	3	6,667	4525.0	67.9
B-171 Meal[3]	1	6,667	5610.0	84.2

[1]IU=International Unit/lb total mixed ration.
[2]Results indicate vitamin E concentrations in the acetate form.
[3]Sample collected prior to the pelleting process.

hours. Feed was delivered twice daily during inclement weather to provide clean, dry feed for a majority of each day.

Cattle were weighed on days 0, 14, 28, and 42 of the study. On d 41 cattle received only one-half of the previous day's ration and were not permitted access to water from 1700 hours until after final processing on d 42. Prior to d 0 processing, a sub-sample of six cattle per pen or twelve cattle per dietary treatment were randomly selected for whole blood sample collection. Upon collection on d 0, 14, 28, and 42, blood samples were allowed to equilibrate to ambient temperature, then stored at 4°C overnight. Serum was separated after the overnight chill and stored at -10°C until laboratory analyses could be performed. Analyses included quantification of serum total cholesterol according to the procedure described by Ravel (1984), and Jenkins et al. (1988), and α-tocopherol according to the procedure described by Njeru et al. (1992). Antibody (IgG) concentrations were determined according to the procedure of Korver et al. (1984), and Pollock et al. (1991).

Cattle were closely observed each morning at approximately 0630 by experienced veterinary personnel (Oklahoma State University College of Veterinary Medicine) for signs of respiratory and other diseases. Two or more clinical signs of disease were required to designate a calf as sick and make that calf eligible for further clinical review and therapeutic antimicrobial treatment. Clinical signs indicating eminent disease included depression, lack of fill, occasional soft cough, physical weakness, altered gait, and ocular or nasal discharge (R.A. Smith, personal communication). Once pulled a calf would be walked to the processing area and restrained in a squeeze chute, its individual weight recorded and rectal temperature assessed. Regardless of health status, all

information was recorded on an individual sick card and filed by pen for future reference. If rectal temperature was greater than 40°C, a required regimen of antimicrobial treatment therapy (Table 8) followed.

After cattle in each load had completed the 42-d receiving period, cattle were transported to one of five commercial feedlots in either the Oklahoma or Texas panhandle for finishing. The feedlot was selected by the cooperating producer/owner who had retained ownership of the cattle since inception of the study. All cattle were fed on a high-energy concentrate diet for approximately 200 days. Upon reaching their final target weights, all cattle were delivered to Farmland National Beef in Liberal, Kan., for carcass harvest. Gross carcass data (hot carcass weight [HCW], quality grade [QG], and yield grade [YG]) were collected on selected loads.

Carcass data were not obtained on four of the original seven loads of cattle. Two loads were taken back to the ranch by the cooperating producer/owner for grazing on wheat pasture during the winter of 1999. These cattle were co-mingled with others, making acquisition of any further data impossible. One load was missed simply due to a scheduling error. In total, carcass data from only two of the seven loads of cattle received at the WSBRC will be reported in this chapter.

Five hundred fifteen serum samples were analyzed at the University of Florida Institute of Food and Agricultural Sciences under the supervision of L.R. McDowell, Ph.D., for vitamin E concentration. Their procedure utilized an ABI Analytical Spectraflow 400 HPLC with a EQC 10 μSi 60A, 4.6 mm x 250 mm column (Whatman International, Ltd., Maidstone, England), a Hitachi L-7485 Fluorescence Detector (excitation wavelength=290nm; emission wavelength=330nm), a Perkin-Elmer ISS-100

Table 8. Antimicrobial treatment protocol.[1]

Pull	Subjective Score[2]	Rectal Temperature	Prescribed Therapy
First	Mild or >	25.8° C or >	Micotil™
No further treatment for at least 48 hrs			
Second	Mild or >	25.8° C or >	Nu-Flor™
No further treatment for at least 72 hrs			
Third	Mild or >	25.8° C or >	Excenel™
Repeat only this therapy in 48 hrs of Subjective Score or Rectal Temperature			

[1]All antimicrobial drugs given under the supervision of a licensed veterinarian.
[2] Indicates severity of disease state.
[3]All antimicrobial drugs given at the recommended label dosages and routes of administration.

auto-sampler, and a Perkin-Elmer LCI-100 integrator. Standards (T-3251 dl-α-Tocopherol, 95%; stock concentration=5mg/ml in 2-propanol) were obtained from Sigma Chemical Co., Inc., St. Louis, MO.

For extracting α-tocopherol, five hundred microliters of serum were pipetted into a 16 x 125 mm glass tube and one ml ethanol was added to precipitate the serum proteins. The sample was then vigorously vortexed. A double-ether extraction procedure followed. Three mL of petroleum-ether (pet-E) was added to the previously described tube, which was then vortexed and centrifuged (1500 rpm x 5 minutes). The pet-E layer was decanted into a separate 16 x 125 mm glass tube already in an ice bath. Three mL of pet-E was added to the tube with the precipitated serum, then vortexed and centrifuged as before. The pet-E layer was removed from this tube and deposited into the second tube in an ice bath. The second tube with the two deposits of pet-E was evaporated to dryness under a nitrogen stream in a water bath (35°C). The remaining residue was then dissolved with 1 mL mobile phase solution (90% iso-octane, 9.5% tetrahydrofuran, and 0.5% acetic acid), and then stored in sealed vials at -10°C until analyzed.

Statistical Analysis. Data were analyzed as a split-plot in a randomized block design where loads were blocks, weight class was the whole plot factor, and dietary treatment was the sub-plot treatment factor. The error term used when testing weight class in the whole plot was the interaction of weight class and load. A three-way interaction between weight class, dietary treatment level, and load was used as the sub-plot error term when variables were tested for dietary treatments and interactions involving dietary treatments. For variables related to animal performance and health, including weight gain, feed conversion, pen was used as the experimental unit. For variables related to incidence of

respiratory disease, drug treatment costs, final live weight, carcass characteristics serum vitamin E, serum cholesterol, where sub-sampling occurred, animal was used as the experimental unit. All models were analyzed using the GLM and MIXED procedures of SAS® (1996).

Antibody Response to KLH. The purpose of this test was to detect and quantify the Ab responses of stressed cattle to a foreign or novel antigen (keyhole limpet hemocyanin [KLH]). Fifty animals in two loads (Loads 6 and 7, Table 1) were injected subcutaneously with an emulsion containing KLH (0.5 mg) and 1.0 mL of Freund's incomplete adjuvant.

Antibodies to KLH were determined by ELISA (Korver et al., 1984; Pollock et al., 1991). ELISA wells were coated with 100 μL of KLH in a concentration of 10 μg mL^{-1} in PBS. Plates were then incubated at 37°C for at least one hour and stored overnight at 4°C. After allowing plates to return to room temperature, they were washed (3x) with PBS/Tween (0.05% Tween 20 (BDH)). Optimal dilutions of sera (100 μl; 1:500) were placed in triplicate wells and incubated at 37°C for two hours. Plates were again washed (3x) in PBS/Tween. A dilute (1:400 in PBS/Tween BSA anti-bovine IgG) conjugated secondary antibody was added (100 μL) and the plate was incubated as before. After a final wash (6x) 100 μL of a color substrate consisting of OPD and H_2O_2. Wells were allowed to color for five minutes, then a stop reagent was added (50 μL) to each well. OD_{490} was determined for each well in an automated plate reader (V Max Kinetic Microplate Reader, Molecular Devices, Inc.).

Results and Discussion

Animal Performance and Health. Performance and health results from data analyzed using SAS® (1996) as described above are shown in Table 9. Typical measures of animal performance, ADG, total gain (TG), and feed conversion (F/G) were not significantly (P >.05) affected by our dietary treatments in this study. During the 42-d receiving period, calves in all treatment groups gained at a similar rate (P>.05) and averaged 0.9 ± 0.3 kg/d. Total gain during any particular feeding period has been a significant indicator of animal performance in some Ranch to Rail experiments recently conducted by Texas A&M University (W.L. Mies, personal communication). However, in this experiment total gain was similar (41.5 ± 1.1 kg; P >.05) across all treatment levels. Total gain and ADG was different (P<.0001) among loads of cattle and appeared to be dependent upon initial BW of cattle at arrival. No significant differences were observed in DMI among our dietary treatments, and therefore, since gains were similar, no differences (5.3 ± 0.3; P >.05) were evident in feed conversion (F/G).

These results are in contrast to those of Gill et al. (1986), who reported improved weight gains and feed conversion among newly arrived stocker cattle under similar geographic and environmental conditions. The diets among these two studies, however, differed. Gill et al. (1986) provided ad libitum access to prairie hay and fed 0.91 kg/d of a soybean-meal based pellet fortified with an additional 800 IU of vitamin E per 0.454 kg of supplement (28-d). Daily gains in their study were lower (Controls=0.43 kg/d, Vitamin E=0.53 kg/d) than those observed in this study. These differences are likely due to the general differences in the two diets fed. The diet fed in our study was developed

Table 9. Animal performance and animal health during the receiving period, estimated animal performance during the finishing period, and carcass data[1].

	Treatments					
	CON (n=183)	E7 (n=180)	E14 (n=178)	E28 (n=174)	S.E.M.	Pr > F
ADG, kg/d	0.95	0.99	0.98	1.0	0.05	.5618
Total Gain, kg	40.0	41.8	41.3	42.2	2.3	.5618
Feed conversion, F/G	5.3	5.2	5.3	5.3	0.32	.8729
% Sick 0-42d	67.8	68.3	61.8	60.3	0.07	.2232
EFLW, kg	483.0	496.1	491.6	486.3	7.58	.5113
EFLADG, kg/d	1.1	1.2	1.2	1.1	0.47	.5196
Medical costs, $/hd	6.29	5.67	5.18	4.88	0.70	.1208
COSTAVWT[2]	0.013	0.012	0.011	0.010	0.002	.1211
AMT/hd	0.92	0.84	0.78	0.76	0.8	.1411
Med0, %	32.3	31.7	38.2	39.7	---	.2829
Med1, %	47.5	53.9	47.2	47.7	---	.6758
Med >1, %	20.2	14.4	14.6	12.6	---	.2088
Carcass Weight, kg	314.0	322.9	319.5	316.1	6.13	.5113
Quality Grade[3]	2.5	2.5	2.5	2.6	0.11	.8790
Yield Grade	2.3	2.3	2.4	2.5	0.12	.1928

[1]Data reported as least squared means, unless otherwise indicated.
[2]Medical costs divided by average weight during the receiving period.
[3]Quality grade: 1=Prime; 2=Choice; 3=Select; 4=Ungraded beef or no-roll.

specifically to achieve a 0.91 kg/d ADG and was fed at a higher DMI level for a longer period (28 d vs 42 d).

The statistical model used in the analyses of these animal performance data included a block for weight class (WTC). Although not of primary interest in this study, this block contained and revealed some predictable results. For TG, WTC as a blocking factor was only numerically improved (P=.1220); performance among the L block was greater (L=42 kg vs H=40 kg). For ADG, an identical pattern was observed also favoring the L class (L=1.0 kg/d vs H=0.9 kg/d).

In order to report on the theoretical synergistic effect of disease and stress on animal performance a combined variable was created, COSTAVWT. This was calculated by determining the average weight on feed during the receiving period (final shrunk weight on d-42 less INWT). Then, total medical cost for each animal was divided by individual animal average weight during the 42-d period. This was an attempt to remove some of the variation in animal size (WTC), and to somehow standardize the effects of performance, stress, and sickness. Some variation remained however, due to the effect of load (n=7); the response within this factor was different (P<.0001) which was similar to the response observed for total gain and ADG. The statistical effect of this variable was that neither WTC (P=.6229) or TRT (P=.1293) were significant. Likewise, when a variable was created to report medical costs on per unit of gain basis (COSTGN), WTC (P=.1161) and TRT (P=.8230) were not significant.

The number and percentage of cattle that were determined to be afflicted with some level of BRD by the veterinarian are reported in Table 9 (% Sick, 0-42d). No significant differences were observed among either WTC (P=.7645) or TRT (P=.2829). Medical

costs were decreased (P=.0802) by vitamin E supplementation. From CON to E28, costs incurred due to respiratory disease were decreased by 28.9% or $1.41/hd treated with antimicrobial drugs. When load x WTC x TRT was used as the error term, medical costs due to TRT were statistically important (P=.0802), but still not significant. On an annual and industry-wide basis, if we could consistently reduce the incidence of respiratory disease by an equivalent amount, the savings might be great. Annual costs to cattle producers from respiratory diseases approach $500 million (NASS, 1996). A reduction by an amount similar to these observations would equal a $62 million saving. These results are generally in agreement with those of Gill et al. (1986), that the addition of vitamin E to the diets of stressed feedlot cattle tends to reduce the incidence of observed sickness.

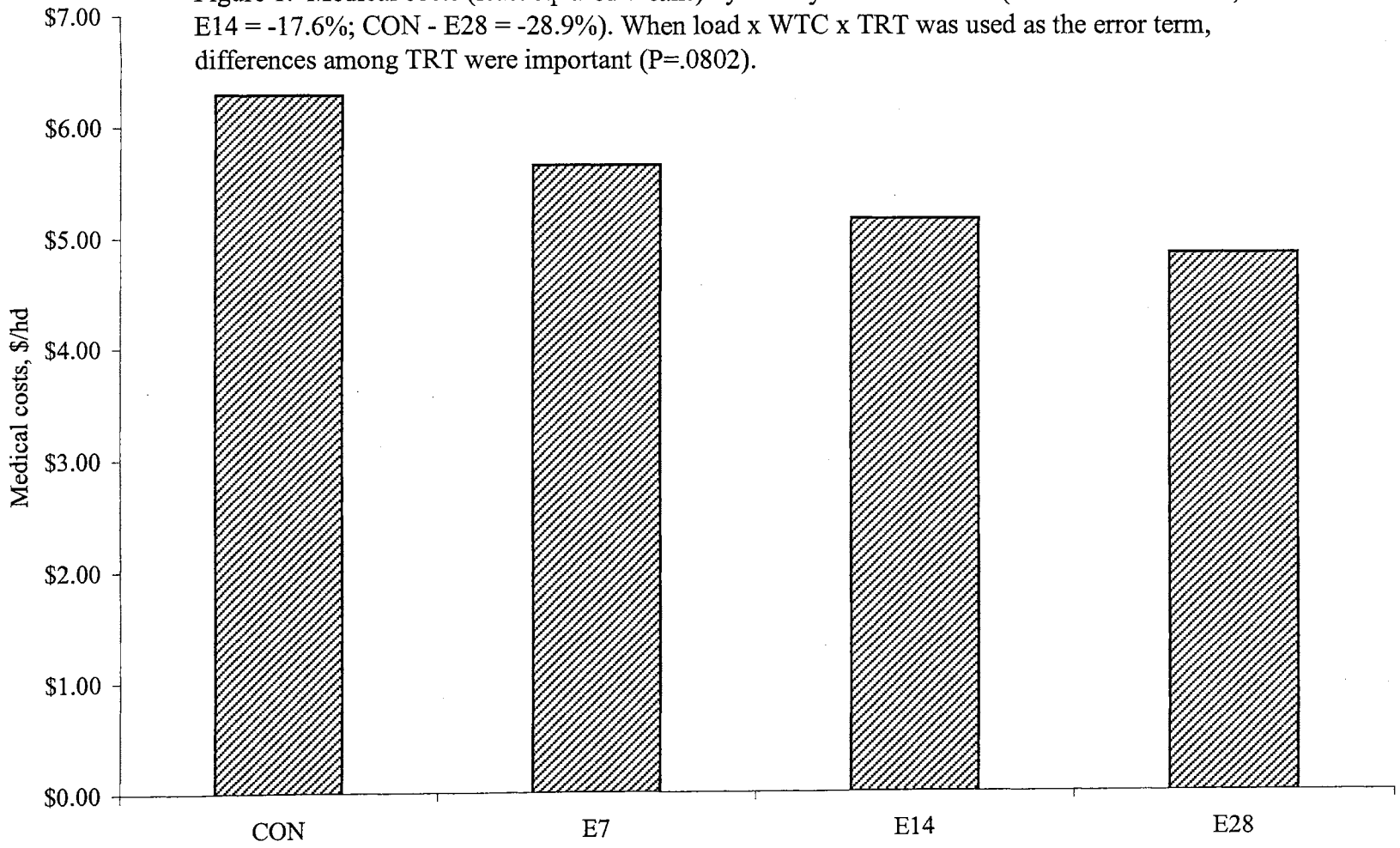
A majority of the calves in this study was observed as sick in the first ten days of the receiving period, similar to results observed by Lofgreen et al. (1975). In our study, on average the first drug treatment was given on d 3, the second on d 9, and all subsequent drug treatments were given on or after d 19. Though no statistical difference was found, CON received their second drug treatment more than three days later and their third drug treatment more than eleven days later than E28. This improvement in "recovery time" induced by our dietary supplementation with vitamin E, especially the E28 level, may indirectly affect animal performance by helping these animals return to a more ideal pattern of feed consumption, and thus a positive level of performance sooner. Lofgreen et al. (1975) found that fewer calves required drug treatment when they were fed a diet with as much as 72 percent concentrates compared with calves consuming a 55 percent concentrate diet. Consequently, however, the calves consuming the 55 percent

concentrate diet required more drug treatments per hd, thus increasing their overall cost of production.

In our study, not only was the incidence of sickness among calves recorded, but the number of drug treatments required per hd were also recorded and analyzed. Those results are also found in Table 9. The number of drug treatments per hd was similar among our dietary treatments (CON=0.93 vs E28=0.78; P=.1411). This decrease is graphically depicted in Figure 1. The difference in medical cost savings (Figure 1) from CON to E28 was $1.41 per head. The cost to provide 2000 IU of vitamin E in the diet for 28 days was approximately one dollar per head. The cost of vitamin E supplements has recently become more affordable, thus the opportunity to include this nutrient in the diet of stressed feedlot cattle seems more practical. The net savings provided by E28 in this study was $0.41/hd. Though this amount may seem trivial, on an industry-wide basis it could have a significant impact on producers' profitability.

Another variable considered regarding animal health was the number and percentage of cattle that received either zero (Med0), exactly one (Med1), or more than one (Med>1) drug treatments per hd (Table 9). No significant differences (P=.2232) were observed in these variables among our dietary treatments. As with other variables, load again accounted for some of the variation in our model (P<.0001) for Med0 and Med1, while Med>1 differences (P=.0052) were not as great. A two-way (WTC x TRT) and a three-way (load x WTC x TRT) interaction occurred for Med1 (P=.0208 and P=.0201, respectively). However, a larger percentage, nearly 23 and 25 percent more than CON and E7, of animals in E28 received no drug treatment during the 42-d receiving period. Therefore, E28 may provide some greater level of protection for the entire duration of a

Figure 1. Medical costs (least squared means) by dietary treatment level (CON - E7 = -10.9%; CON - E14 = -17.6%; CON - E28 = -28.9%). When load x WTC x TRT was used as the error term, differences among TRT were important (P=.0802).



receiving period. Garber et al. (1996) reported results similar to the results found in our study, in that performance was not changed but immune response was improved when a high level of vitamin E was added to the diets of beef steers.

Although individual animal weights could not be obtained prior to slaughter, an estimation of performance in the finishing phase was made based on the actual dressing percentage (DP) of total weights obtained from Farmland National Beef. As previously mentioned, at the writing of this thesis, carcass data from only two of the seven loads of cattle have been obtained. Therefore, the following results represent only approximately one-quarter of the cattle initially received at the WSBRC. An estimate of the final live weight prior to carcass harvest (EFLW) was calculated by dividing the carcass weight (HCW) by the overall dressing percentage (DP). The EFLW was similar (490.2 ± 6.9 kg) among all dietary treatment levels in our study. However, H cattle had heavier (P=.0915) EFLW (502.2 kg) than cattle in the L class (476.3 kg). Performance in the feedlot (EFLADG) was also estimated using the EFLW and the known days on feed (DOF). Only a WTC x TRT interaction (P=.0274) was observed for this variable. Data for both of these variables are reported in Table 9.

Carcass Characteristics. Gross carcass data (carcass weight [HCW], quality grade [QG], and yield grade [YG]) were obtained on 185 heifers originally received as Loads 3 and 6 at the WSBRC (Table 1). The results are shown in Table 9. A WTC difference was observed for HCW; H (326 kg) carcasses were larger (P=.0915) than L (309 kg) carcasses. No differences were observed among WTC or TRT for QG; 51.1% of all carcasses graded Choice or higher. Although WTC alone did not affect YG results, a

significant WTC x TRT interaction (P=.0475) was observed. Among all carcasses graded in this study, YG averaged 2.4 and was numerically higher from CON (2.3) to E28 (2.5).

Carcass data were arranged in a format to display the frequency of QG and YG among the four dietary treatment levels (Table 10). Though these data were statistically analyzed, no differences were found. In 1996, according to the American Meat Institute in Washington, DC, 60.4 percent of all carcasses graded in the United States during that year were given a QG of low Choice, or better. Thirty-seven percent of the carcasses graded received the Select QG, and only 2.4 percent of carcasses graded were graded as Prime (Carter, 1998). Comparatively, our carcass data do not appear to represent the industry average distribution. The number of carcasses graded inferior to Select and with a YG of greater than 3.0 would certainly have a detrimental effect on carcass value. It is interesting to note that 20% of carcasses in E28 were given a YG of 4, while CON and E7 treatment groups produced only 1% YG 4 carcasses. Further investigation may be required to determine if vitamin E can impact YG, and ultimately carcass value.Stovall et al. (2000) observed a negative relationship between marbling score, and thus carcass value because of one or more than one treatment with an antimicrobial drug. Carcass values were reduced by nearly $20/hd when heifers in their study received more than one drug treatment. Carcass values were not calculated in our study. However, because our data revealed no statistical differences in the attributes that would effect carcass value, no differences in carcass value would be expected. To exclude any possibility of the relationship observed by Stovall et al. (2000) among the data in our study, a regression comparison was developed to evaluate the potential of the contributing variables. The test was conducted using QG and YG as dependent variables.

Table 10. Distribution of carcass quality and yield grades by dietary treatment.

	CON	E7	E14	E28
Quality Grade				
Prime, n (%)	2 (4.4)	--	--	1 (2.2)
Choice, n (%)	22 (48.9)	23 (54.8)	24 (52.2)	19 (42.3)
Select, n (%)	18 (40.0)	18 (42.8)	19 (41.3)	23 (51.1)
Ungraded, n (%)	3 (6.7)	1 (2.4)	3 (6.5)	2 (4.4)
Premium Brand[1]				
FAB, CAB, n (%)	7 (15.5)	7 (16.7)	7 (15.2)	6 (13.3)
Yield Grade				
1, n (%)	--	--	6 (13.0)	3 (6.7)
2, n (%)	31 (68.9)	32 (76.2)	22 (47.8)	26 (57.7)
3, n (%)	13 (28.9)	9 (21.4)	13 (28.3)	7 (15.6)
4, n (%)	1 (2.2)	1 (2.4)	5 (10.9)	9 (20.0)

[1]Premium Brands include Farmland Angus Beef (FAB) and Certified Angus Beef (CAB).

The number of drug treatments per hd were the independent variable. Neither model was significant, nor was any linear, quadratic, or cubic relationship was detected. Table 11 does however present QG data distributed by frequency of drug treatment. Although the linear regression model was not significant (P=.1637), a trend is evident in this distribution that may be in direct contrast to the results of Stovall et al. (2000). The data suggests a higher frequency of Choice or better carcasses as the number of drug treatments increases to exactly one, or more than one drug treatment per hd. This difference is likely not real due to the relatively few number of carcasses graded (n=178) in comparison to the more than 700 health records reported and analyzed.

Serum Cholesterol. Vitamin E is a fat-soluble vitamin and as with many other biological compounds, requires a lipid carrier for transport throughout the body.

Lipoproteins, or serum lipids, are molecules with variable proportions of both lipid and proteins (Lehninger, 1993). We hypothesized, based on previous physiologic work by Han et al. (1999) studying the mechanisms of tocopherol metabolism, that the decrease in serum antioxidant concentrations (α-tocopherol) may coincide with, or even be precluded by a decrease in serum lipid values when animals are exposed to stress and disease.

Total serum cholesterol is determined by measuring the gross amount of cholesterol found in circulating lipids, such as LDLs (low-density lipoproteins), VLDLs (very low-density lipoproteins), HDLs (high-density lipoproteins), chylomicrons, and other such lipid molecules (Ravel, 1984). Cholesterol is a major component of LDL molecules, and is also found in HDLs and VLDLs. The level of difficulty and expense to measure serum LDL is high. Therefore, laboratory methods to measure total serum cholesterol have

Table 11. The relationship between the number of medical treatments and quality grade (QG)[1].

Number of Medical Treatments	Quality Grade			
	Prime	Choice	Select	Ungraded
0	2 (2.4%)	38 (44.7%)	41 (48.2%)	4 (4.7%)
1	--	39 (52.7%)	30 (40.5%)	5 (6.8%)
2	1 (5.9%)	9 (52.9%)	7 (41.2%)	--
3 or more	--	2 (100%)	--	--

[1]This relationship was statistically tested using a regression model. No significant ($P<.05$) linear ($P=.1637$) or quadratic ($P=.3776$) relationship was found. Similarly, no significant relationship was found with regard to yield grade (YG).

been widely used over time as an accurate and reliable indicator of serum lipid concentration (Ravel, 1984). To measure and quantify serum lipid values in our study, we utilized such a procedure (Sigma Diagnostics, Procedure No. 352). The accuracy and reliability of a procedure such as the one used in this study was verified against published literature (Havel et al., 1955; Friedewald, et al., 1972; Allain et al., 1974; Ravel, 1984; Jenkins, et al., 1988); the procedure was found to be valid and reliable.

Results of serum sample analyses are shown in Table 12. Among the cattle in our four dietary treatment levels, quantification of serum total cholesterol indicates no significant differences. However, when the results are considered over time (0, 14, 28, or 42 d), the reduction of serum cholesterol is severe and significant (P=.0001). Figure 2 shows the pattern by dietary treatment level over time. The means of all samples indicate that these cattle had cholesterol values well within the normal range (60-240 mg/dl for juvenile bovine) (Jenkins et al., 1988) on d 0. However, a sharp decrease was observed in average values by d 14. Although the means indicate that only animals in the E14 treatment group fell below the minimum threshold level, in reality some animals in all treatments reported values well below 60 mg/dL. Some recovery of cholesterol values was observed by d 28 and d 42. However, the final values on d 42 were still substantially lower than values at d 0.

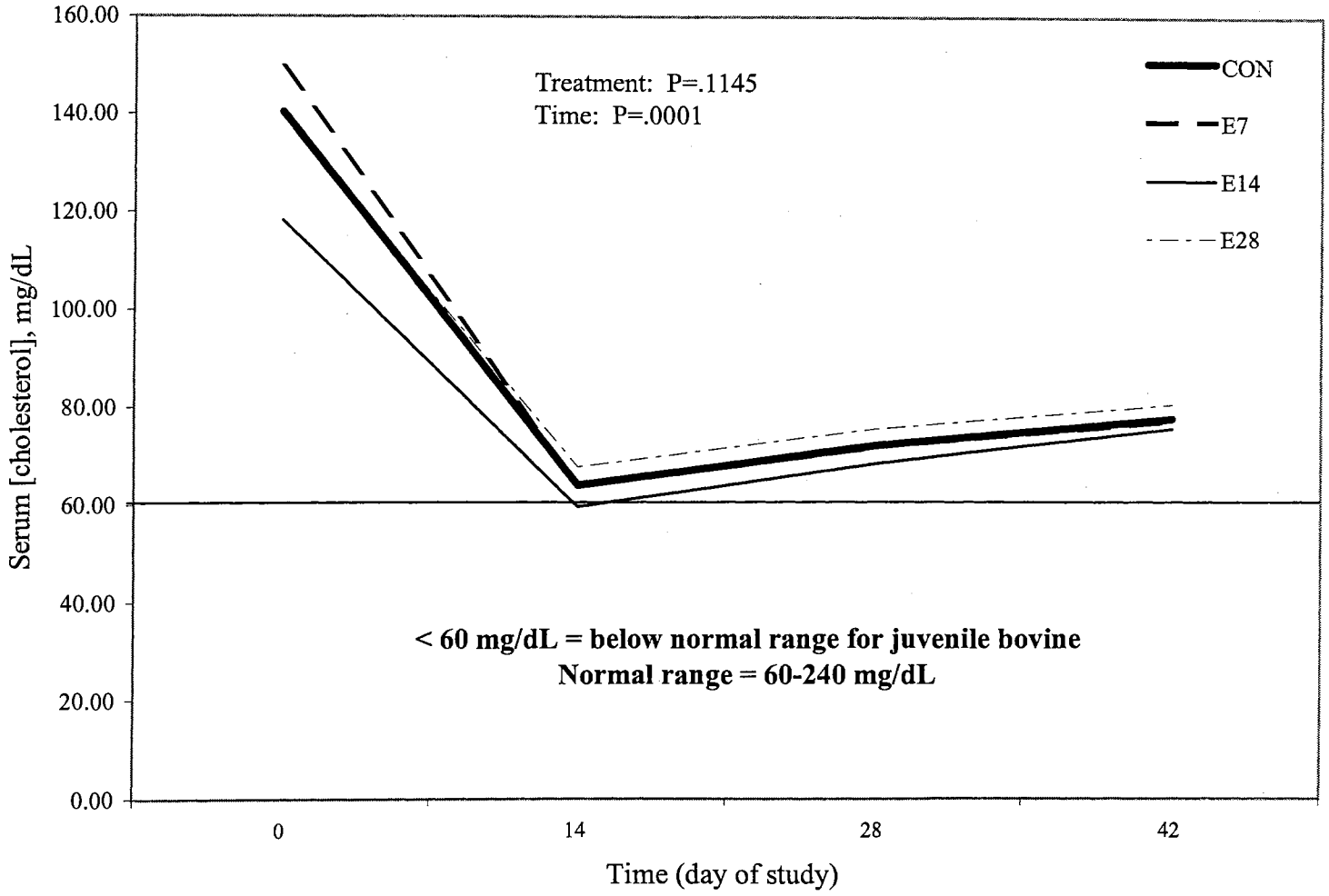
Serum Vitamin E. The normal serum concentration of vitamin E or tocopherol in cattle is variable depending on diet (Caravaggi, 1969; McMurray et al., 1983). Han et al. (1998) described various levels of tocopherol status in cattle: < 2.0 μg/mL = deficient; 2.0 – 3.0 μg/mL = marginal; 3.0 – 4.0 μg/mL = minimal, but adequate. These values agree with those reported by Gill et al. (2000) from previous years' experiments. Results

Table 12. Results of laboratory analyses for serum concentrations of cholesterol and vitamin E[1].

	Treatments					TRT	Time
	CON	E7	E14	E28	S.E.M.	Pr > F	Pr > F
Cholesterol-d0, mg/dl	130.35	129.48	118.09	128.09	3.51	.9596	
Cholesterol-d14, mg/dl	61.03	61.33	60.34	63.96	3.52	.9489	†
Cholesterol-d28, mg/dl	66.24	66.65	66.69	70.30	3.52	.9719	†
Cholesterol-d42, mg/dl	78.51	80.54	76.95	82.61	3.52	.9289	†
Vitamin E-d0, mg/ml	5.72	4.95	5.05	5.22	0.76	.4492	
Vitamin E-d28, mg/ml	1.11[a]	1.20[a]	1.48[a]	3.38[b]	0.22	.0001	†
Vitamin E-d42, mg/ml	1.32	1.46	1.47	1.67	0.16	.1860	†

[1]Results taken from PROC MIXED analysis and represent least squared means (error term: load x WTC x TRT).
[a, b]Means within a row with common superscripts do not differ.
†Means within a column differ (P=.0001) from values at d0.

Figure 2. Serum [cholesterol] measured by dietary treatment level at four time intervals.



of serum vitamin E analyses from our study are reported in Table 12. In support of our hypothesis expressed in the previous section, the pattern of serum vitamin disappearance is similar to that of serum cholesterol (Figure 3). Among the cattle sampled at d 0, no significant differences were observed among WTC or TRT. Subsequently, E28 had higher (P=.0001) α-tocopherol concentrations by d 28 (TRT was significant when load x WTC x TRT was used as the error term) than other treatment groups, especially CON (CON=1.08 μg/mL vs E28=3.37 μg/mL; P=.0001); these results were similar to results published by Garber et al. (1996). By d 42, the serum concentration α-tocopherol of had decreased to levels similar (P>.05) to CON, E7, and E14; no differences were observed among WTC. Load was once again significant (P<.005) at every measurement point suggesting that previous management and nutritional status likely had a direct impact on α-tocopherol concentrations once cattle were confined in the feedlot. Considering these higher serum α-tocopherol concentrations for E28 relative to medical treatment cost and the incidence of respiratory disease, E28 may provide a "zone of protection" against at least a portion of the detrimental effects of stress and disease. The shape and magnitude of this "zone" is graphically represented in Figure 3.

The relationship between total serum cholesterol and serum vitamin E was tested using a statistical regression model in SAS® (1996). The results of these comparisons are shown in Figure 4. Dependent variables were set as Lip0 (concentration of cholesterol on d0), Lip28 (concentration of cholesterol on d28), and Lip42 (concentration of cholesterol on d42) and these were compared to the respective concentrations of α-tocopherol. Linear and quadratic responses were examined. For Lip0, the linear response was significant (P=.0001) indicating a strong and direct linear relationship. Similar results

Figure 3. Serum alpha-tocopherol concentration measured by dietary treatment level at three time intervals.

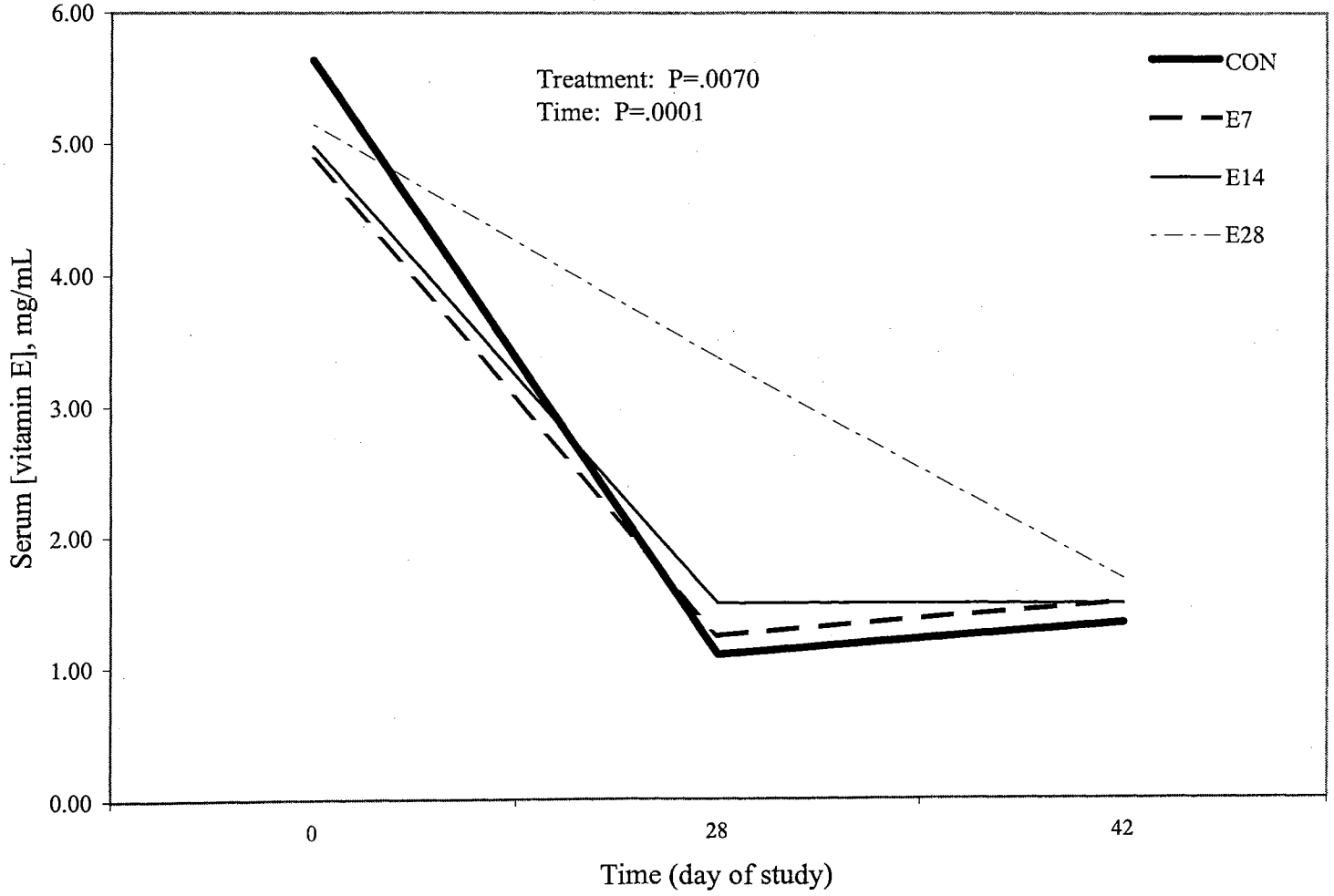
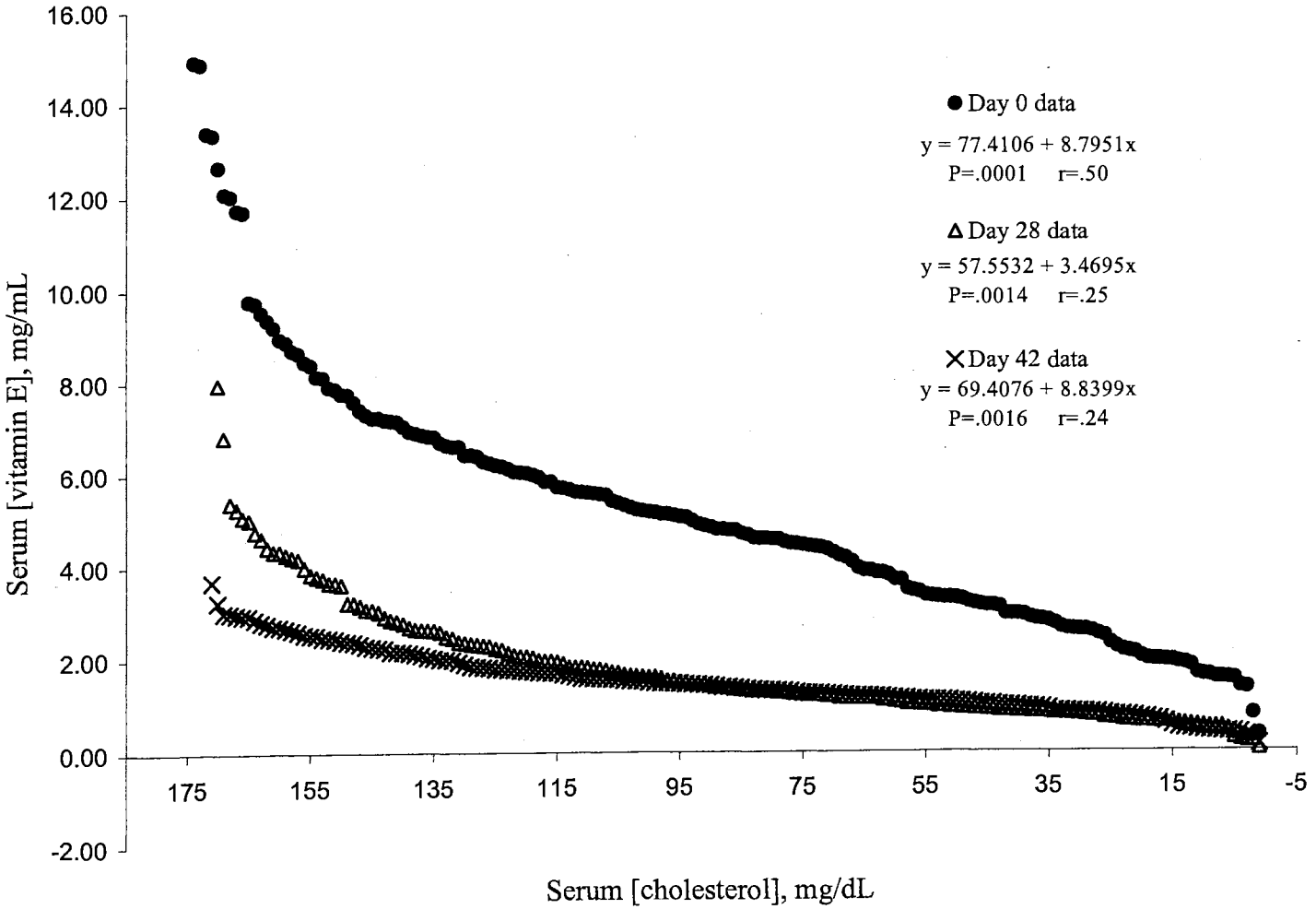


Figure 4. Regression of serum cholesterol values on serum alpha-tocopherol concentration at d0.

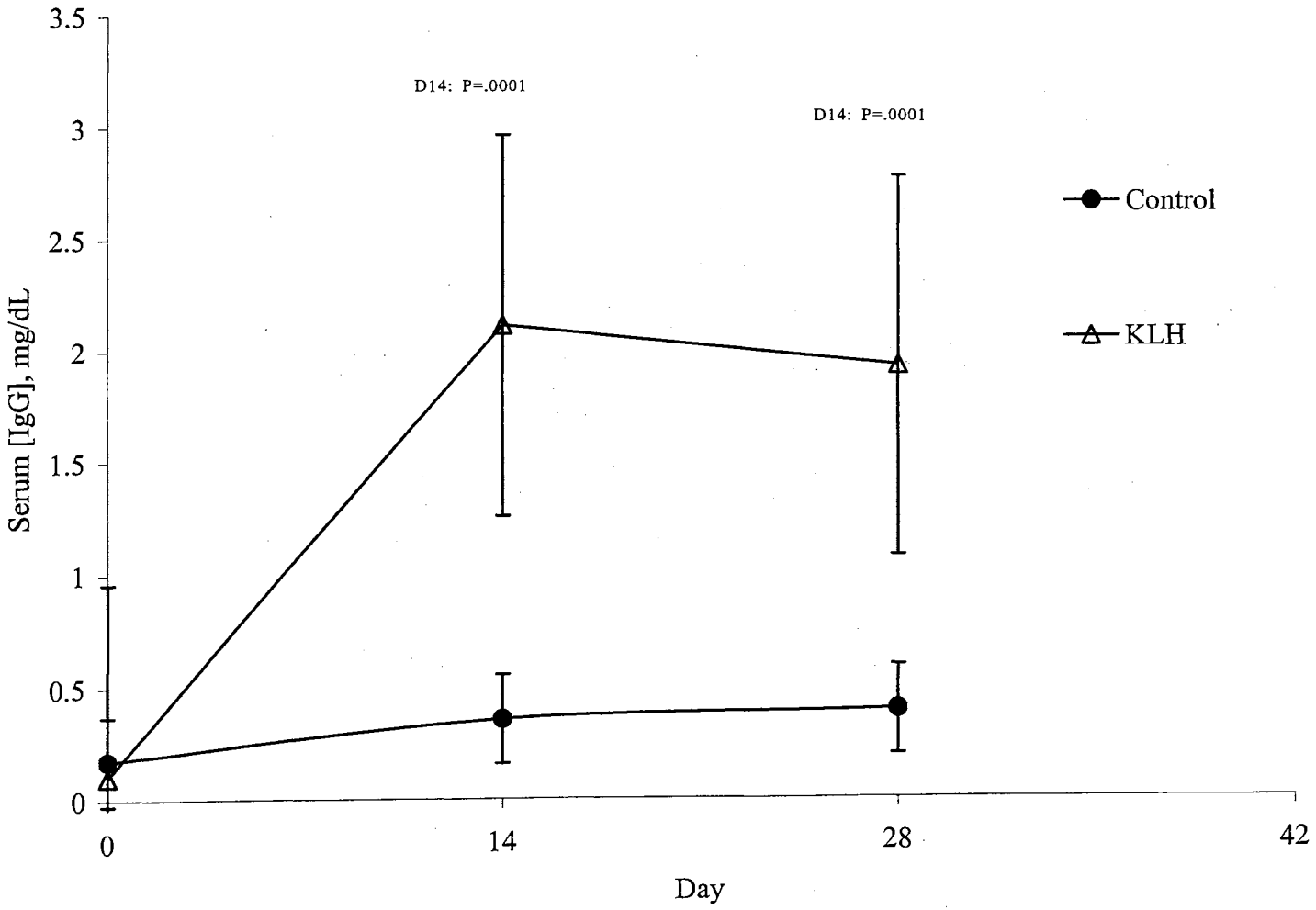


were observed for Lip28 (P=.0014) and Lip42 (P=.0016). These analyses clarify and enforce the hypothesized notion that serum vitamin E concentrations are reliant on some mode of transport, namely lipid molecules, to be carried throughout the body. One important question remains unanswered: how much vitamin E, or α-tocopherol is required physiologically, i.e., at the tissue level, or by immune cells such as macrophages to effectively stave off the negative effects of an infectious or inflammatory insult and promote a greater and more positive response by the immune system?

Other comparisons were made via regression analysis using serum α-tocopherol concentration values as the dependent variable and drug treatment as the independent variable. No meaningful linear, quadratic, or cubic relationships were detected using this analysis. A quadratic relationship (P=.0498), however, was detected when SICK (the number of sick animals per treatment group) was set as the dependent variable and E28 (serum concentration of α-tocopherol at d 28) was set as the independent variable. This relationship may simply indicate that of those animals categorized as sick by our criteria, serum α-tocopherol concentrations were lower than those of their non-sick counterparts. These results should not necessarily be interpreted as a predictive tool for the onset of infectious disease.

IgG Anti-body Response to KLH. The serum antibody responses of the cattle vaccinated against KLH in this study was significantly increased (P=0.0001) on d 14 and d 28. Control cattle at d 14 averaged only 0.37 mg/dL IgG, while KLH cattle averaged 2.13 mg/dL IgG (P=0.0001). On d 28, controls averaged 0.40 mg/dL IgG, and KLH vaccinated cattle averaged 1.94 mg/dL (P=0.0001). Figure 5 shows the magnitude of difference between these two variables at three different measurement points. These data

Figure 5. Serum IgG anitobody response to KLH.



strongly suggest that the immune systems of the cattle in this experiment were fully responsive to a foreign antigen, and thus considered functional. Further, these results add strength to other variables discussed (medical costs, RxTrtX) because of the immune system status proven by this test.

Conclusions

In this study, we did not realize any benefit among the traditional measures of animal performance, ADG, TG, DMI, or F/G, by adding a high level of vitamin E to the receiving diet of these cattle. Two factors may have impacted and even muted the performance expectations. First, these cattle may have had a history of adequate or a high plane of nutrition. If they had recently been grazing forage that was not drought stressed, or in some other way depleted of nutrients, their vitamin status would have been far from deficient. Second, after much consideration and review of the data from which significant statistical separation was not always clear, it may be that the control diet provided a sufficient quantity of vitamin E from natural and added sources. A small amount of supplemental vitamin E was added to the B-171 pellet; however, the majority of the vitamin E in the control diet occurred naturally in the feedstuffs utilized. The elusiveness surrounding the determination of an absolute vitamin E requirement again presents itself. It may be that this absolute requirement is a moving target among cattle types and the determination of an actual requirement may be futile. A better approach may be to find an optimal level and ascertain a reasonable amount by which to provide excess during stressful conditions or when disease may be preeminent, especially with consideration to the monetary value of such profusion.

The direct costs associated with the treatment of respiratory disease in cattle remain large. Indirectly, this disease complex may be even more costly as it affects animal performance and can ultimately even deplete the economic salvage value of some chronic animals. Addition of supplemental vitamin E in this study effectively decreased the direct costs associated with antimicrobial drugs by as much as 28.9 percent. On an industry-wide basis, this could amount to an overwhelming improvement to the bottom line of many beef producers. As the global population has grown to more than six billion people, more food resources are and will be required to meet the demand for sources of protein. Improving the industry-wide scope of beef production is essential.

Among the carcass data evaluated in this study, none of the economically important carcass characteristics were improved by dietary supplementation with vitamin E. However, data does exist that indicates vitamin E and other natural and synthetic antioxidants can help to improve shelf life, red-meat color, and thus consumer acceptability of beef products. Indirectly, vitamin E in beef cattle diets may have an impact on carcass quality and value by improving the health of the animal during the feeding phase. The data in this study indicated otherwise. However, these data are likely inconclusive due to the small number of carcasses evaluated in relation to the number of animal health records analyzed.

It seems clear that there is a significant physiological response of both serum lipid and vitamin E concentrations in response to stress and disease. The decrease in serum lipid concentrations in this study from d 0 to 14 was not affected by dietary supplementation with vitamin E. In an experiment subsequent to our study, this same diet was supplemented with fat to determine if this severe physiologic decrease in lipid

concentration could be manipulated. Health records were again kept to determine if the immune response was improved by a greater availability of vitamin E to tissues and cells. The results of that study are not yet available. The differences in serum α-tocopherol concentrations in this study were significant. Based on normal serum values described, serum concentrations of α-tocopherol from cattle in all experimental treatment groups fell below the deficiency threshold. Since the majority of disease occurred between d 0 and d 14 in this study, serum α-tocopherol appeared to recover in conjunction with a decrease in the incidence of BRD after d 14. When a high level of vitamin E remained in the diet during beyond 14 d , as in the E28 treatment group, a reduction in medical costs was observed. Regardless of any dispute that may surround the reliability of the E28 results for serum α-tocopherol concentration, the concentrations of serum α-tocopherol for the E7 and E14 treatment groups were both improved over CON at their lowest point. Further investigation of these physiological responses during stress and disease will be required to adequately explain these patterns of change.

Vitamin E appeared to improve the overall health status and responsiveness of the immune system of the cattle in this study. Although previous nutritional status of an animal entering the feedlot can have an affect on the influence that a vitamin supplement may have on performance or animal health, this information may not always be accessible. Therefore, it seems reasonable and prudent to include vitamin E at some level, preferably above the stated minimum requirement, in the receiving diets of all feedlot cattle.

Implications

Research has provided evidence that supplemental vitamin E can be beneficial to stressed and disease-prone feedlot cattle. Efficacy of vitamin E supplementation may be a function of prior nutritional status combined with management induced stress and exposure to unfamiliar animals and pathogens. In the present study, an attempt was made to describe the physiological aspects of α-tocopherol flux due to stress and disease. Further investigations and analyses are required to determine how the body and immune system uses α-tocopherol during stress events.

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CHAPTER IV

BENEFITS OF FEEDING A PELLETED SUPPLEMENT MANUFACTURED FROM NORTH ATLANTIC SEAWEED TO TRANSIT-STRESSED FEEDLOT CATTLE

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ABSTRACT

An experiment was conducted to examine the effects of a novel dietary supplement (Tasco™) fed in the receiving period on animal performance, the incidence of bovine respiratory disease (BRD), and medical costs. Carcass data were also collected and analyzed. Mixed breed heifers (mostly British crosses) from southern Oklahoma and northern Texas auction barns were used (220 kg initial BW). In this experiment, feed was delivered once daily to each pen during the receiving period; cattle were fed ad libitum. Tasco™ was included in the diet at the rate of 0.27 kg/hd/d for the first 14 d only. Experienced veterinary personnel evaluated all cattle each day for signs of respiratory and other diseases; all incidences of disease and antimicrobial treatments were closely monitored and recorded. No differences were observed in daily gains, total gain and feed conversion between the Control diet and the diet supplemented with Tasco™. Estimates of daily gain in the finishing phase were also similar among the two dietary treatments. Tasco-fed cattle required more treatments with antimicrobial drugs, and thus medical costs overall were greater in these cattle compared to Controls. Tasco™ clearly did not improve the immune function and overall health response of the cattle in this study and provided only isolated benefits to animal performance. Among all carcass

characteristics including carcass weight, marbling score, fat thickness, rib-eye area, kidney, pelvic and heart fat, yield grade, quality grade, and carcass value no significant differences were observed as a result of our dietary treatments.

Key Words: Tasco™, Seaweed, Shipping Fever, Feedlot

Introduction

Animal agriculture has been criticized recently of contributing to the development of resistant strains of bacteria by either over-use or inappropriate use of antimicrobial drugs that ultimately could effect human populations (Angulo et al., 1999; Copeland et al., 2000). Therefore, developing other means of minimizing the effects of BRD may be prudent.

Many dietary supplements are now considered effective at improving the immune response. One particular product receiving attention in animal nutrition recently is seaweed meal. The utilization of marine plants is not new to this millennium. Hoie and Sandvik (1956) fed seaweed meal in place of grass meal to chicks and found it to be a better source of riboflavin and an adequate source of iron for egg production. Seaweed meal also impacted the turkey industry in Canada when seaweed supplements significantly reduced mortality in young poults (Thivy, 1960). Patil (1960) clarified the many nutritional benefits of seaweed; in countries where competition for food among man and beast is great, the motivation to make use of alternate sources of nutrients for livestock should be great, as well. Arzel (1984) noted that seaweed use along European coastal regions was so common that some sea plants came to be known as "cattle-weed."

However, little empirical data are available with regard to the effects of seaweed in ruminant nutrition.

Numerous researchers (Booth, 1965; Brain et al., 1973; and Abetz, 1980) have suggested that commercial seaweed products contain kinins as an active constituent. Kinins are considered a subset of many mediators that contribute to the inflammatory response (Swenson and Reece, 1993) and could have a negative impact on the immune system.

The objective of this research was to determine the effects that a novel supplement (Tasco™) manufactured from North Atlantic seaweed might have on animal performance and response to the normal challenge of BRD induced by co-mingling and transit.

Materials and Methods

Mixed breed beef heifers were purchased by order buyers in several southern Oklahoma and northern Texas auction barns in late October 1999. They were trucked to a facility near Purcell, Okla., held overnight and subsequently organized into load lots the next morning. Two semi-truck loads of heifers (n=175; initial BW=208 kg) were then delivered to the Willard Sparks Beef Research Center (WSBRC) in Stillwater, Okla. On arrival, cattle were held in a return alley for approximately 1 h to rest and acclimate to their new surroundings. The cattle were then pre-processed during which individual weights were obtained and individually numbered ear tags were applied. The cattle were allowed to rest overnight before initiation of the study the next day; feed and water was provided (.90 kg/hd of prairie hay and 1.4 kg/hd of the control diet described in Table 1). Cattle were randomly and evenly distributed to eight pens; four pens received the Control

Table 1. Composition of control diet on a dry matter basis[a]

Ingredient	%DM
Soybean hulls	33.0
Corn, whole shelled	26.5
Wheat middlings	16.9
Supplement[b]	13.6
Cottonseed hulls	10.0

[a]Crude Protein = 14.8%.

[b]Supplement composition: Cottonseed meal 55.5%, soybean meal (47.5%) 31.5%, limestone 8.75%, pellet partner 5.0, salt 1.75%, vitamin A (30,000 IU/gm) .14%, vitamin E-50 Adsorbate .02%, Bovatec 68™ .17%, selenium (0.02) .08%.

diet and four pens received the Control diet plus Tasco™ (Acadian Seaplants, Ltd., Dartmouth, Nova Scotia, Canada). On d 15, all cattle received the Control diet only for the remainder of the 42-d receiving period. Tasco pellets consisted of 50% seaweed meal and 50% wheat middlings. Tasco was added to the Control diet and fed to one-half the cattle (4 pens) for the first 14 d only at the rate of 0.27 kg/hd (6% of total diet if intake was 4.54 kg/d). The amount of wheat middlings in the diet was decreased at low levels of feed intake and Tasco was substituted accordingly by percentage to maintain a constant intake level of the experimental product. Feed was delivered once daily to each pen; eight pens (12.2 m x 30.5 m) were used with 11-12 hd/pen. Cattle were started on feed at 2.27 kg/hd/d on d 0 and were fed ad libitum, but were required to have a slick bunk at 0600 each day to merit an increase in feed for that day. Hay was fed at .90 kg/hd for the first 5 d, then reduced by half on each of d 6 and d 7; no hay was fed after d 7.

At 0600 the day after arrival, cattle were processed and allocated to their treatment pens. Processing included vaccination for viral respiratory (BRSV-Vac 4™, 2 ml via intramuscular injection [IM]) and clostridial (Vision-7™, 2 ml via subcutaneous injection [SubQ]) diseases, as well as treatment for internal and external parasites (Ivomec-Plus™, 1.0 ml/110 lb SubQ). Viral respiratory vaccines were boostered on d 14. Cattle were weighed on d 0, 14, 28, and 42. Day-0 weights were averaged with off-truck weights and d-42 weights were obtained after an overnight shrink with no access to feed or water.

A hospital card was initiated for each animal that was either suspect or confirmed as morbid. Information recorded included identification, date, weight, rectal temperature, severity score, and treatment regimen engaged. This information was not made available to veterinary personnel making each day's evaluation of cattle for morbidity. Health

records were monitored by WSBRC personnel to determine if an animal had been previously treated. If so, eligibility for re-treatment was determined based on the label instructions of the relevant antimicrobial drug and the veterinary prescribed treatment regimen.

At the conclusion of the 42-d receiving period, cattle were transported to a commercial feedyard for finishing; cattle were on feed for an average of 190 d The finishing diet consisted primarily of high energy concentrates (corn, wheat, milo, or wheat middlings) with added roughage (chopped alfalfa hay and corn silage), animal fat, and a protein supplement that typically contained a vitamin and mineral pack, and monensin and tylosin. Once cattle reached their final target weight (determined by feed yard manager and cooperating producer), the cattle were delivered to commercial beef processing facility in Liberal, Kan., for carcass harvest. Detailed carcass data were collected, which included hot carcass weight (HCW), marbling score (MB), backfat thickness (FT), rib-eye area (REA), kidney-pelvic-heart fat (KPH), quality grade (QG), and yield grade (YG). Additionally, carcasses qualifying for premium beef programs such as Farmland Angus Beef (FAB) or Certified Angus Beef (CAB) were designated.

Statistical Analysis. Data were analyzed by ANOVA in a completely randomized design using ordinary least squares procedures of SAS (SAS Inst. Inc., Cary, NC). Sources of variation included load, dietary treatment (TRT), and a load x TRT interaction; load x TRT within pen was used as the error term. Differences for TRT were reported when tests of hypotheses (Type III mean square) using load x TRT within pen as the error term resulted in a probability of a greater F-value of less than .10 ($P<.10$). For daily gain (ADG), total gain and feed conversion (F/G) pen was used as the experimental

unit. Variables related to medical treatments, carcass characteristics, and carcass value were analyzed using animal as experimental unit.

Regression analyses were performed to examine the relationships between carcass value and total and daily gain in the receiving period, drug treatments in the receiving period, as well as estimated final live weight and daily gain in the receiving period. Linear, quadratic and cubic relationships were tested. When quadratic and cubic results were not significant (P<.10), those terms were omitted.

Results

Animal performance, health parameters, and carcass data results by dietary treatment are displayed Table 2. Cattle fed Tasco consumed less feed overall and gained at a rate similar to Controls, thus F/G was slightly improved (5.7 vs 5.5) in those treatments, but only numerically (P=.4252). Daily gains were lower (P=.3374) on average among cattle supplemented with Tasco; performance measured by total gain was similarly improved (P=.3374) among Controls.

The difference in medical costs was important ($4.35 vs $8.24; P=.0673), which coincided with the increased incidence of BRD (% Sick, 0-42d) in Tasco fed cattle compared to Controls (42.6% vs 65.9%; P=.1114). When the percentage of cattle receiving medical treatments was compared across dietary treatments, more cattle in the Control group required no treatment (P=.1119) compared with Tasco-fed cattle, whereas a greater percentage of cattle requiring two or more treatments (P=.0289) were among the Tasco-fed cattle. In total, AMT per head for Controls was lower (P=.0782) than cattle whose diets were supplemented with Tasco.

Table 2. Performance data comparing results from dietary treatments: Control compared with Tasco™.

	Control	Tasco™	S.E.M.	Pr > F
ADG, kg/d	0.87	0.80	0.05	.3374
Total Gain, kg	36.4	33.4	2.2	.3374
Feed conversion, F/G	5.7	5.5	0.4	.4252
% Sick 0-42d	42.6	65.9	9.7	.1119
Est. final live weight, kg	502.1	505.3	5.3	.6751
Est. feedlot ADG, kg/d	1.3	1.4	0.02	.6751
Medical costs, $/hd	4.35	8.24	1.38	.0673
AMT per hd	0.6	1.0	0.17	.0782
Med0, %	57.4	34.1	10.0	.1119
Med1, %	31.4	35.9	6.0	.6091
Med >1, %	11.2	30.0	5.0	.0289
Carcass Weight, kg	333.5	335.6	3.5	.6751
Marbling Score[1]	284.4	264.1	10.2	.1818
Fat thickness, in.	0.63	0.60	0.01	.2329
Ribeye area, sq. in.	14.2	14.4	0.16	.5869
KPH	2.1	2.0	0.04	.5338
Quality Grade[2]	2.7	2.8	0.07	.4548
Yield Grade	2.2	2.6	0.08	.8483

[1]Marbling Score: 200=Sl^{00-49} ($Select^{-}$), Sl^{50-99} ($Select^{+}$); 300=Sm^{00-99} (Ch^{-}); 400=Mt^{00-99} (Ch°); 500=Md^{00-99} (Ch^{+}); 600=$SlAb^{00-99}$ (Pr^{-}) (USDA, 1997).

[2]Quality grade: 1=Pime; 2=Choice; 3=Select; 4=Ungraded beef or no-roll.

Estimated final live weight and estimated feedlot ADG were only slightly greater among Tasco-fed cattle compared with Controls (P=.6751). Carcass weight (334.3 kg) was not different (P=.6751) among Controls and Tasco-fed cattle. Fat thickness (.62 in.) was not different (P=.2329) among our treatments, nor was marbling score (273.6; P=.1818), rib-eye area (14.3 sq. in.; P=.5869), KPH (2.1; P=.5338), QG (2.7; P=.4548), or YG (2.3; P=.8483).

Discussion

The increased level of morbidity and related medical costs experienced in both experiments is puzzling, especially when one considers the claims made in popular press and other recent reports regarding the ability of Tasco to improve the overall health and immune response of animals. As previously mentioned, little information exists in the literature to uphold, refute, or explain this claim. However, the kinin-like activity inherent in seaweed products discussed earlier in this chapter may indeed have a serious affect on the immune system, albeit negative.

Briner et al. (1979) indicated that other compounds in seaweed extracts exist to inhibit the cytokinin-like activity previously described. They suggest that the inhibitory factor could be related to the extremely high level of sodium or salt in most seaweed product. We analyzed the chemical composition of the Tasco supplement for sodium (Na) and chloride (Cl) concentrations. Table 3 shows the extremely high sodium and chloride analyzed values for Tasco compared to other feedstuffs used in the diets in this study.

Table 3. Results of Tasco chemical analysis for sodium and chloride: Comparison with other feedstuff values.

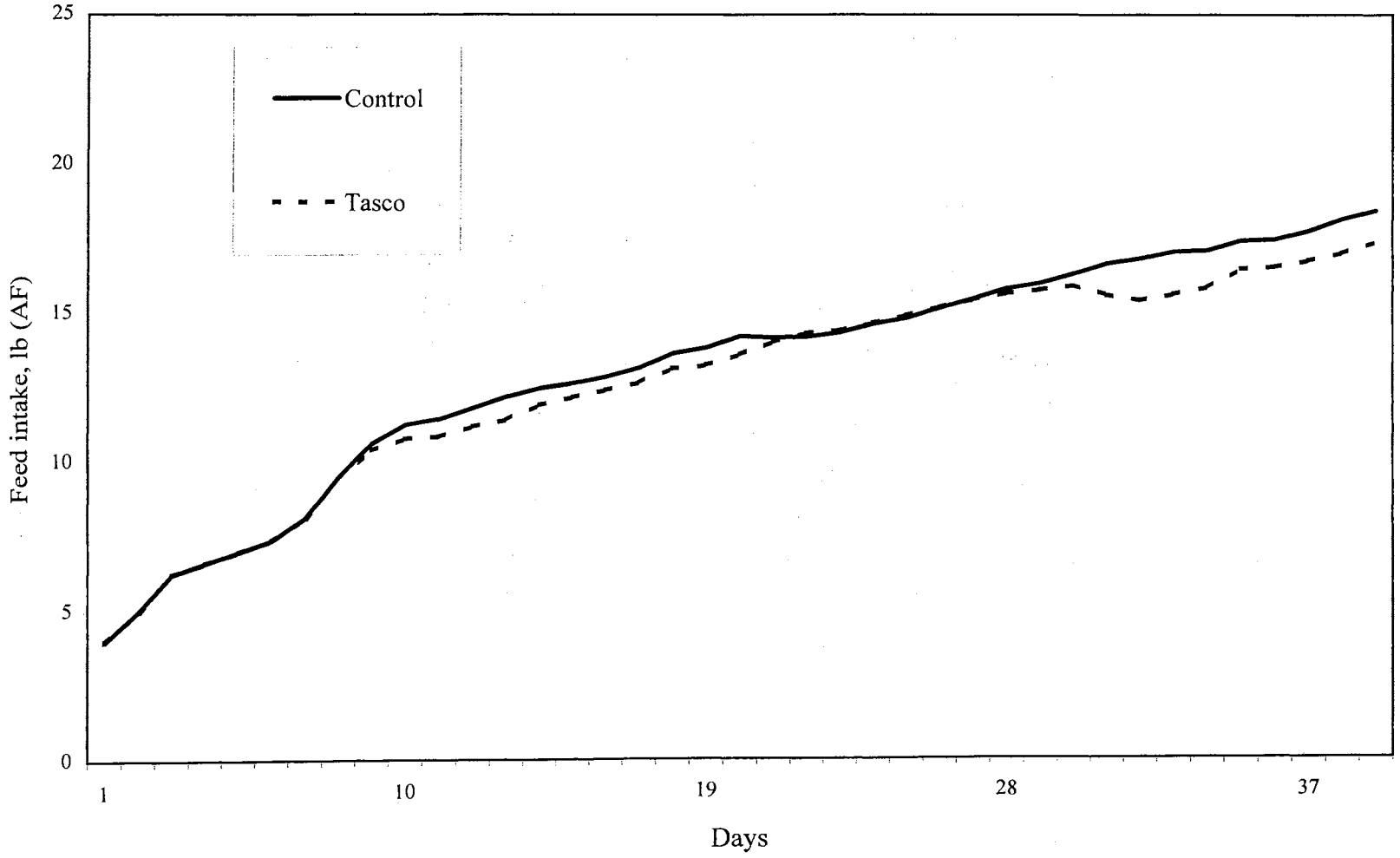
	Sodium (%DM)	Chloride (%DM)
Tasco	1.91	1.04
Corn, dry grain[1]	0.01	0.06
Cottonseed meal (43%)[1]	0.03	0.00
Soybean hulls[1]	0.03	0.00
Soybean meal (49%)[1]	0.01	0.08
Cottonseed hulls[1]	0.02	--
Wheat middlings[1]	0.01	0.04
Limestone[1]	0.06	0.03

[1]Tabled values from NRC, 1996.

Salt (NaCl) fed to cattle at high levels has been used to regulate feed intake (NRC, 1996). Further, cattle can usually tolerate rather high levels of salt in the diet, as long as a good supply of water is not limited. Figure 1 represents the pattern of feed intake of cattle in the two dietary treatments. As with many of the other results in this study, intakes were variable, though identical for the first approximately eight days. Tasco-fed cattle consumed less feed than Controls after d 10, and similar amounts from d 21 to d 27, after which consumption for the remainder of the 42-d receiving period dropped sharply, falling below the intake level of Controls. The high dietary concentration of sodium and chloride likely depressed feed intake and thus performance during the receiving period of these cattle.

Another observed, though unmeasured, phenomenon in this study was the incidence of diarrhea and a strongly acidic odor in and among the cattle consuming the Tasco diet. Possibly related to the salt content of the supplement and a logical increase in water consumption, no clear explanation exists in the literature relevant to feedlot cattle. LaManna et al. (1999) showed that high dietary salt concentrations increased percentages of some ruminal volatile fatty acids (butyrate and isobutyrate) in a linear fashion, thus leading to an increased susceptibility to acidosis and other digestive upsets. Orpin et al. (1985) provided a description of ruminal function and microbial changes when seaweed was fed to Orkney sheep. Their findings indicated qualitative, as well as quantitative differences in the microbial populations dominating the ruminal environments of seaweed-fed sheep compared with grass-fed sheep. Data regarding digestibility, either ruminal or total tract, were not provided; very few early digestibility studies have been performed (Woodward, 1951). Whittemore and Percival (1975) fed a seaweed residue to

Figure 1. Pattern of feed intakes during the receiving period.



pigs and found the nutritive value to be low and not beneficial to growth, but that it may provide some positive effect as a vitamin or mineral supplement. Some of the pigs in their study were withdrawn entirely due to acute diarrhea leading these scientists to conclude that seaweed is an excellent and effective laxative for pigs. Physiologically, they attribute the diarrhea to the low digestibility coefficients of the product, or the inability of the pigs to digest the unusual polysaccharides found in the seaweed. Woodward (1951) indicated that seaweed products, based on previous research, was likely more beneficial in the diets of ruminants than of horses and pigs. Most importantly, he proclaimed its use purely as a supplementary feed with predictive values for energy, vitamins, and minerals.

Orpin et al. (1985) described the chemical composition of sea plants to be very different from that of land plants. They indicated that while land plants have cell walls composed primarily of cellulose, hemicellulose, and lignin, the composition of sea plants' cell walls are made mostly of alginates with some cellulose and xylan, or xylaglucan. Storage polysaccharides of land plants are typically starch and fructosan, while storage forms in sea plants are mostly mannitol, fucoidin, floridean starch, and laminarian; laminarian is a β-1,3 linked glucan.

Carcass values (Table 4) were determined using a standard grid-pricing system for all carcasses to make these comparisons uniform and thus, more meaningful. Carcasses were sold on the same packer-pricing grid, harvested at the same facility, and generally treated and exposed to identical conditions. Slaughter dates were within one week of each other, making these comparisons logical and reasonable. Table 5 represents carcass data and shows the distribution of quality and yield grades between the two dietary

Table 4. Distribution of carcass value by dietary treatment.

	Control	Control with Tasco™
Carcass value[1]	$768.29 ± 84.72	$774.79 ± 82.13
Minimum (Min)	$580.16	$579.54
Maximum (Max)	$952.95	$957.38
Max - Min	$372.79	$377.84

[1]Carcass value based on real prices obtained during the week of June 19, 2000; values are reported as raw means plus or minus one standard deviation. Value was determined according to a single Farmland National Beef pricing grid and calculated by J.N. Carter in SAS®. Actual prices paid by Farmland National Beef may have been different.

Table 5. Distribution of carcass quality and yield grade by dietary treatment.

	Control	Tasco™
Experiment 1:		
Quality Grade		
Prime, n (%)	---	1 (1.2)
Choice, n (%)	33 (38.8)	29 (34.9)
Select, n (%)	46 (54.1)	42 (50.6)
Ungraded, n (%)	6 (7.1)	11 (13.3)
Premium Brand[1]		
FAB, CAB, n (%)	14 (16.5)	7 (8.5)
Yield Grade		
1, n (%)	3 (3.4)	3 (3.5)
2, n (%)	62 (69.7)	58 (67.4)
3, n (%)	8 (9.0)	12 (14.0)
4, n (%)	12 (13.5)	10 (11.6)
Ungraded, n (%)	4 (4.5)	3 (3.5)

[1]Premium Brands include Farmland Angus Beef (FAB) and Certified Angus Beef (CAB).

treatments. These figures were not examined statistically, but simply provide a reference from which subjective comparisons can be made on at least two parameters that can affect carcass values. With regard to carcass values, Table 6 displays the relationship between animal health during the receiving period and carcass values.

Previous research (Stovall et al., 2000) indicated that carcass characteristics, including monetary value, can be affected by the severity of disease experienced during the receiving period. A regression comparison was made comparing carcass value to the number of AMT received during the receiving period. Both linear and quadratic effects were tested; however, no significance was found in this relationship. Careful review of the data in Table 6 shows no clear and consistent pattern among these parameters.

In order to test the regression model used for sufficiency, comparisons between carcass value and estimated final live weight, as well as estimated feedlot ADG were made. These parameters were chosen because of the predictability of the expected results, and the accepted relationship between feedlot gain, final live weight of the animal, and gross carcass value. Both of these models resulted in significant linear responses ($P=0.0001$; both linear and quadratic were tested, quadratic was never significant ($P>0.10$) and was therefore removed from the model). Figures 2 and 3 display the linear relationships of these data.

Conclusions

In some cases, especially with regard to medical costs and health-related parameters, the results of Tasco supplementation were often more costly and severe. Morbidity was increased in Tasco-fed cattle over Controls by 54.6 percent. No improvements were

Table 6. The relationship between the number of medical treatments given and carcass value[1].

Number of Medical Treatments	Carcass Value ($/hd)	
	Controls	Tasco
0	769.01 ± 77.46	773.39 ± 71.17
1	770.65 ± 97.67	781.31 ± 85.23
2	766.29 ± 104.69	766.67 ± 90.86
3 or more	726.55 ± 30.64	790.03 ± 144.32

[1]This relationship was statistically tested using a regression model. No significant ($P<.05$) linear or quadratic relationship was found.

Figure 2. Regression of estimated final live weight on carcass value.

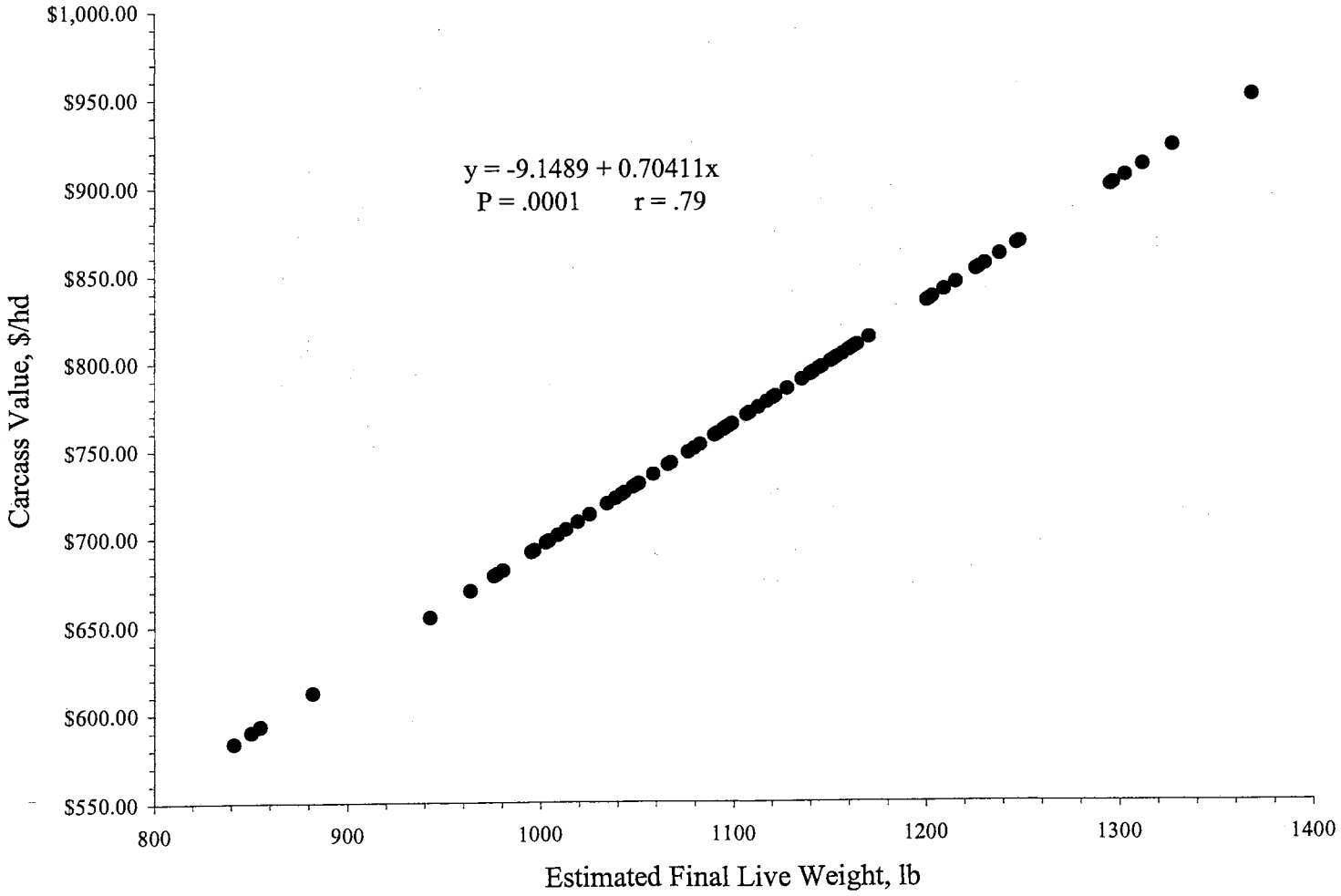
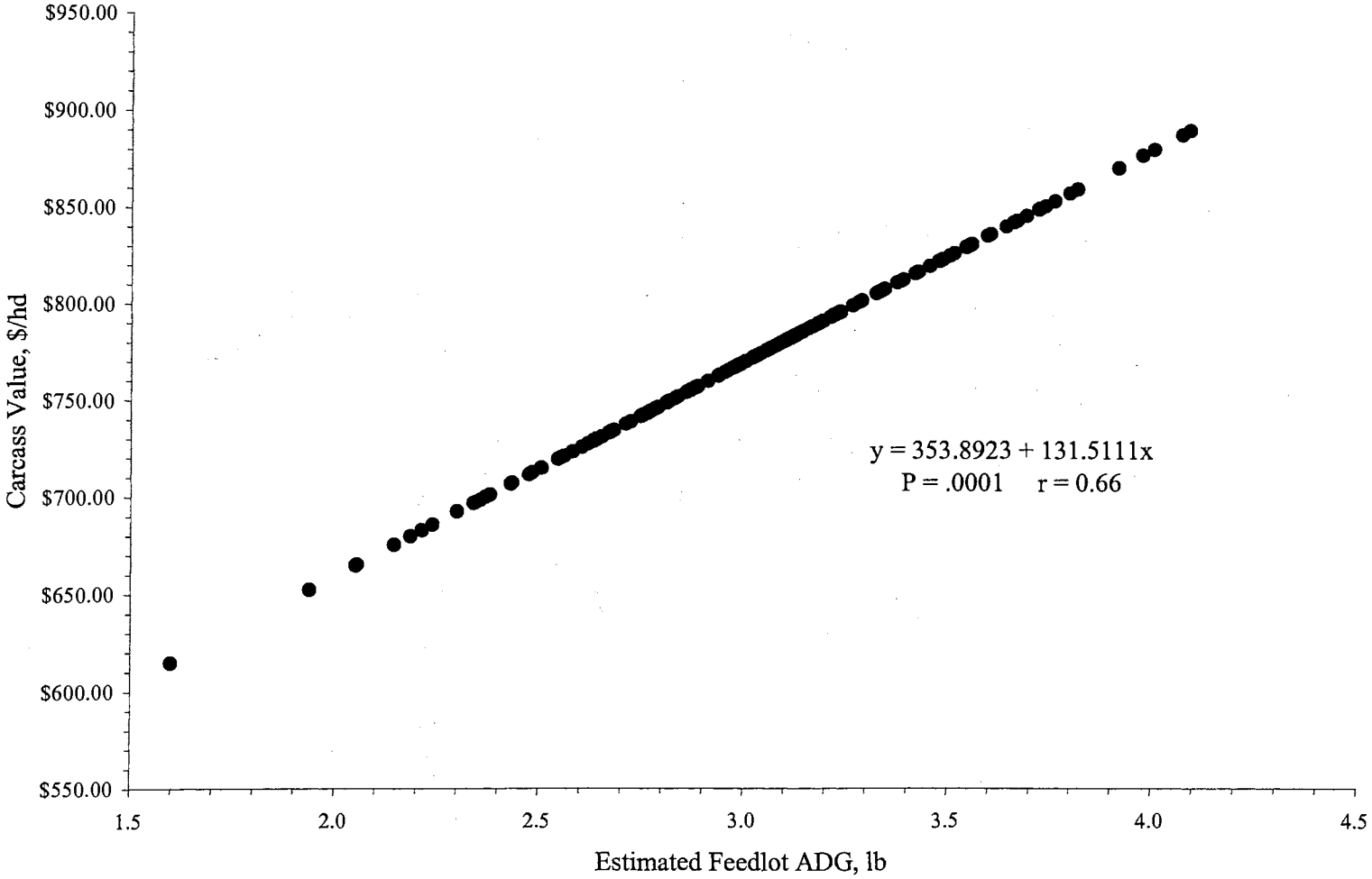


Figure 3. Regression of estimated feedlot ADG on carcass value.



observed regarding performance in the receiving period as measured by daily gain, total gain, and feed conversion among cattle fed the experimental diet compared with Controls. The data clearly show that the incidence of BRD was more frequent, and medical costs were greater in cattle supplemented with Tasco. Carcass marbling score or quality grade was not affected positively or negatively by Tasco. Carcass values did not appear to be largely different among our dietary treatments.

Implications

There is little evidence in scientific literature currently that conclusively shows consistent positive results from directly feeding Tasco at the levels used in the present study. Among unpublished sources, variable results have been observed. Based on the results of our study, this product was detrimental to the performance and health of these cattle. Further investigations and analyses are required in order to substantiate claims made by the product manufacturer.

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CHAPTER V

COMPARISON OF THE ACUTE PHASE PROTEIN RESPONSES OF NEWLY ARRIVED FEEDLOT CATTLE WITH RESPIRATORY DISEASE: RELATIONSHIPS TO TREATMENT RESPONSE

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Abstract

Seven truckloads of shipping stressed feedlot cattle were received at the Willard Sparks Beef Research Center near Stillwater, Okla., and used to study the impact that dietary vitamin E supplementation can have on animal performance, medical treatment costs, and the immune response. Plasma and serum samples were collected from a subset of animals in each truckload lot and used to quantify selected acute phase proteins, as well as *Pasteurella multocida* outer membrane proteins, *Pasteurella haemolytica* whole cell cultures, and antibody (IgG) response to a novel antigen (keyhole limpet haemocyanin [KLH]).

These parameters were used to describe the immune response among both healthy animals and those afflicted with a respiratory infection (bovine respiratory disease [BRD]) in four dietary treatment groups. The ultimate objective of this research was to determine if one or more acute phase proteins can lead to the development of a prognostic tool for use in veterinary medicine. These tests may also be effective in providing a method to monitor the efficacy of therapeutic treatment for respiratory diseases and to improve diagnostic success. Four acute phase proteins were quantified: Fibrinogen (Fb), haptoglobin (Hp), serum amyloid-A (SaA), and α-1-acid glycoprotein

(AGP). A direct immune system challenge was employed to test the responsiveness to a foreign antigen; IgG antibodies to KLH were determined.

Serum concentrations of Fb, Hp, SaA, and AGP were all similar among the four dietary TRT levels on d 0. Vitamin E supplementation (E7, E14, and E28) decreased ($P<.05$) the serum concentrations of SaA and AGP by d 14 compared to controls (CON). Serum concentrations of Hp, SaA, and AGP were also decreased ($P<.05$) by vitamin E among samples collected at d 28, also compared to CON. These decreases may have minimized the systemic inflammatory effect typically observed in BRD, thus reducing the severity of the disease overall. Antibody (IgG) response to KLH were greater ($P<.05$) among those cattle immunized with KLH antigen compared to those given PBS, thus indicating functional and responsive immune systems among our test animals.

The cost of medical treatment with antimicrobial drugs is an important factor in the profitability of beef cattle production. Determination of cattle unable to respond to routine therapy could be useful to beef cattle producers and veterinarians. No significant differences in the serum concentration of SaA or AGP were observed at the three time-periods (d 0, 14, or 28) sampled regardless of the number of antimicrobial drug treatments given. However, a significant ($P<.01$) pattern was observed in the serum concentrations of Hp. For cattle receiving zero drug treatments, Hp was significantly lower on d 0 and d 14. Serum concentrations of Hp were more than two-fold greater on d 0 when cattle required exactly one treatment, and more than three-fold greater for those cattle eventually requiring more than one treatment. By d 14, the Hp concentration of cattle never treated was elevated and similar to cattle that had already received exactly one treatment. However, the cattle requiring more than one treatment still had

significantly higher ($P<.01$) Hp concentrations. These results indicate that Hp may be used as a prognostic tool to determine the severity of BRD that may affect a group of cattle, and could also be used to monitor the efficacy of a therapeutic treatment regimen.

Introduction

Bovine respiratory disease (BRD) continues to disturb the economic viability of the beef cattle industry, as it has for many years (Gill and Smith, 1992; Duff et al., 1992; Godson et al., 1996; Young et al., 1996). This occurs despite numerous vaccines and antimicrobial drugs designed to prevent or eradicate the pathogens that make this disease complex so significant. A complete understanding of the pathogenesis and etiology of the BRD complex is yet to be mastered (Roth and Perino, 1998). Significant amounts of epidemiological data exist showing the relationship between viral and bacterial pathogens and their combined contribution to the incidence of bacterial pneumonia in cattle (Roth, 1984; Roth and Perino, 1998). This pathogenic synergism can make accurate diagnoses, and thus treatment difficult (Merck, 1991; Godson et al., 1996). According to NASS (1996), respiratory diseases among cattle diverted nearly $500 million from beef cattle producer's bottom lines in 1995.

Development of an analytical tool to identify transient and often self-limiting infections, which are frequently undetectable by even expertly trained personnel, would be particularly valuable to food animal veterinarians, as well as the beef cattle industry overall (Hirvonen et al., 1996). The measurement of acute phase proteins (APP) as a screening test to indicate the overall health status of groups of cattle has potential as a diagnostic and even a prognostic tool (Young et al., 1996). Serum proteins of the acute phase are synthesized and secreted by the liver in response to certain systemic infections,

toxic insult, or other immune system injury (Faulkner et al, 1992; Kent, 1992; Wright et al., 1995). Their function is to minimize the cellular damage caused by viral and bacterial pathogens and to promote the repair of already damaged tissue (Faulkner et al, 1992). Once an infection is recognized by the immune system, the secretion of, and thus the serum concentration of, APP increase very rapidly (Roitt et al., 1998). In extreme cases, the total hepatic synthesis of APP can be as high as 50 gm per day (Guyton, 1987).

Of interest in both human and veterinary medicine, the proteins most frequently measured and discussed are fibrinogen (Fb), haptoglobin (Hp), serum amyloid-A (SaA), α-1-acid glycoprotein (AGP), ceruloplasmin, and C-reactive protein (Kent, 1992; Hirvonen et al., 1996; Kumar et al., 1997; Roitt et al., 1998). Also included in the category of APP are specific cytokines (interleukin-1, interleukin-6 and tumor-necrosis factors), which tend to modulate the function of other immune cells and can stimulate the acute-phase response (Werling, 1996; Hochepied et al., 2000). Previously, there has been a lack of reliable laboratory procedures for measuring the acute phase proteins Fb and Hp for use in disease prognosis and diagnosis. Improvements in these procedures has elevated their status among clinicians (Pfeffers and Rogers, 1989; Cross et al., 1991). Characteristics of a reliable and high quality prognostic and diagnostic tool like APP include basal values that are low, or essentially undetectable, quick response times with great magnitude (>100x) changes in response to infection or inflammation, and to be unaffected by age, sex, or genetics (Kent, 1992). Stability of the compound of interest is also a beneficial characteristic, so that the sample may be stored frozen for later analysis or reference (Horadagoda et al., 1999).

The concentrations of many APP found in healthy cattle are negligible, at best, but are easily detectable during an acute infection (Eckersall and Conner, 1988). Horadagoda et al. (1999) and Kent (1992) both suggested that Hp is a predominant APP in cattle, and its serum concentration is clearly increased in response to clinical infection in stressed feedlot cattle (Conner et al., 1989; Wright et al., 1995). Godson et al. (1996) further suggested that Hp might be highly effective as a prognostic tool, while Wittum et al. (1996) and Young et al. (1996) proposed the use of Hp to monitor antimicrobial drug efficacy.

Eckersall and Connor (1988) described Fb as the most recognized and most commonly used APP assay in bovine; however, Hirvonen et al. (1996) suggested that Fb has distinct limitations as a prognostic tool. Alsemgeest et al. (1994) and Alsemgeest et al. (1995) described SaA as clinically useful, especially when a ratio of Hp and SaA (Hp/SaA) was developed. Alsemgeest et al. (1995) ultimately categorized SaA as a better indicator of physical stress than of disease, while Hp was not affected by experimentally imposed physical stressors. Horadagoda et al. (1999) disputes the previous findings, and suggests that any deficiency of SaA to detect disease was more in the laboratory procedure than in the actual response of SaA *in vivo*.

Our objective in this study was to compare serum concentrations of four APP (Fb, Hp, SaA, and AGP) as either a prognostic or diagnostic tool regarding the occurrence of BRD in marketing and shipping stressed feedlot cattle.

Materials and Methods

Experimental Design. An extensive study using more than 700 shipping stressed calves calves (568 heifers, 197 kg of BW initially; 126 bulls and steers, 151 kg of BW initially)

was initiated at the Willard Sparks Beef Research Center (WSBRC) near the campus of Oklahoma State University, Stillwater, Okla., in July, 1999. The primary focus of this research project was to determine if a high-level of vitamin E added to the receiving diets of these cattle for either 0, 7, 14, or 28 d would have an effect on animal performance, medical treatment costs, and the responsiveness of the immune system. It was determined that since detailed individual health records would be kept on each animal being treated for respiratory disease, concurrent sampling for APP would be a logical and valuable component to include in this study.

The cattle were received in truckload lots from mid-July 1999, to late December, 1999 (Table 1). This season of the year, especially from late September through December, is typically when the greatest number of stocker and feedlot cattle are diagnosed and treated for respiratory disease in Oklahoma (D.R. Gill—personal communication). On arrival, calves were allowed to co-mingle and rest for at least one hour in a processing facility alley prior to a pre-processing procedure. This procedure included assessment of overall health, individual weight of each calf and application of a sequentially numbered identification tag in the left ear. Calves were randomly distributed to six holding pens for no more than 36 hours before inception of the study. While in these holding pens, 0.9 kg of prairie hay and 1.4 kg of the control diet (Table 2) were fed per head. On d 0, calves were processed at approximately 0600 hours prior to feeding. Processing included: individual weight, vaccination for viral respiratory diseases (BRSV-Vac 4™, 2 mL via intramuscular injection [IM]; Vision-7™, 2 mL via subcutaneous injection [Sub-Q] (heifers); or Covexin 8™, 5 mL Sub-Q (bulls and steers)), and treatment with anthelmintics for internal and external parasites (Ivomec-

Table 1. General information regarding cattle received at Willard Sparks Beef Research Center for this study.

Load #	Origin	Date Received	# Head in Load[1]	Average Weight, kg
1	Texas-Oklahoma	July 15, 1999	86	234 ± 33
2	Texas-Oklahoma	August 12, 1999	85	233 ± 33
3	Texas-Oklahoma	August 20, 1999	85	227 ± 33
4	Texas-Oklahoma	September 4, 1999	130[2]	151 ± 11
5	Texas-Oklahoma	October 4, 1999	111	168 ± 33
6	Texas-Oklahoma	December 8, 1999	106	172 ± 33
7	Texas-Oklahoma	December 16, 1999	112	174 ± 33

[1]Total cattle received=715. Not all cattle completed the 42-d receiving trial due to mortality or morbidity. In total, twenty-one head were removed due to morbidity and were chronic, or due to death loss (<1%).

[2]Bulls and steers; all other loads were heifers.

Table 2. Control diet composition.[1,2]

Ingredient	% of Diet (DM basis)
Soybean hulls	32.5
Whole corn	27.0
Wheat middlings	17.0
Sparks 99 supplement	13.5
Cottonseed hulls	10.0

[1]Fed ad-libitum.

[2]Control diet provided 127 IU vitamin E per 4.5 kg feed from added and natural sources.

Plus™, 1.0 ml/110 lb Sub-Q); viral respiratory was boostered at d 14. Bulls were retained as in-tact males until after d 42. Castration likely would have initiated an acute phase response not representative of any respiratory infection, and would have altered the APP activity and results. Each truckload lot received was fed at WSBRC for a 42 d receiving period. Dietary treatments are represented by the number of days that the control diet was supplemented with 2000 IU of vitamin E (Table 3): 0 days=Control (CON), 7 days=E7, 14 days=E14, or 28 days=E28. Pen size was uniform across all treatments (12.2 m x 30.5 m) and alternating pens shared automatic water basins. Feed was delivered once daily at approximately 0700 hours. Feed was delivered twice daily during inclement weather to provide clean, dry feed for a majority of each day. Cattle were weighed on days 0, 14, 28, and 42 of the study; blood samples to be used in the analysis of APP were also collected from designated animals at those intervals.

Prior to d 0 processing, a sub-sample of at least six cattle per pen or twelve cattle per dietary treatment were randomly selected for whole blood sample (3 serum samples, and 1 plasma sample) collection. Blood samples were allowed to equilibrate to ambient temperature, then samples for serum separation were stored at 4°C overnight. One plasma sample from each animal was collected (10mL Vacutainer® tube with sodium heparin); plasma was separated within six hours of collection, stored overnight at 4°C and fibrinogen (Fb) concentration determined the next day. Three serum samples were collected (10mL Vacutainer® tube with no additive). Serum was separated after the overnight chill and stored at -10°C until laboratory analyses could be performed. Analyses included serum cholesterol, serum vitamin E, and serum APP (Hp, SaA, and AGP), as well as antibody responses to *Mannheimia* (formerly *Pasteurella*) *haemolytica*

Table 3. Experimental diet (Vitamin E-2000) composition.[1,2]

Ingredient	% of Diet (DM basis)
Soybean hulls	32.5
Whole corn	27.0
Wheat middlings[3]	15.0
Sparks 99 supplement	13.5
Cottonseed hulls	10.0
B-171 Supplement[3]	2.0

[1]Fed ad-libitum.

[2]Control diet provided 127 IU vitamin E per 4.5 kg feed from added and natural sources.

[3]A pelleted supplement (for composition of B-171, see Table 3) replaced wheat middlings in experimental diets and was added to provide 2000 IU per day of vitamin E. Inclusion in the diet was based on average daily feed intake: 2.3 kg = 6%; 4.5 kg = 4%; 6.8 kg = 2%).

whole-cell proteins (WCP), and to *Pasteurella multocida* outer-membrane proteins (OMP).

Cattle were closely observed each morning at approximately 0630 hours by experienced veterinary personnel (Oklahoma State University College of Veterinary Medicine) for signs of respiratory and other diseases. Two or more clinical signs of disease were required to designate a calf as sick and make that calf eligible for further clinical review and therapeutic antimicrobial drug treatment. Once pulled a calf would be walked to the processing area and restrained in a squeeze chute, its individual weight recorded and rectal temperature assessed. Regardless of health status, all information was recorded on an individual sick card and filed by pen for future reference. If rectal temperature was greater than 40°C, a required regimen of antimicrobial treatment therapy followed.

More than seven thousand plasma and serum samples were collected over the course of this experiment. One-half of the samples collected on routine weigh dates were dedicated for the determination of serum cholesterol and vitamin E concentrations, the other half for APP determination. Additionally, whenever a calf was designated as sick, one plasma and one serum sample was collected for APP determination. Approximately two weeks after the initial antimicrobial treatment for respiratory disease, an evaluation of the animal's health status was conducted. If the animal showed no further clinical signs during that two-week period, additional blood samples were drawn for APP analysis. Although we have a multitude of samples from which an enormous amount of APP data could be derived, we were not equivalently funded, nor would there have been enough time to analyze every sample. Therefore, it was determined that the following

analyses would be performed on Loads 2, 3, 6, and 7. We believe these cattle to be truly representative (> 50%) of all the cattle received at the WSBRC during this experiment.

Fibrinogen (Fb). After the overnight chill as described above, plasma samples were delivered to the Boren Veterinary Teaching Hospital Clinical Pathology laboratory on the campus of Oklahoma State University in Stillwater for determination of plasma Fb concentration. After vortexing, a 100μl sample of plasma was deposited into a micro-hematocrit tube and the Fb concentration determined using heat precipitation (60°C for three minutes) and refractometry, as previously described (Duncan and Prasse, 1977). Results are reported as mg/dl of Fb.

Haptoglobin (Hp). Bovine serum Haptoglobin radial immunodiffusion kits (Code No. P0105-1, Cardiotech Services, Inc., Louisville, Ken.), were used to determine the serum Hp concentrations in samples. Results are reported as μg/ml of Hp.

The test procedure for Hp includes the following steps. Our bovine serum samples (100 μl) were treated with a equal amount of 40mM 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. Both Standards A and B are loaded (5 μl) into specified wells in the test plate. Similarly, 5 μl samples of the cysteine-treated bovine serum is loaded into identity recorded wells. Only one sample was used per well. The plate was covered and incubated in a horizontal position at 37°C for at least 24 h. After the 24-h incubation, results could be determined. A plastic scale included with the kit was used to measure the outer, or external diameter of each precipitin ring to the nearest .01 mm.; standards were measured first. The results were plotted on the vertical axis of a semi-logarithmic graph (also provided in the kit), and a Hp concentration was identified on the

corresponding horizontal axis. A reference curve was developed from the ring diameters and an actual Hp concentration could then be determined from the known dilution factor; in this case, the dilution factor used was 2x. Absence of a precipitin ring altogether would indicate a Hp concentration of less than 10 μg/ml, which is normal for healthy cattle. This test kit provided ease of use and a limited amount of variation. The coefficient of variation stated was less than four percent for repeated, identical measurements of the same test specimen. These procedures were derived from, supported by, or otherwise validated in scientific literature (Makimura et al., 1982; Van Rijn et al., 1987; Conner and Eckersall, 1988; Conner et al., 1989; Makimura et al., 1990; Morimatu et al., 1991; Skinner et al., 1991; Morimatu et al., 1992).

Serum Amyloid-A (SaA). Commercial enzyme-linked immunosorbant assay kits (The Tridelta Phase™ Range kit, Tri-Delta Development, Ltd., Wicklow, Ireland) were used to determine the SaA concentrations following the manufacturer's recommendations. Included in the kit are microtiter strips with microwells (12 x 8, identical to a 96-well plate) coated with a monoclonal antibody (Ab) specific for SaA. Serum samples from test animals were vortexed and added (50 μl diluted 1:500; only one sample per well—no replicates due to high cost of this test) to each well along with biotinylated anti-SaA monoclonal Ab (50 μl; diluted 1:100 in 1x diluent buffer). The plate was covered and incubated at 37°C for at least one hour, after which, the plate is thoroughly washed (6x with PBS) to remove all unbound material and tapped dry. One hundred microliters of streptavidin-horse radish peroxidase diluted 1:4000 was added to each well and the plate incubated at room temperature in darkness for 30 minutes. Again, the wells were washed (4-6x) and tapped dry. One hundred microliters of TMB substrate was added, and the

plate was incubated at room temperature in darkness for another 30 minutes. Stop solution (50 µl) was then added. The plate was then read in an automated plate reader (V Max Kinetic Microplate Reader, Molecular Devices, Inc.) at OD_{490}. Results are reported as µg/ml of SaA.

α-1-Acid Glycoprotein (AGP). Bovine α-1-Acid Glycoprotein radial immunodiffusion kits (Code No. P0101-1, Cardiotech Services, Inc., Louisville, Ken.) were used to determine the serum α-1-Acid Glycoprotein concentration of our samples. Standards and serum samples (5 µl) were added to individual test wells in agar gel plates containing anti-AGP antibodies. Results were determined by the formation of a visible precipitin ring after incubation at 37°C for 24-48 hours. After incubation, results were determined by measuring the external diameter of each precipitin ring formed to the nearest 0.1 mm. Standards were again measured first, and resulting diameters plotted onto the vertical axis of the provided semi-logarithmic graph paper. A corresponding AGP value was found on the horizontal axis of the graph paper and a reference curve developed. The concentration of each test sample was plotted, and an AGP value calculated from the reference curve. Results are reported as µg/ml of AGP.

Statistics. All data were analyzed using the GLM procedures of SAS (1996). Least squared means were examined for differences among our dietary vitamin E treatments (TRT) and among the total number of antimicrobial drug treatments each sick animal received. Paired-t tests were also used to examine the data for patterns of significant change among the three standard time periods (0, 7, and 28 d). Where appropriate, linear, quadratic, and cubic regression equations were fitted to the data.

Results and Discussion

Fibrinogen (Fb). Means of serum Fb concentrations by dietary TRT are shown in Table 4. Fibrinogen concentration decreased as the duration of supplemental dietary vitamin E increased; however, none of these decreases were significant over the times measured (d 0, 28, 42). Serum Fb concentrations were lower on d 0 (P=0.0154) and coincided with more drug treatments among those cattle (Table 5). These differences were also evident, but less significant (P=0.08) by d 14. Table 6 displays means across dietary TRT for Fb concentration when cattle received the first drug treatment (RxIn) and when they were determined to have recovered. Vitamin E supplementation did not affect Fb concentrations at either measurement point. When samples were taken from cattle concurrent with the first drug treatment (RxIn), Fb was elevated in animals requiring more than one drug treatment (Table 7), but not different (P=0.18). Samples taken at recovery (RxOut) were also similar (P=0.32).

Paired t-tests compared differences in serum [Fb] among the three time periods when samples were analyzed (Table 8). A trend was observed in the difference (P=0.0060) of Fb values and the number of treatments given between d 0 and 28 (Table 9). As well, the differences in values on d 0 and 42 were also significant (P=0.0316). While the statistical model was significant (P=0.0001), the block for dietary TRT level was not (P=0.2464), except for the change among cattle in the E14 TRT group, compared to CON. The decrease of Fb in this group showed a trend towards significance (P=.06). Similar results were observed for the comparisons of day 28 to 42, and of day 0 to 42.

While the Fb assay was easy to perform and results could be obtained rapidly, the test by itself did not provide consistent results with regard to the severity of BRD, nor to

Table 4. Results of laboratory analyses for plasma and serum acute phase protein concentrations.

Factor Measured	D0	D7	D28
Fibrinogen (Fb)			
CON	600.0 ± 57.9	316.7 ± 37.5	252.3 ± 27.1
E7	559.5 ± 59.9	211.4 ± 36.6	218.2 ± 26.8
E14	686.4 ± 58.5	214.8 ± 36.6	265.1 ± 27.1
E28	567.0 ± 58.5	212.7 ± 36.6	245.5 ± 26.7
Haptoglobin (Hp)			
CON	403.0 ± 61.7	492.4 ± 87.6	139.7^{a} ± 34.4
E7	400.7 ± 61.7	386.6 ± 87.6	36.3^{b} ± 34.4
E14	407.4 ± 67.7	407.6 ± 96.2	71.7^{a} ± 37.7
E28	290.9 ± 67.7	362.3 ± 97.9	45.2^{b} ± 37.7
Amyolid-A (SaA)			
CON	25.2 ± 12.7	19.6^{a} ± 14.0	12.3^{a} ± 13.4
E7	26.7 ± 10.6	14.0^{b} ± 11.4	6.1^{b} ± 9.8
E14	25.8 ± 9.5	11.1^{bc} ± 10.4	7.6^{a} ± 10.0
E28	20.9 ± 13.1	11.5^{bc} ± 13.5	7.2^{b} ± 9.6
α-1-acid Glycoprotein (AGP)			
CON	620.2 ± 60.2	953.4^{a} ± 78.3	827.5^{a} ± 73.3
E7	639.7 ± 60.2	747.6^{b} ± 78.3	597.4^{b} ± 73.3
E14	580.5 ± 59.3	729.1^{b} ± 77.1	616.7^{b} ± 72.2
E28	549.2 ± 60.2	585.8^{c} ± 78.3	630.9^{b} ± 73.3
Hp / SaA Ratio			
CON	16.1 ± 3.3	40.8 ± 10.8	15.1 ± 4.1
E7	15.3 ± 3.4	48.6 ± 11.4	5.3 ± 4.3
E14	15.7 ± 3.5	28.7 ± 11.6	5.2 ± 4.4
E28	14.4 ± 3.5	56.1 ± 11.6	4.8 ± 4.4

$^{a, b,c}$Means within a column with different superscripts differ (P<.05).

Table 5. Results of paired t-tests for serum acute phase protein concentrations.

Factor Measured	D0	D7	D28
Fibrinogen (Fb)[1]			
Med0	$711.3^{a} \pm 48.4$	$219.8^{a} \pm 31.1$	258.2 ± 22.6
Med1	$528.0^{b} \pm 39.4$	$271.2^{b} \pm 25.3$	246.8 ± 18.3
Med>1	$613.6^{a} \pm 81.2$	$145.2^{a} \pm 52.9$	197.5 ± 39.5
Haptoglobin (Hp)			
Med0	$154.5^{a} \pm 50.2$	$310.3^{a} \pm 80.4$	43.4 ± 32.0
Med1	$441.4^{b} \pm 37.3$	$427.2^{a} \pm 59.1$	98.8 ± 23.8
Med>1	$721.9^{bc} \pm 89.1$	$670.0^{b} \pm 141.0$	35.4 ± 56.8
Amyolid-A (SaA)			
Med0	23.7 ± 1.7	13.9 ± 1.9	8.9 ± 1.6
Med1	24.5 ± 1.3	14.3 ± 1.5	8.6 ± 1.3
Med>1	29.7 ± 3.5	13.3 ± 4.1	3.7 ± 3.5
α-1-acid Glycoprotein (AGP)			
Med0	559.2 ± 50.1	689.1 ± 67.4	683.6 ± 62.0
Med1	623.5 ± 39.8	777.6 ± 53.5	682.9 ± 49.2
Med>1	581.0 ± 107.4	877.0 ± 144.6	484.0 ± 132.8
Hp / SaA Ratio			
Med0	$5.0^{a} \pm 2.6$	$21.1^{a} \pm 9.3$	5.0 ± 3.7
Med1	$19.6^{b} \pm 2.0$	$52.1^{b} \pm 7.3$	9.8 ± 2.9
Med>1	$28.6^{b} \pm 5.1$	$76.0^{b} \pm 18.4$	6.5 ± 7.2

[1]Fb means are reported from D0, D28, and D42.

[a, b,c]Means within a column with different superscripts differ ($P<0.01$), except for Fb and Hp D14 where $P=0.08$.

Table 6. Results of laboratory analyses for serum acute phase proteins when cattle were first observed as sick (RxIn) and when cattle were determined to be recovered (RxOut).

Factor Measured	RxIn	RxOut
Fibrinogen (Fb)		
CON	575.7 ± 40.7	358.7 ± 31.9
E7	593.6 ± 46.8	321.6 ± 34.7
E14	638.2 ± 44.7	346.5 ± 35.0
E28	559.3 ± 50.2	288.9 ± 38.3
Haptoglobin (Hp)		
CON	456.4 ± 56.7	82.3^{a} ± 32.8
E7	459.7 ± 61.7	89.8^{a} ± 35.2
E14	503.3 ± 64.3	68.3^{a} ± 37.6
E28	522.0 ± 71.9	194.8^{b} ± 39.9
Amyolid-A (SaA)		
CON	24.7 ± 11.2	8.9 ± 10.8
E7	26.0 ± 9.7	8.6 ± 9.0
E14	25.5 ± 9.7	9.9 ± 11.9
E28	24.0 ± 12.3	9.0 ± 10.4
α-1-acid Glycoprotein (AGP)		
CON	673.7 ± 414.4	566.1 ± 350.2
E7	694.6 ± 398.4	530.0 ± 275.9
E14	571.8 ± 275.5	504.5 ± 243.0
E28	582.9 ± 271.7	556.7 ± 222.7

$^{a, b,c}$Means within a column with different superscripts differ ($P<.05$).

Table 7. Results of laboratory analyses for serum acute phase proteins when cattle were first observed as sick (RxIn) and when cattle were determined to be recovered (RxOut).

Factor Measured	RxIn	RxOut
Fibrinogen (Fb)		
Med1	575.0 ± 25.4	325.3 ± 18.6
Med>1	648.3 ± 48.0	376.1 ± 47.8
Haptoglobin (Hp)		
Med1	444.7[a] ± 34.3	108.6 ± 20.5
Med>1	628.1[b] ± 69.0	86.0 ± 40.5
Amyolid-A (SaA)		
Med1	24.9 ± 0.9	10.0[a] ± 0.9
Med>1	26.0 ± 2.0	4.9[b] ± 1.9
α-1-acid Glycoprotein (AGP)		
Med1	638.8 ± 31.4	549.5 ± 24.0
Med>1	627.9 ± 62.2	505.5 ± 49.2

[a, b,c]Means within a column with different superscripts differ (P<0.02).

Table 8. Results of paired t-test statistical analyses for plasma and serum acute phase protein concentrations.

Factor Measured	Diff 0-7	Diff 7-28	Diff 0-28
Fibrinogen (Fb)[1]			
CON	-293.3 ± 61.4	-75.0 ± 43.2	-353.8 ± 56.6
E7	-323.5 ± 61.4	-4.0 ± 42.3	-335.0 ± 53.2
E14	-454.2 ± 60.0	40.0 ± 42.7	-405.5 ± 52.6
E28	-340.0 ± 60.0	30.0 ± 42.3	-310.0 ± 52.1
Haptoglobin (Hp)			
CON	88.8 ± 90.2	-335.0 ± 89.6	-246.1 ± 65.3
E7	-22.0 ± 91.5	-346.1 ± 90.8	-368.1 ± 66.1
E14	14.5 ± 106.7	-311.3 ± 105.9	-296.8 ± 77.1
E28	68.6 ± 104.6	-302.4 ± 103.8	-239.0 ± 74.8
Amyolid-A (SaA)			
CON	-7.0 ± 2.2	-7.6 ± 1.9	-14.6 ± 2.1
E7	-12.9 ± 2.3	-7.5 ± 2.0	-20.5 ± 2.2
E14	-15.4 ± 2.2	-3.5 ± 1.9	-18.9 ± 2.0
E28	-9.7 ± 2.2	-4.9 ± 1.9	-14.6 ± 2.1
α-1-acid Glycoprotein (AGP)			
CON	334.4[a] ± 57.0	-144.4 ± 60.5	190.0[a] ± 56.7
E7	96.3[b] ± 55.8	-139.5 ± 59.2	-43.2[b] ± 55.5
E14	147.3[b] ± 55.2	-102.2 ± 58.6	45.1[b] ± 54.9
E28	38.0[b] ± 55.8	34.2 ± 59.2	72.2[b] ± 55.5
Hp / SaA Ratio			
CON	24.4 ± 10.0	-23.5 ± 10.6	0.9 ± 4.2
E7	27.0 ± 11.0	-35.9 ± 11.6	-8.9 ± 4.6
E14	10.7 ± 12.8	-20.3 ± 13.5	-9.6 ± 5.3
E28	37.8 ± 11.4	-45.8 ± 12.0	-8.0 ± 4.7

[1]Fb means are reported for Diff 0-28, Diff 28-42, and Diff 0-42.

[a, b,c]Means within a column with different superscripts differ (P<.05).

Table 9. Results of paired t-tests for serum acute phase protein concentrations.

Factor Measured	Diff 0-7	Diff 7-28	Diff 0-28
Fibrinogen (Fb)[1]			
Med0	-494.9[a] ± 59.9	38.4 ± 38.5	-456.6[a] ± 49.9
Med1	-255.6[b] ± 49.3	-26.1 ± 31.4	-285.7[b] ± 40.9
Med>1	-464.3[a] ± 102.1	47.5 ± 67.3	-372.5[b] ± 87.2
Haptoglobin (Hp)			
Med0	159.1 ± 89.9	-265.8 ± 86.8	-111.1[a] ± 54.3
Med1	-14.2 ± 66.1	-328.4 ± 63.9	-342.6[b] ± 40.4
Med>1	-51.9 ± 157.6	-634.6 ± 152.4	-686.5[c] ± 96.4
Amyolid-A (SaA)			
Med0	-9.8 ± 1.8	-4.9 ± 1.5	-14.8[a] ± 1.7
Med1	-10.9 ± 1.5	-5.7 ± 1.2	-16.6[a] ± 1.4
Med>1	-16.3 ± 3.9	-9.6 ± 3.3	-26.0[b] ± 3.7
α-1-acid Glycoprotein (AGP)			
Med0	130.0 ± 47.9	-5.5[a] ± 48.5	124.5 ± 48.4
Med1	154.2 ± 38.0	-94.8[a] ± 38.5	59.4 ± 38.5
Med>1	296.0 ± 102.8	-393.0[b] ± 103.9	-97.0 ± 103.9
Hp / SaA Ratio			
Med0	16.1 ± 8.9	-16.1[a] ± 9.1	-0.1[a] ± 3.4
Med1	32.4 ± 7.0	-42.3[b] ± 7.1	-9.8[b] ± 2.7
Med>1	47.4 ± 17.6	-69.5[b] ± 18.1	-22.1[b] ± 6.8

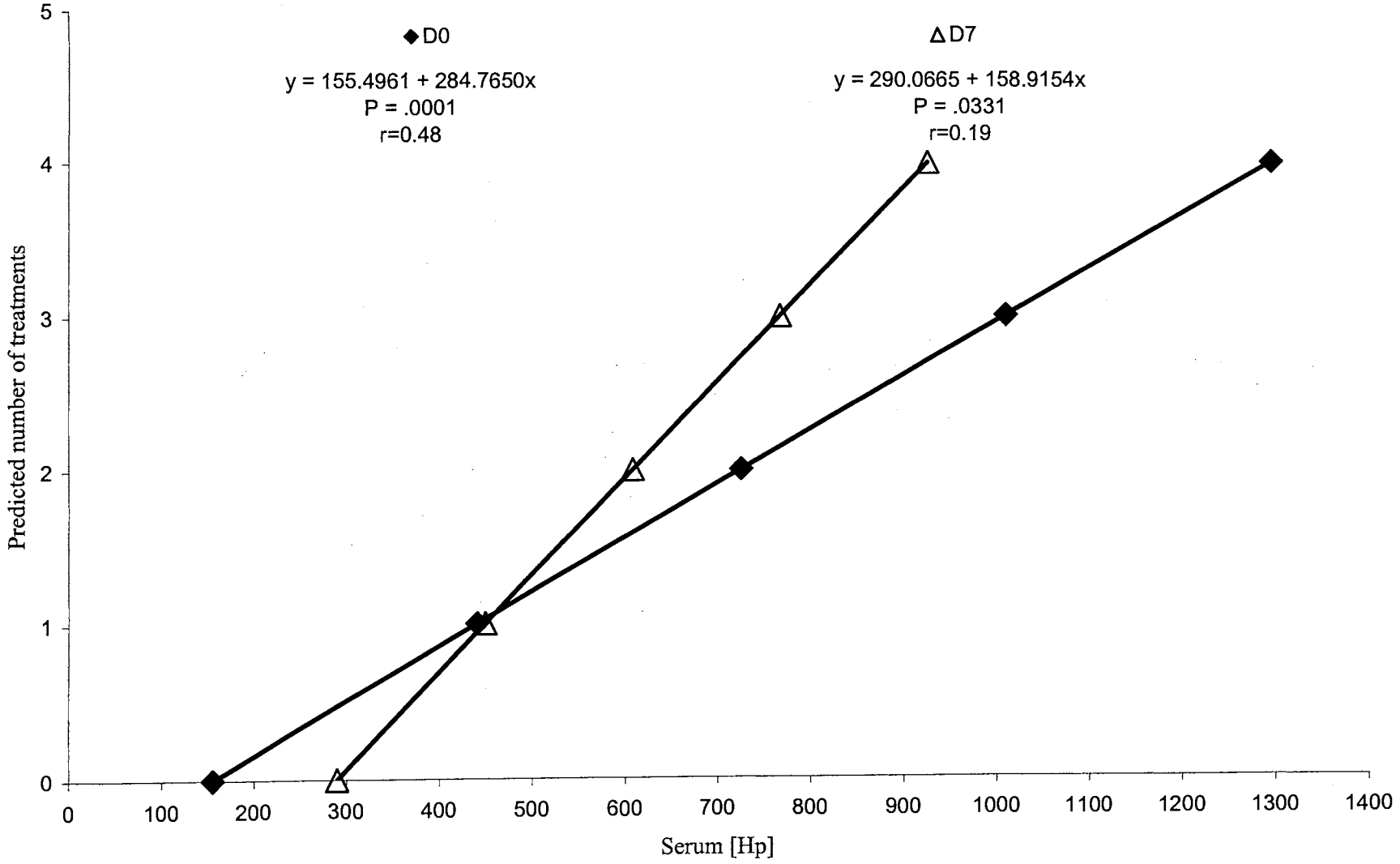
[1]Fb means are reported from Diff 0-28, Diff 28-42, and Diff 0-42.
[a, b,c]Means within a column with different superscripts differ (P<0.01).

the number of drug treatments that would be required. Therefore, the inconsistent results did not lead to a conclusion that Fb would be useful as a stand alone prognostic or diagnostic tool.

Haptoglobin (Hp). No significant differences were observed among the four dietary TRT (CON, E7, E14, and E28) levels at either day 0 or day 7. Vitamin E supplementation did, however, reduce the serum [Hp] observed at day 28 for the E7 (P=0.04) and E28 (P=0.07) TRT levels compared to CON. The means at each period are shown in Table 4. When a comparison was made by the total number of antimicrobial drug treatments given, both d 0 (P=0.0001) and d 7 (P=0.03) results were significant (Table 5). On d 0, Hp levels were elevated (P=0.0001) in those cattle eventually requiring more than one treatment. A similar pattern remained on d 7 (P=0.08). Paired t-tests revealed no significant differences by TRT among the three time-periods (Table 8). Results of linear regression analysis among the Hp data and the number of drug treatments given indicated that a strong linear relationship exists for both day 0 values (P=0.0001) and day 7 values (P=0.0331). Figure 1 displays these relationships graphically, along with the relevant linear equations and r-values. These data indicate that Hp can be a reliable predictor of disease in cattle when samples are collected on arrival at the feeding facility.

Data derived from serum samples of sick cattle were also statistically analyzed (Table 6). The Hp concentration was higher (P=0.03) in E28 compared to our other dietary TRT levels when cattle were determined to be well, or recovered from their initial diseased state. The serum concentration of Hp (Table 7) was observed to be significantly lower (P=0.02) in cattle receiving only one drug treatment (444.7 μg/ml) compared to

Figure 1. Regression of [Hp] on the number of antimicrobial drug treatments required at D0 and D7.



cattle receiving more than one drug treatment (628.1 μg/ml). Therefore, not only can Hp be a reliable predictor of disease; these data will help to establish a range above which chronic cattle, or at least cattle with a greater need for therapeutic treatment procedures such as metaphylaxis, may be predetermined.

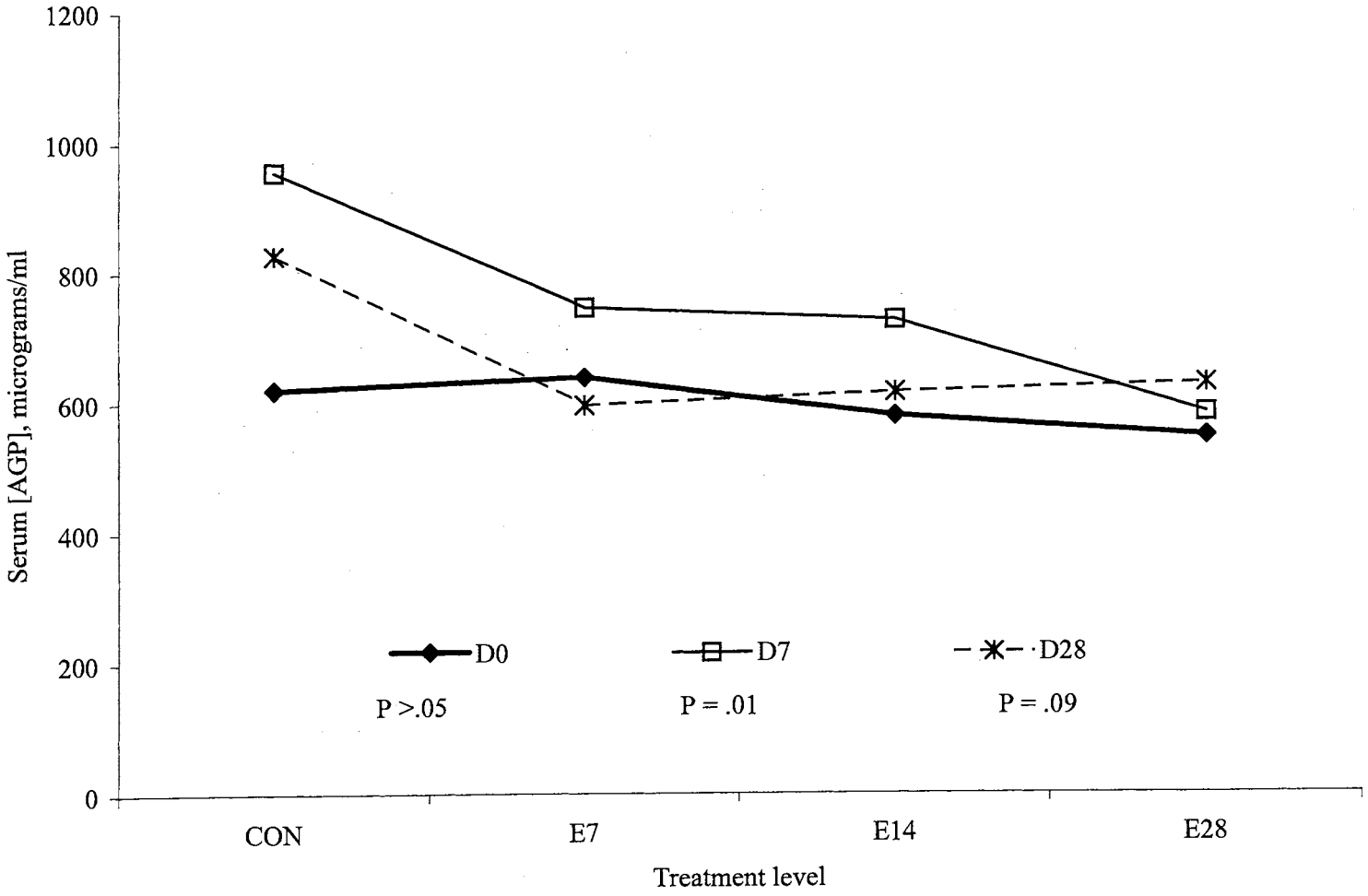
Linear regression analysis of Hp values from sick cattle taken at d 0 (r=0.44; P=0.0001), or when cattle received their first drug treatment (r=0.19; P=0.0186) indicated that the number of drug treatments, and thus the severity of respiratory disease, may be predicted from an initial serum [Hp] determination. Paired t-tests comparing the differences in [Hp] among the three time-periods (Table 8) and between RxIn and RxOut (Table 9) produced no significant differences.

Serum Amyloid-A (SaA). Supplementation with vitamin E decreased [SaA] at d 7 (P=0.02; Table 4). When cattle required more than one treatment (Table 5) SaA was higher on d 0 compared to cattle that were never treated. Among the cattle in our study that were determined to be clinically ill and eligible for antimicrobial drug treatment therapy, SaA concentrations were not different by dietary TRT level when cattle were first sick (25.1 μg/mL; Table 6). Similarly, values were not different when cattle were considered to be recovered (9.1 μg/mL). Only the improving health status of the cattle over time, which could be due to the success of treatments given, decreased the concentration of SaA in these cattle. Unlike the Hp results observed in these same cattle, SaA values alone are likely not a reliable predictor of disease, or severity of disease. However, SaA may be a very reliable indicator of drug efficacy. This relationship was observed to be linear (r = .19; P=0.0140).

α-1-Acid Glycoprotein (AGP). As mentioned previously, AGP functions differently in the acute phase response than other proteins. Our data support that notion. Among the four dietary TRT levels (Table 4), the AGP response at d 0 was not different. However, serum concentrations of AGP were decreased as the dietary level of vitamin E increased (Figure 2). The use of AGP as a predictive tool did not appear to be validated by these data. All serum AGP concentrations were similar regardless of severity of disease (Table 5 and 7). Only when the differences between AGP concentrations on day 7 and day 28 compared were any differences realized. The change of serum [AGP] was much greater (P=0.004) for cattle receiving zero (-5.50 mg/dl), or exactly one treatment (-94.78 mg/dl) compared to those receiving more than one treatment (393.0 mg/dl). Paired t-tests (Table 8) were significantly different (P=0.04, 0.10, 0.009) at all three comparisons examined and displayed changes in serum concentrations among the dietary TRT levels as great as 296 μg/ml between d 0 and d 7. No linear, quadratic, or cubic relationships were observed among these data. Likewise, no significant differences were detected when the data for only sick cattle were analyzed (Table 7).

Hp/SaA Ratio. According to the procedure described by Alsemgeest et al. (1994), a ratio of Hp and SaA values was created and analyzed statistically. Among the four dietary TRT (Table 4), Hp/SaA was similar (P>0.05) at all three measurement points. No prominent or consistent pattern of change was evident. The Hp/SaA ratio was very effective in matching the severity of BRD infection (Table 5) on d 0 and d 7 with the number of drug treatments eventually given. When cattle required exactly one (Med1), or more than one (Med>1) treatment, the ratio on d 0 was greater (P=0.0001) than the value observed from cattle never sick (Med0). On d 7, Hp/SaA values increased

Figure 2. Serum alpha-1-acid glycoprotein (AGP) is decreased by dietary vitamin E.



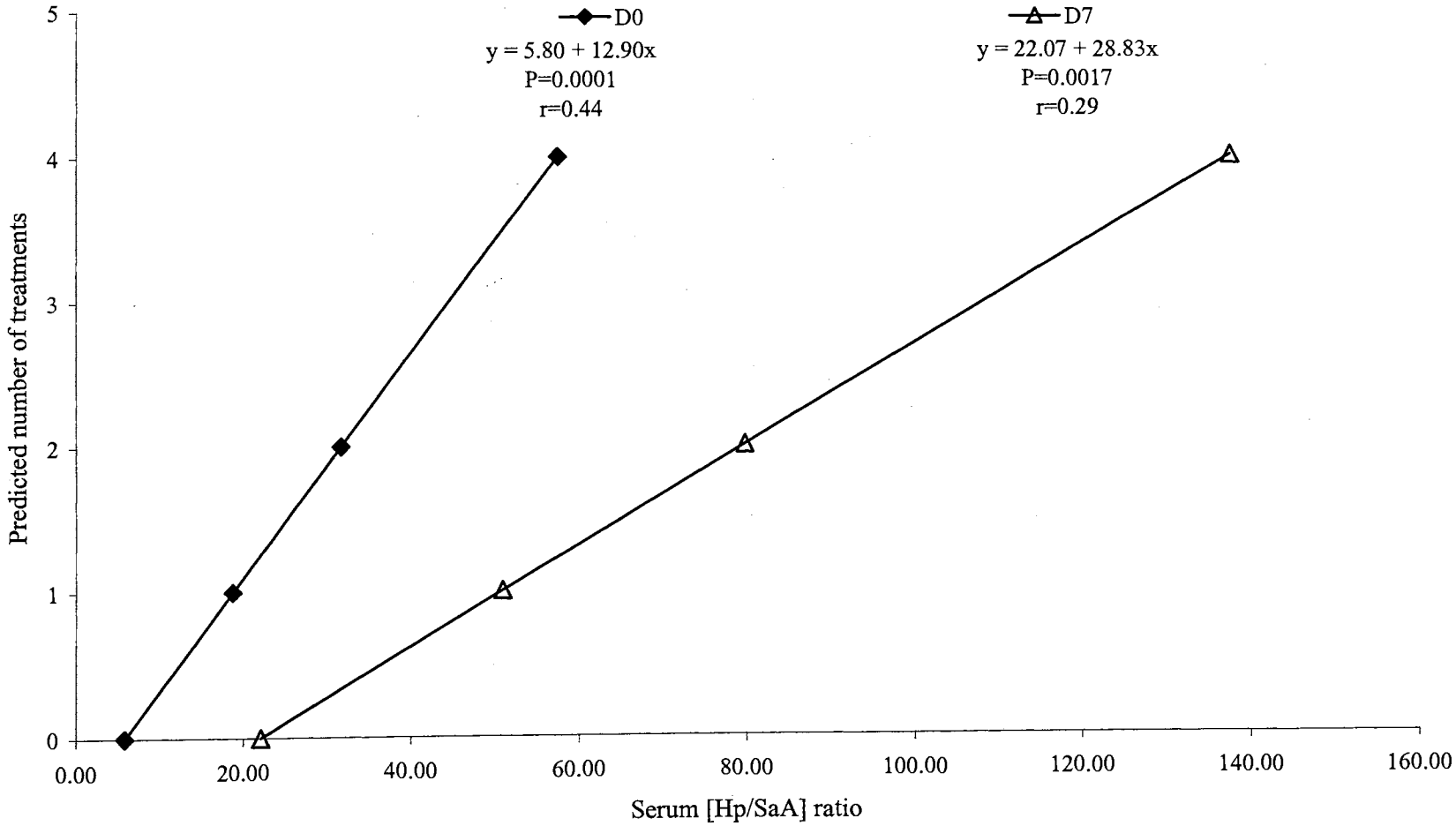
altogether for both healthy and sick cattle, but the difference in values of cattle requiring one or more than one treatment was still greater (P=0.0073). The majority of cattle observed as sick by our identification techniques, and thus given treatment, were observed as such primarily in the first 14-21 days of this study. By d 28, Hp/SaA ratios confirmed this observation. The values at this measurement point were not different (P>0.05), and were similar to d 0 values in cattle that were never sick (μ=7.8 μg/ml). Thus, this method could be used as a predictive tool with reliability.

Paired t-tests (Table 8) analyzing the differences between measurement points revealed no significant differences (P>0.05). Linear regression analysis was performed and a significant linear relationship was found (r=0.44; P=0.0001). Based on these data at d 0, the number of treatments could be predicted from the Hp/SaA values obtained (Figure 3). Likewise, at d 7 the number of treatments in a given group of cattle could also be predicted (r=0.29; P=0.0017).

Conclusions

It is obvious that the circulating concentration of proteins in cattle becomes altered during the acute phase response to infection, inflammation, disease, or even physical stress. Each acute phase protein has a characteristic flux during the acute phase, thus we were able to associate their increase and decrease in concentration with observed physical conditions. While the gold standard of these proteins—Fb, can provide some information about the onset of disease, others appear to be more precise and reliable. Haptoglobin values provided clear and consistent evidence that beef cattle producers and veterinarians could use to make practical management decisions regarding any particular group of

Figure 3. Regression of [Hp/SaA] ratio on the number of treatments required on d 0 and d 7



feedlot cattle. When Hp values were combined with SaA values to create a ratio, the results were especially reliable.

Vitamin E supplementation appeared to decrease the concentrations of all acute phase proteins measured. However, only the concentrations of SaA and AGP were reduced significantly by d 14, the point after which most cattle did not become sick or receive drug treatment. This pattern emphasizes the variable functions and responses of these proteins.

Although the reliability of the predictions derived from the Hp/SaA were good, the cost of SaA determination is rather high. Therefore, the most practical determination to pursue for the development of a prognostic tool is likely Hp. Results of Hp determination were consistent with previous literature, as well as showing consistent patterns relative to our subjective determination of disease in these cattle. Alternately, if cost is not an issue, SaA especially when combined in a ratio with Hp could provide more than adequate information for disease prognosis or diagnosis. Other APP, particularly AGP, could be extremely useful in the monitoring recovery progress, or the efficacy of treatment for disease.

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VITA

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Doctor of Philosophy

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