Effects of dietary energy level and intake of corn by-product based diets on newly received growing cattle

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Abstract

Four pen studies and one digestibility trial were conducted to evaluate the effects of energy level and intake of corn by-product based diets on newly received growing cattle. In Exp. 1 there were four diets where one was offered for ad libitum intake and formulated to supply 0.99 Mcal NE_g/kg DM (0.99/100) and the other three treatments were fed at 95, 90, and 85% of the ad libitum treatment and to supply 1.10 (1.10/95), 1.21 (1.21/90), and 1.32 Mcal NE_g/kg DM (1.32/85), respectively. ADG was unaffected by treatment ($P = 0.32$). However, G:F increased linearly with increasing energy and decreasing intake level ($P < 0.01$). In Exp. 2, a digestibility trial was conducted to study diets from Exp. 1. Ruminal propionate linearly increased with increasing dietary energy and decreasing intake ($P < 0.01$). Total tract DM digestibility increased linearly with increasing energy and decreasing intake ($P < 0.01$), whereas passage rate decreased ($P < 0.01$). Experiment 3 validated results from Exp. 1 feeding the 1.10/95 treatment at 2.40% of BW daily and the 1.32/85 treatment at 2.2% of BW daily and studied a DNA-immunostimulant (Zelnate, Bayer Animal Health, Shawnee Mission, KS). Zelnate had no effect on parameters measured. ADG was not different between energy treatments ($P = 0.75$), but efficiency was greater for the 1.32/85 treatment ($P = 0.03$). Experiment 4 was designed to observe effects of the 1.32 Mcal NE_g/kg DM diet fed at four intake levels of 1.9, 2.2, 2.5, and 2.8 % of BW daily. ADG increased linearly with increasing intake ($P < 0.01$), however G:F was not affected ($P = 0.98$). In Exp. 5 a factorial design was employed to evaluate the effects of two by-products; wet corn gluten feed and wet distiller's grains plus solubles, and two levels of corn processing; whole corn or dry-rolled corn. Final ADG and G:F were not affected by by-product, corn processing, or their interaction ($P > 0.30$). Additionally, animals and diets from Exp. 1 were used to study effects on antibody production, acute phase protein response, stress, and immunocompetency of

healthy and morbid cattle. Diet had no effect on the parameters measured ($P > 0.10$). A quadratic response to time ($P < 0.01$) was detected for haptoglobin, titers for bovine viral diarrhea type 1 (BVD-1), and infectious bovine rhinotracheitis (IBR). Haptoglobin was highest on d 14, and close to baseline levels by d 27. Titer levels for BVD-1 and IBR were higher on d 14, and significantly higher on d 27. Titers for bovine viral diarrhea type 2 (BVD- II) responded linearly ($P < 0.05$) to time with the highest levels on d 27. Haptoglobin was elevated in morbid animals compared to healthy pen mates ($P < 0.05$). Titer levels for BVD-I and IBR were higher in healthy animals ($P < 0.01$). Fecal cortisol was higher on arrival than on d 14 ($P < 0.05$). In summary, high-energy limit-fed diets based on corn by-products do not affect health and are more efficient than when roughage-based growing diets are fed.

Key words: stocker cattle, limit-feeding, programmed-feeding, by-products, immunity

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Chapter 1 - Review of Literature

Introduction

Purchasing lightweight stocker cattle for entry into the feedlot is common practice in the United States' beef industry due to low initial costs involved and potential for large returns on investment. However, there are many risks producers' must consider when choosing to invest in this sector of the industry. These animals are often newly weaned and subjected to extensive transport stress leading to dehydration, malnourishment, sickness, and other forms of stress. In addition, commingling at auction markets and unfamiliarity with new feeding facilities further exaggerates these issues. Appreciably, the extensive stress load to which stocker cattle can be exposed affects many physiological and psychological processes such as the immune system and the animal's willingness to consume feed on arrival to feeding facilities. Because intakes are generally low, conditions are suitable for large energetic deficiencies expanding overall stress further and causing metabolic and pathogenic disease. Utilizing higher energy diets early in the feeding period to combat low total intakes and prevent disorders is one strategy used by producers and nutritionists. However, excessive amounts of the incorrect source of energy could exacerbate the problem. Non-structural carbohydrate sources like starch in cereal grains are often used to increase dietary energy, but too much of this readily fermentable carbohydrate can lead to acidosis and other metabolic disorders and sicknesses. The use of high fiber low starch corn by-products as a primary energy source in high-energy diets to be utilized on arrival to feeding facilities and limit-feeding such diets to control over consumption and increase efficiency may be an economical means to manage risk and increase productivity in this sector of the cattle feeding industry.

The Newly Received Stocker Calf

Procurement of lightweight stocker cattle is an attractive and financially incentivized business decision employed by many cattle feeders in the United States. However, substantial risk is incurred and management of that risk is paramount. Characterizing the newly received stocker calf is the first step of many in optimizing performance, limiting morbidity and mortality, and ultimately maximizing the economical return on investment of young lightweight cattle entering the feedlot. Several aspects must be considered when deciding on management schemes having to do with such animals. These animals are faced with a plethora of stressors associated with duration of transport, dehydration, starvation, unfamiliarity with new feeding facilities, commingling at auction markets, and weaning status. After recognizing the risks associated with the stress of procurement and transport of newly received stocker cattle, the health status upon arrival must then be considered. Previous vaccination protocols, their ability to mount a robust immune response to vaccines and natural pathogen insults, as well as the potential of already morbid animals all play important roles. Centered in the manifest of unknowns associated with the management and handling of high-risk stocker cattle is their previous plane of nutrition and how this may affect the animal's ability to adapt from a diet of low-quality fibrous forages or even milk, to one based primarily on concentrate feeds like cereal grains and by-products. In summary, producers are faced with many challenges affecting the management practices needed to optimize performance, minimize health issues, and maximize the return on investment of high-risk stocker cattle.

Weaning management and preconditioning

There has been substantial research over the last 40 years analyzing the effects of stress due to weaning status, commingling at auction market facilities, unfamiliarity with new feeding facilities, and duration of transport to those facilities on health and performance of newly-received stocker cattle.

Weaning is one of the most stressful events in the course of life for a feeder calf (Loerch and Fluharty, 1999). Calves can be weaned from their mothers and shipped to auction market facilities or retained where they may be preconditioned. In most cases, weaning cattle before they are shipped to feeding facilities has shown benefits in terms of health, performance, and economical returns. Typical preconditioning programs involve introducing the animals to concentrate feeds soon after weaning in a bunk-fed scenario thereby decreasing the chance of low-energy intakes due to unfamiliarity. Also, it is commonplace to vaccinate cattle in the preconditioning phase to prepare the otherwise naive immune system for insult from the pathogens associated with the feedlot. One study conducted by Arthington et al. (2008) compared performance in the receiving period of calves that were weaned on the day of shipping, creep-fed, where they were allowed access to concentrate feeds on pasture, preweaned and provided supplemental concentrate on pasture, or early weaned at 70 to 90 days of age. These workers found calves that were early weaned exhibited the highest ADG and G:F ($P < 0.01$). Alternatively, Step et al. (2008) reported conflicting results to the prior study in regard to performance in the receiving period as it relates to weaning management. In this study, auction market derived calves were compared to calves that were weaned immediately prior to shipping, weaned for 45 days before shipping, or weaned for 45 days before shipping and vaccinated. Over the course of the 42-d study period, G:F and ADG were unaffected by weaning

management practice ($P = 0.17$ and $P = 0.46$, respectively). One explanation for the differences detected, or not detected, in performance for these two studies may be the length of the receiving period. Arthington et al. (2008) used a 28-d receiving period compared to 42 days used by Step et al. (2008). Over time, any performance benefit may be nullified as the cattle adapt to the current environment and management practices.

Although the effects of weaning management on performance in the receiving period may vary, the economical return from pre-conditioned calves is often times still higher when the costs of health issues are taken into account. Richeson et al. (2012) conducted a study using 528 calves to determine the effects of preconditioning vs. auction market derived on health and the resulting costs associated with increased morbidity. In this study, total morbidity due to BRD was 70.45% for auction market derived calves vs. 6.7% ($P < 0.001$) for cattle that were preconditioned with the protocol involving vaccination with modified live virus before entering the feedlot. Furthermore, 4.4% of treated auction market derived calves became chronically ill vs. 0.7% of preconditioned cattle ($P = 0.03$). The resulting impacts on total antibiotic costs from the two management practices were evident with auction market derived calves costing $20.51 vs. $2.51 for cattle that were preconditioned ($P < 0.0001$). However, it is important to note that in this study calves were exposed to calves persistently infected with BVD (BVD-PI). However the main effects of exposure and the interaction between exposure and management technique were not significant ($P > 0.05$). Agreeing with the results of Richeson et al. (2012), Boyles et al. (2007) used three different weaning strategies to determine their effects on health on performance of newly received stocker cattle. The treatments in this study were weaning immediately prior to shipping, weaning 30 d before trucking and placing in a dry lot, or weaning 30 d before shipping and pasturing with fence-line contact to their dams. Calves that were

weaned in the pasture had an overall initial morbidity of 15% vs. 28% for calves weaned onto the truck and 38% for calves weaned in a dry lot. The effect of treatment in this case was significant ($P = 0.03$). Calves from all treatments were vaccinated against common bacterial and viral diseases 45 d before shipping, and calves that were weaned onto the truck received a booster 30 d before shipping with another modified live vaccine. This could explain the higher morbidity associated with the dry-lot calves, as they did not receive the booster leading to levels of antibodies below that needed to protect the animals from disease.

Weaning management and preconditioning practices vary widely amongst producers. Differences in performance due to these strategies may differ between experiments as the environment, time of year, disposition, and perhaps most importantly the diets offered in this crucial time are often very different. Granting the inherent variability from those factors, it is likely the return on investment of stocker cattle entering the feedlot is increased by weaning cattle in some form and highest when preconditioning vaccination is involved by decreasing overall morbidity and total health costs per calf. Both of these techniques may better prepare the calf for their journey through the production cycle.

Time in transport

Often, the distance traveled from origin to feeding facilities is extensive and highly variable, ranging from 0 to over 2000 km in extreme cases. Cernicchiaro et al. (2012) conducted an observational study involving data gathered from 21 U.S. commercial feedlots in the U.S. between the years of 1997 to 2009. Cattle were analyzed as cohorts in a multivariable mixed-effects negative binomial model to predict BRD morbidity, overall mortality, ADG, and HCW based on distance traveled (DTV), mean arrival BW, cohort size, and several other demographic

variables. These workers found that DTV was associated with BRD morbidity and overall mortality with the highest of both occurring in cattle traveling over 1000 km ($P < 0.05$). Across all categories of cohorts, ADG was less in cattle traveling the longer distances than in cattle traveling ≤ 250 km. Negative effects on HCW were also detected across cohorts with the largest effect realized when cattle were transported between 501 to 750 km. In addition, negative effects on all response variables, excluding HCW, were greatest when the interaction of DTV x BW was considered ($P < 0.05$), with the largest effect present in longer distances traveled and lighter weight cattle. These results are in agreement with the findings of Sanderson et al. (2008) who conducted a similar observational study using a survey instrument administered by the USDA in 1999 that involved 102 feedlots in Midwest U.S. comprised of 122 pens and 20,136 cattle. Their objective was to use distance traveled to feeding facilities along with arrival BW and other cattle attributes to predict morbidity in feeder cattle over a 12-week observation period. These workers concluded that initial morbidity associated with respiratory disease increased by 10% across all categories of cohorts for every 160 km increase in transport distance ($P = 0.00$) with the highest morbidity observed in lighter weight cattle (< 250 kg).

Feed intake on arrival

The stressors involved with procurement and travel to feeding facilities often have a profound effect on intakes early in the feeding period. Hutcheson and Cole (1986) observed highly variable feed intakes in cattle received at the Texas Agricultural Experiment Station in 1980. Calves consumed 0.5 to 1.5%, 1.5 to 2.5% and 2.5 to 3.5% of their body weight on days 1 to 7, 8 to 14, and 15 to 28, respectively. These researchers noted that in the first 7 days after arrival calves consuming 0.5% of their body weight were from one source and unfamiliar with

eating from a bunk, while calves that consumed 1.5% of their bodyweight had been exposed to a preconditioning program where they had been bunk fed. Furthermore, these workers found that only 38.9% of calves were observed feeding on d 1 and it was not until d 7 that 88.1% of calves were observed eating from the bunk. To further compound the problem, only 27% of sick animals were observed eating on d 1 and 70% on d 7. Fluharty et al. (1994) designed an experiment to determine whether the low intakes on arrival were due to limited diet digestibility or other factors. Using 60 Angus crossbred steers the experiment was a 2 x 2 factorial arrangement of treatments with 2 levels of energy (high and low) and two different protein sources (blood meal and soybean meal). The results of their experiment showed total tract digestion was maximized on d 7 due to the low intakes during the first week after arrival. Therefore, digestion is not hindered early in the receiving period but rather maximized, and decreased intakes are due to other factors. Today, it is generally accepted that low intakes on arrival are due to the stress associated with procurement and travel with little association with digestion.

The consequences of low intakes on arrival can be harsh. It is at this time the animal's immune system is expected to mount potent and timely responses to vaccines and pathogens (Loerch and Fluharty, 2000). If energy is limited, the immune system will not function properly, which leads to increased morbidity, mortality, and decreased performance.

Understanding the risks associated with newly received lightweight stocker cattle is undeniably one of the most determining factors of profitability in this sector of the industry.

Stress and Immune Function: A Complex Interaction

Stress is defined by Stott et al. (1981) as the "external body forces that tend to displace homeostasis in the animal." There are a variety of factors contributing to the stress of newly received feedlot cattle such as environment, health issues, handling, transportation, and weaning. Stress from these stimuli have an effect on the animal's immune system. However, recently it is more commonly debated whether or not these effects are more immuno-stimulatory or immuno-modulatory in nature.

The stress response and the immune system interact through what is known as the hypothalamic-pituitary-adrenal axis (HPA) (Smith and Vale, 2006). This complex set of organs involving the hypothalamus, pituitary, and adrenal glands is responsible for the maintenance of homeostasis, which is chiefly carried out by a potent family of steroidal hormones known as glucocorticoids (Bellevalence and Rivest, 2014). Receptors for glucocorticoids are in most of the cells belonging to the immune system, and therefore are directly impacted by the secretion of GCs from the adrenal glands during stress.

The immune system

The immune system is wholly described as the host's defense against external pathogens such as bacteria, viruses, parasites and also internal sources such as cancer cells and those associated with autoimmunity. The immune system is separated into two sub parts; innate immunity and adaptive immunity that work together in concert to protect the host.

Innate immunity describes the first line of defense for the host and includes physical barriers such as the integument and mucosal barriers in the respiratory and digestive systems as well as the cellular innate immunity brought on by natural killer cells and phagocytic cells. Also

included in innate immunity is the complement system. After physical barriers are compromised, phagocytic cells like macrophages ingest pathogens and cellular debris, which then activate PAMPs (pathogen associated molecular pathways) and DAMPs (damage associated molecular pathways) receptors. The initial stimulation of these receptors along with TLRs (toll-like receptors) stimulate cells of the innate immune system to secrete cytokines responsible for recruitment and activation of the adaptive immune system (Vivier et al., 2011).

Adaptive immunity is further broken down into humoral immunity and cell-mediated immunity. Humoral immunity consists of the antibodies produced by B-cells during infection. Adaptive immunity is associated with T-cells that become activated by macrophages and other antigen-presenting cells to specifically aid in the destruction of pathogens present at the time of insult. Unlike the non-specific nature of innate immunity, adaptive immunity has evolved with a high level of specificity to pathogens present. Activated T-cells secrete cytokines that insure the proliferation of B-cells specific to antigens associated with the pathogen present. Innate and adaptive immunity work in concert in a complex and highly regulated biochemical system to eliminate internal and external threats to the host while minimizing negative effects (Hoebe et al., 2004).

Immuno-modulatory effects of stress

Traditionally, the HPA has been considered as a potent inhibitor of the immune system. Norrman et al. (2003) studied the effects of a synthetic glucocorticoid (dexamethasone) treatment on immune function of calves that received colostrum and those that received a nutrient-balanced formula with or without IgG. These workers found that dexamethasone decreased B-cell proliferation in Peyer's Patches in the small intestine of calves regardless of

receiving colostrum or formula with or without IgG. However, serum IgG was not affected by dexamethasone in those individuals that received colostrum-derived IgG in formula or those receiving colostrum. The authors from this study speculated that simulated stress in this experiment by dexamethasone inhibits immune function in calves not receiving colostrum. Those calves receiving colostrum obtained the antibody through normal gut absorption, briefly bypassing the need for the calf's immune system to produce antibodies. This research highlights the importance of successful passive transfer as stress is abundant at this point in life. In agreement, Anderson et al. (1999) conducted a study to analyze the effects of a dexamethasone on immune function. They treated 6 holstein steers with a short acting dexamethasone injection followed 37 hours later by a long acting version of the drug. Dexamethasone caused leukocytosis in the treated animals but decreased lymphocyte proliferation upon exposure to phytohaemagglutanin. Serum concentrations of IgM were decreased. In a more realistic setting Stanger et al. (2005) designed an experiment to study the effects of transportation on the immune system. Steers (n=10) were shipped a total of 72 hours and blood was drawn 48 h before shipping, at arrival, and 6 d later. Total leukocyte numbers were decreased by transportation ($P < 0.05$). Pytohaemagglutanin was also used to stimulate lymphocyte proliferation, which was decreased by transportation ($P < 0.05$). On the contrary, antibody titers to tetanus toxoid were unaffected by shipping. It is important to note that all measured immune-related function returned to baseline levels 6 d post-arrival. Coinciding with the previous study, a similar experiment was conducted by Blecha et al. (1984). In this experiment, steers were shipped 700 km to a feedlot. On arrival, lymphocyte blastogenic responses were decreased but, contrary to the study above, total leukocyte numbers were increased due to shipping. One cause for this

difference could be the cattle in the second study were exposed to transient infection during their journey stimulating leukocytosis.

Research indicates that stress has a profound effect on the immune system due to the activation of the HPA axis. The examples above indicate that in some cases the effects may be more immuo-modulatory than they are stimulatory regardless of whether or not the stressor is naturally or artificially induced. On the other hand, most of the same studies indicate at least some aspect of immuno-stimulation specifically with leukocytes.

Immuno-stimulatory effects of stress

As in the above examples, stress has been shown in numerous cases to hinder or suppress at least some element of the immune system. For many years, it was believed that stress only has negative effects on the immune system but more recent research suggests otherwise. Minton (1994) published a review introducing another feature of the activation of the HPA. Going one step deeper than glucocorticoids mentioned formerly in this review, he describes the role of ACTH (adrenocorticotropic hormone) as the chief regulator of the production and release of adrenal glucocorticoids. Minton et al. (1993) conducted a study to measure the effects on the immunes system of cortisol produced during a stressful event vs. similar levels of circulating cortisol induced through injections. The model attempted to show differences between HPA activation alone through the injections of cortisol and also cortisol produced naturally during a stressful event through restraint of sheep and the accompanying tone of the sympathetic nervous system. Restraint decreased the proliferation of lymphocytes in response to pokeweed mitogen, phytohaemagglutanin, and concanavalin A, all of which stimulate proliferation in control animals. Alternatively, cortisol alone, which only activated the HPA, did not negatively affect

lymphocyte proliferation. Other research has shown more positive effects than negative on the immune system from stress. These results agree with a study cited earlier by Anderson et al. (1999) where stress induced by dexamethasone caused leukocytosis but decreased lymphocyte proliferation.

Recent research shows that stress affects the two branches of the immune system differently. On one hand, the innate immunity comprised of phagocytic cells, natural killer cells, and other leukocytes seems to be stimulated by stress. On the other, in the case of adaptive immunity as measured by immuno-stimulatory compounds like pokeweed mitogen and Con A, stress appears to hinder lymphocyte proliferation. From an evolutionary view it makes sense that at least part of the immune system should be inhibited by stress, specifically adaptive immunity, because without tight regulation the immune system can destroy the host through autoimmunity and other detrimental cascades. Stimulatory effects of stress on parts of the innate immune system may better prepare and protect the animal for various pathogenic and environmental insults while the adaptive arm of the process is suppressed as to not inflict damage. Research is ongoing in the field of stress and immunology, but most authors would agree that less stress corresponds to increased productivity for domestic livestock.

Receiving Diets to Meet the Needs of Stressed Calves

It is known that feed intakes of calves upon arrival to feeding facilities are generally low, thus setting the animal up for a plethora of metabolic and pathogenic diseases brought on by energetic deficiencies. Diet formulation at this point in the feeding period is crucial, as it should allow for adequate energy intakes in the face of low total feed intake to fuel the challenged immune system, which could already be hindered by stress, and ensure adequate performance

throughout the receiving and growing period. Formulating diets with sufficient energy, but not so much to cause metabolic issues is a common problem facing producers in this sector of the cattle industry.

Concentrates as an energy source

Generally speaking, the total mixed diet contains ingredients that fall into one of two categories; roughages and concentrates. Concentrates describe the grains and processed feeds that are often higher in energy density and total digestible nutrients (TDN). As their name would imply, the energy and other important nutrients are "concentrated" in these feedstuffs. Of the concentrates fed to cattle today, cereal grains and by-products produced during their refinement are often the most common sources of dietary energy due to their availability and relatively low cost. The economic benefit cereal grains bring to the diet stems from the chemical structure of starch, its vulnerability to microbial degradation, and the energy yielding end products of their fermentation in the form of volatile fatty acids. Cereal grains contain between 57 and 77% starch on a DM basis depending on the specific grain with wheat being at the top of the spectrum, oats at the bottom, and corn falling somewhere in the middle (Huntington et al., 2006). Of course these characteristics would vary depending on environmental factors and agricultural management practices related to planting and harvest. For the purpose of this review corn and its by-products will be the focus of discussion.

Dietary characteristics of corn grain

Corn grain is used in the majority of the livestock feeding industry due to its relatively high concentration of starch, overall availability, as well as financial benefits when considering

other energy sources. Starch is a polysaccharide made from glucose monomers of amylose and amylopectin joined by α1-4 and α1-6 linkages. In the rumen, starch is mostly fermented by what are known as amylolytic bacteria although other organisms like protozoa and fungi can also influence the process. Protozoa can encapsulate starch granules preventing bacterial degradation while the hyphae of fungi can promote microbial attack by mechanical disruption of the outer coatings of plant material (McAllister et al., 1994). Of the three major volatile-fatty-acids produced in ruminal fermentation (acetate, propionate, and butyrate) proportions of propionate increase during grain fermentation (35 to 45 moles/100 moles VFA) (Ørskov, 1986). Propionate is beneficial to ruminant metabolism for several reasons. More hydrogen is captured in the production of propionic acid that can be used by the animal and less lost through the production of methane characterizing it as a hydrogen sink. In addition, more hydrogen going toward propionic acid synthesis means less available H^+ ions to contribute to decreases in ruminal pH. Perhaps most importantly, propionic acid production is also the most energy yielding VFA to the animal metabolically as 43 to 67% of carbon skeletons used by the liver to synthesize glucose during gluconeogenesis originate from propionic acid (Huntington, 1981).

Aside from ruminal digestion, starch can also be metabolized in the small intestine. After entering the small intestine, pancreatic α-amylase begins to hydrolyze the α1-4 and α1-6 linkages described above. Following initial breakdown into simpler units, oligosaccharidases in the brush border of the small intestine further degrade the monomers into glucose units for absorption into the blood stream. SGLT1 transporters are responsible for facilitating the absorption of one glucose molecule with two NA^+ ions (Huntington et al., 1997). There is accepted conjecture that starch digestion taking place in the small intestine could represent an energy savings when one considers the costs of heat increment and gas production during fermentation (Ørskov, 1986).

However, this idea has been tested and the amount of starch digested in the small intestine is more a function of pancreatic amylase availability rather than actual amounts of starch reaching the lower gastrointestinal tract (Kreikemeier et al., 1991). These workers found the capacity of oligiosachharidases to digest, and SGLT1 transporters to facilitate absorption, exceed apparent starch digestion when it was infused into the small intestine leaving pancreatic amylase as the controlling factor of postruminal starch digestion. Nonetheless, of the 5 to 20% of starch that could reach the lower GI tract for digestion, the majority of that digestion takes place in the small intestine, emphasizing that the rumen is not the only site of digestion (Streeter et al., 1989).

Fiber in concentrate-based diets

The rumen has evolved to be a favorable site for microbial fermentation of fibrous forages due to its aqueous and anaerobic environment in addition to the constant inflow of substrate and removal of end products (VFAs). Digestion of fibrous feedstuffs requires extensive cud regurgitation, mastication, and rumination that lead to more steady saliva flow to the rumen. Components in saliva such as bicarbonates and phosphates help to prevent acidosis by increasing osmolality in the rumen thus forcing more water in, diluting organic acids, and also neutralizing excess protons from fermentation. Normally, fibrous forages are fermented to acetate, propionate, and butyrate. Sub-acute ruminal acidosis is possible simply by the accumulation of these VFAs, but the far more insidious form of the disease known as lactic acidosis results when grain fermentation and lactate production exceed the level by which lactic-acid utilizing bacteria are able to convert lactic acid to more harmless organic acids. When lactic acid accumulates, microbial populations responsible for the fermentation of structural carbohydrate are negatively affected but lactic acid producing bacteria multiply rapidly (Hungate

et al., 1952). At this point, total VFA production begins to decline while lactic acid concentrations continue to rise. When ruminal pH is below the threshold to activate chemostatic receptors located in the rumen, contractions begin to slow and eventually stop in attempt to slow fermentation in the rumen and decrease the level of fermentation (Crichlow and Chaplin, 1985). The collective symptoms and physiological responses to lactic acid accumulation in the rumen make up what is known as acute acidosis. Acute acidosis can lead to decreased performance, increased morbidity, and even death. In contrast, sub-acute ruminal acidosis or SARA occurs when organic acids accumulate due to organic acid production but not from excess lactic acid production. Sub-acute ruminal acidosis is generally the point where negative effects on fiber digestion begin to occur and non-structural carbohydrate fermenting bacteria proliferate (Mackie and Gilchrist et al., 1979). Generally, saliva production and VFA absorption by ruminal epithelium correct SARA. However, decreased ruminal pH and acidosis have been shown to decrease VFA absorption (Wilson et al., 2012). The pH thresholds for these two diseases vary depending on whether or not time spent at a low pH is considered or the lowest pH is considered (nadir pH). In either case, the threshold for SARA is generally considered to be a ruminal pH below 5.5 and for lactic acidosis or acute acidosis a ruminal pH below 5.0 (Aschenbach et al., 2011). Balancing roughage in concentrate-based diets is crucial in limiting the occurrence and duration of metabolic disorders brought on by excessive grain fermentation. SARA and acute acidosis are usually thought to be the cause of increased morbidity in high-energy diets due to the local inflammation and resulting immunosuppression by the irritated ruminal epithelium.

Fiber from roughages plays an important role in the formulation of beef cattle diets. The environment of the rumen is such to favor fiber digestion and often times high roughage diets lead to less metabolic disorders such as ruminal acidosis when compared to diets with excessive

readily fermentable carbohydrate. Unfortunately, feeding increased levels of roughage is not economically feasible as performance decreases with decreased energy (Rivera et al., 2005). Today, with the use of by-products and intensive programmed feeding systems, there could be alternatives to traditional sources of fiber and their inclusion into cattle diets could maintain dietary energy at elevated levels without ruminal acidosis becoming an issue.

Balancing energy in receiving diets

A common paradigm amongst ruminant nutritionists dealing with young, stressed calves is as dietary energy is increased in the receiving diet, health issues also increase. Most of this ideology stems from work done by Lofgreen et al. (1975) where several experiments were carried out to determine the effects of dietary energy on health and performance of calves subjected to marketing stress. Dietary treatments based on rolled barley concentrations of 38, 46, 50, and 54% (0.84, 1.01, 1.1 and 1.19 Mcal NE_g/kg DM, respectively) were used and concentrate levels were 20, 55, 72, and 90%, respectively. Results from the trials indicated decreased intakes in the first week after arrival followed by rapid compensation. However, more calves were treated for respiratory disease in the first weeks after arrival in one of the trials thus incurring higher medication costs. Another trial included in the analysis showed no differences in overall morbidity among the concentrate levels. The authors did note that performance throughout was increased by increasing dietary energy as animal weight gain was comparable and achieved more efficiently on the higher concentrate diets. Still, the authors cautioned readers about adopting higher energy receiving protocols in light of possible increases in morbidity. More recently, Rivera et al. (2005) used data from a number of experiments carried out in one location in a mixed model regression setting to plot dietary roughage against

performance parameters and health data. Morbidity decreased with increasing roughage levels such that morbidity % = 49.59 – 0.0675 x roughage %, which agrees with Lofgreen et al. (1975). Also in agreement with Lofgreen et al. (1975) data were the negative effects on performance when roughage increased: ADG (kg) = 1.17 – 0.0089 x roughage (%), DMI (kg/d) = 5.34 – 0.0135 x roughage (%). However, characteristics of concentrates and roughages used in these trials such as extent of processing could have supplied variation that was not controlled for in the meta-analysis (Rivera et al., 2005). In a review published by Galyean et al. (1999) more of Lofgreen et al. (1975) work was cited, noting that calves subjected to stress selected for a higher energy diet over a lower roughage based diet when given the choice.

Berry et al. (2004a,b) designed trials to further examine the effects of energy on overall health, immune function, and performance of growing cattle subjected to stress. In Berry et al. (2004a) diets containing two energy levels of 0.85 or 1.07 Mcal NE_g/kg DM and two starch levels of 34 or 48% of ME from starch were fed to growing cattle to analyze their effects on health and performance in a 2 x 2 factorial design. The objectives were to establish whether effects on health and performance were a result of increased energy alone or increased energy from starch. Dried distiller's grains without solubles were used to replace corn in the low starch diets. These researchers found that there were no differences in performance in ADG or G:F for cattle receiving the different energy levels or starch levels. The results are in contrast to Lofgreen et al. (1975) who observed increased ADG with increased energy but are in agreement with Fluharty and Loerch (1996) who also saw no differences in performance with increased energy. Berry et al. (2004a) did report increased DMI with the lower energy diets possibly due to the animals compensating with higher intakes to obtain energy needed. There were no significant differences in cattle being treated once, twice, or three times for respiratory disease

among treatments. However, there was a tendency ($P = 0.06$) for fewer cattle being treated three times on the lower energy diets. Interestingly, these workers also found that cattle treated for respiratory disease on the higher energy diets had fewer *P. multocida* and *H. somnus* pathogens when nasal swabs were analyzed. Berry et al. (2004b) used the same diets to test their effects on the acute phase protein response as measured by haptoglobin, serum amyloid A, and fibrinogen. The results from this study suggested that energy levels as high as 1.07 Mcal NE_g/kg and dietary starch concentrations of 48% do not affect the acute phase protein response. They also found haptoglobin concentrations at day 0 could be a useful predictor of antimicrobial treatments required.

Limit-feeding and Corn By-products

As mentioned earlier in this review, there tends to be a paradigm amongst ruminant nutritionists that as dietary energy is increased in the diet, health issues generally become more common. Most scientists would agree that ruminal acidosis caused by excessive grain fermentation could be the leading cause of the uptick in morbidity when caloric limits are approached. The easiest and most economical way to increase energy in the diet is to remove roughage and replace with concentrate and in many instances the concentrate of choice is a cereal grain high in starch. Rather than feeding high-energy diets free-choice to ensure ad libitum intakes it makes sense that feeding higher energy diets at controlled intakes could maintain total energy intake at levels adequate for acceptable performance without causing negative health issues brought on by overconsumption of readily fermentable carbohydrate. Alternatively, the ethanol and corn wet milling industries in the United States produce corn by-

products that are high in energy, but the energy is derived from fermentable fiber and protein, not starch.

Overview of distiller's grains and wet corn gluten feed

Ethanol production and the corn wet-milling process have started a new era in cattle feeding in the United States. During ethanol production corn grain is processed and mixed with yeast in a large fermentation vat where ethanol is produced and distilled as fuel from the starch within the grain. After the starch is removed, a digestible fiber-based product high in protein and dietary energy is produced. This product is known as distiller's grains and can be obtained as a dried product with the solubles from the mash added back as distiller's grains with solubles. The feed can also be purchased wet, which aids in the ration conditioning characteristics of the feedstuff (Stock et al., 1999). There are many advantages to feeding distiller's grains. It is usually a cheap source of energy and protein and also can be incorporated at high levels because the starch has been removed. In addition, because the protein in corn exists mostly as undegradable intake protein, more amino acids are available for use by the animal as the pass through they rumen undegraded.

Aside from the distillation process during the production of distiller's grains, corn grain is also refined in a process known as corn wet milling. The end products of the process include corn syrups that are used in many artificial sweeteners in the food industry as well as a product called wet corn gluten feed or simply gluten feed. The process of wet corn milling differs from ethanol distillation mostly by the fact that ethanol is produced during fermentation of starch in cereal grains while wet corn milling utilizes the steeping of corn in a dilute sulfurous dioxide solution. The components of the kernel are steeped and separated to produce the products above.

What is left is the bran from the kernel and the gluten protein (Stock et al., 1999). This product can be obtained dry as corn gluten meal or the bran, gluten meal, and the fluid from the steep can be added to produce wet corn gluten feed. Like the distilling process, the starch is again removed and highly digestible fiber-based products high in energy and protein are produced. Dietary energy intake can be increased greatly by the inclusion of these co-products because the dietary energy derived is protein-and fiber-based rather than starch, which can lead to metabolic issues.

Effects of limit-feeding

Limit-feeding, or what is sometimes referred to as programmed feeding utilizing high energy diets, is one approach used to solve the issue of maintaining energy intake in the face of low total intake, and restricting the animals ability to over-consume and self-inflict potential digestion-related health issues. This strategy involves programming energy in diets under the assumption that less total feed will be offered than what the animal is capable of consuming in terms of gastro-intestinal fill or energy. Most often this approach is used to target specific gains early in the feeding period when cattle are grown and also to increase efficiency because less total feed is required to achieve similar gains. Advantages and disadvantages are observed with the strategy.

Schoonmaker et al. (2004) conducted a trial that involved four diets to observe the effects of energy source and concentration on overall performance in the growing period of Angus x Simmental steers. The diets were a 50% concentrate diet fed *ad libitum* (ALC), a 70% concentrate diet limit-fed to achieve 0.8 kg/d ADG from days 119 to 192 and 1.2 kg/d from days 193 to 254 (LFC), a forage based diet fed *ad libitum* (ALF) and a positive control silage diet fed

ad libitum. Also compared in the study were two different weaning protocols where cattle fed the ALC, LFC, and ALF diets were weaned at 119 days of age and the cattle fed the silage diet were weaned at 204 days of age. They observed that in the growing period, limit-fed cattle were the most efficient in terms of G:F but final BW was lower in limit-fed steers. Quality grade distribution and marbling scores were unaffected by any of the treatments indicating that physiological maturity was hastened by limit-feeding. Because the cattle deposited adipose tissue more quickly they reached physiological maturity at lighter BW compared to cattle fed in more traditional scenarios. These results can be mostly explained by Schoonmaker et al. (2003) where circulating insulin was monitored in cattle limit-fed a similar ration to obtain 1.2 kg/d ADG. In this study insulin was increased by limit-fed high concentrate diets, which would in turn increase uptake of glucose by adipocytes in tissues. Propionate is one of the predominant VFA produced during starch fermentation and an important precursor for gluconeogenesis by the liver. In this trial propionate was increased by the high-energy limit-fed diet that would in theory accelerate gluconeogenesis and thus insulin production. Also in agreement with the first trial, the cattle fed the high-energy limit-fed ration were more efficient than cattle fed a diet *ad libitum* based on fiber. More work conducted by Schoonmaker et al. (2004) illustrates that carcass characteristics may differ after the growing phase but these differences are diminished after cattle are finished on the same diet such that growing cattle diets fed to achieve 1.2 kg/d ADG do not have drastically different carcass characteristics. This same study showed that hypertrophy of adipocytes had more of an effect on lipid deposition than did hyperplasia and that hypertrophy was affected more by level of energy intake than energy source.

The work above illustrates that limit-feeding high concentrate diets to restrict gain can at times extend the growth curve by allowing cattle to experience compensatory growth after the

growing phase. When intake is restricted on high-energy diets it is possible to achieve satisfactory gains without over-fleshing cattle from free choice access to the diets. This same effect was not realized when growth was limited through decreased energy intake in forage based diets most likely because there was not enough energy in the forage based diets to satisfy growth requirements. Across the trials conducted by Schoonmaker et al. (2003, 2004) cattle that were limit-fed high-energy diets were more efficient in the growing phase and the effect was still realized in the finishing phase. These results are in partial agreement with Knoblich et al. (1997) where the same diet was used at different levels of intake to program gain. After the predetermined target weight was achieved, the cattle were switched to an ad libitum diet. Because some cattle were put on the diet offered free choice quicker than others, the effects on efficiency seen in Schoonmaker et al. (2003, 2004) work may have been diluted. Nonetheless, economic analysis by Knoblich et al. (1997) showed that offering diets *ad libitum* in the growing phase was not more economical when compared to limit-feeding systems.

In regard to cattle health, as aforementioned, many nutritionists would be inclined to not recommend high-energy diets to newly arrived cattle or those in the growing phase of production. Trials conducted by Lofgreen et al. (1975, 1980, and 1981) do show some correlation between respiratory sickness and dietary energy but the response is not repeatable amongst trials, and Lofgreen et al. (1975) showed that stressed cattle often prefer higher energy diets. In addition, Fluharty and Loerch (1996) showed that diets containing between 70 and 85% concentrate yield similar performance and little effects on health. These authors concluded that high-energy diets were beneficial in newly received feedlot cattle.

Research since Lofgreen's work does not definitively correlate increased dietary energy in the growing and receiving phase with negative effects on health and oftentimes shows

increases in performance. Limit-feeding high-energy rations has been shown to be more efficient in the growing and receiving phase and in some instances more efficient throughout the entire feeding period due to carry-over effects. When health issues do occur, they are most often related to over consumption of the rations and metabolic disorders from excessive non-structural carbohydrate fermentation. Diets formulated to be high in dietary energy without excessive starch are now possible with the ready availability of by-products.

Utilizing corn by-products in limit-feeding protocols

Because corn by-products are generally high in energy and protein they have been the focus of recent research in the area of limit-feeding. Montgomery et al. (2003) used two experiments to evaluate to use of wet corn gluten feed in diets containing varying levels of alfalfa. In experiment 1, three diets containing steam flaked corn and 40% wet corn gluten feed (WCGF) with 0, 10, and 20% ground alfalfa (0AH, 10AH, and 20AH respectively) were offered 1.8% of BW once daily on a dry matter basis. A fourth diet containing steam-flaked corn and 20% alfalfa served as a control. These workers found that ADG and gain efficiency decreased linearly ($P < 0.05$) with increasing alfalfa in diets containing WCGF. They concluded that increasing alfalfa in the diet decreased NE values of the diet from the dilution of energy. In addition DMI increased as alfalfa increased in the diet. In a second experiment, treatments consisted of steam-flaked corn with 10, 20, and 30% alfalfa and 0, 40, or 68% WCGF fed at 1.6% of BW daily in a 3 x 3 factorial study. Results indicated ADG and efficiency decreased with increasing WCGF or alfalfa with the exception of a WCGF x Alfalfa interaction detected in the 40% WCGF 30% alfalfa diet. Cattle consuming this diet had similar ADG ($P > 0.10$) to those consuming 30% alfalfa and 0% WCGF or 20% alfalfa 0% WCGF but had higher

efficiencies of gain ($P < 0.05$) because DMI was decreased with decreasing roughage from the alfalfa. In conclusion, WCGF in diets containing alfalfa could replace some of the energy supplied by steam-flaked corn.

The increased performance due to WCFG can be explained by Montgomery et al. (2004) where digestion of the by-product and intake's effects on digestion were analyzed. Ruminal pH was increased by WCGF inclusion along with total VFA concentration presumably due to WCGF replacing steam flaked corn. However, when the same diets were fed ad libitum and limit-fed then compared, total tract OM digestion was decreased by limit feeding. These findings were in contrast to others who reported increased total tract organic matter digestion (Galyean et al., 1979; Murphy et al., 1994). Montgomery et al. (2004) hypothesized the difference in their findings to be due to meal eating behavior of the limit-fed diets producing an unstable ruminal environment. Because the calves in this trial were limit-fed at 1.6% of BW daily regardless of the diet, the meals were consumed rapidly thus increasing passage rate and presumably decreasing digestion. Increased digestion by limit-feeding has been highlighted as one of the advantages of such protocols, but the level of starch in these diets can still cause a problem in the face of meal-eating behavior.

Felix et al. (2011) conducted another limit-feeding trial to analyze the effects corn distiller's grains and corn fed at two intakes in the growing period on performance through the finishing phase. Limit-fed diets were fed to obtain 0.9 kg/d or 1.4 kg/d ADG. In the growing phase, DMI, ADG, and G:F were increased with corn compared to distiller's grains. In addition, the diets fed to obtain lower gains decreased DMI and ADG but had no effect on G:F. An energy source x intake was reported for DMI and Felix et al. (2007) speculated several reasons due to adverse effects of increased dietary N and sulfur concentrations in the DDGS which have

both been shown to have negative effects on digestion (Gunn et al., 2009; Gould et al., 1997, respectively). In the finishing phase however G:F and ADG were increased by limit-feeding whereas energy source had no effect. These results can be explained by the carry-over effect of limit-feeding in the growing phase as a result of compensatory growth in the finishing phase (Hicks et al., 1990). Overall, energy source and intake did not affect performance in terms of ADG, DMI, or G:F.

Conclusions

Managing the risks involved with lightweight stressed cattle entering the feedlot is a difficult task facing many producers in the United States. Animals subjected to shipping stress are often dehydrated, malnourished, and at times already ill upon entry to the feedlot. To compound the problem, feed intakes are typically low compromising the animal's immune system and setting the stage for a list of other diseases and metabolic disorders all potentially negatively affecting performance throughout the feeding period. Advances toward increasing energy intakes on arrival through the use of corn by-products and intensive programmed feeding designs could aid in the management and overall productivity of feeding lightweight cattle subjected to stress early in the feeding period.

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Chapter 2 - Effects of dietary energy and intake of corn by-product based diets on newly received growing cattle: I. Performance, health, and digestion

INTRODUCTION

Increasing dietary energy in diets for newly arrived cattle that have been subjected to marketing stress has been shown in some cases to increase morbidity early in the feeding period (Lofgreen et al., 1975, Rivera et al., 2005). One possible reason for the increase could be the increased incidence and severity of metabolic disorders such as acidosis initiated by the excessive fermentation of readily available carbohydrates such as starch. Nonetheless, increasing energy in diets to be fed to such animals is an often-considered strategy, because total DMI in the receiving period is generally low or at least sporadic (Hutcheson and Cole, 1986). Energetic deficiencies are possible when typical low-energy high-roughage receiving diets are utilized.

Limit-feeding, sometimes referred to as programmed feeding, involves high-energy diets offered at specific intakes to program gain and often times produces gain more efficiently (Schoonmaker et al., 2003, 2004) because less total feed is consumed. Most often, diet formulation still involves primary energy sources based on cereal grains. To our knowledge, there has not been work done addressing a large range of dietary energy levels and intakes to determine their effects on health and performance of stocker cattle. In addition, little work has been done in this area with diets primarily based on corn by-products like distillers's grains and wet corn gluten feed in limit- or programmed-fed receiving systems.

MATERIALS AND METHODS

All procedures involving the use of animals were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC # 3745 and 3696).

Experiment 1. Performance and Health Study

A total of 354 crossbred heifers (BW = 217 ± 4 kg) were purchased at auction markets in Alabama and Tennessee, assembled at an order buyer's facility in Dickson, TN then shipped 1,086 km to the Kansas State University Beef Stocker Unit over a 10-d period from May 24 to June 3, 2016. The heifers were used in a randomized complete block design to analyze the effects of 4 energy levels and intakes of fibrous by-product based diets on health and performance of stocker cattle in a 55-d receiving and growing study. Calves were blocked by load (4), stratified by individual arrival weight within load and assigned to pens containing 11 or 12 heifers. Pens within each block were randomly assigned to 1 of 4 treatments that equaled 8 pens/treatment for a total of 32 pens. The pens were soil surfaced and of equal size (9.1 x 15.2 m). Concrete bunks were 9.1 m in length and attached to a 3.6-m apron. Experimental diets (Table 2.1) were formulated to provide 0.99, 1.10, 1.21, or 1.32 Mcal NE_g/kg DM and were offered for ad libitum intake (0.99/100), 95 (1.1/95), 90 (1.21/90), or 85% (1.32/85) of ad libitum intakes. All diets were formulated to contain 40% wet corn gluten feed (Sweet Bran; Cargill Animal Nutrition, Blair, NE) on a DM basis.

At the time of arrival, calves were individually weighed, given an individual identification ear tag, and grossly assessed for disease and lameness. All animals were ear-notched, and the samples placed on ice until shipped following processing to the Kansas State University Veterinary Diagnostics Laboratory to identify animals persistently infected with

Bovine Viral Diarrhea by a commercial kit utilizing a polymerase chain reaction assay (7500 Fast Real-Time PCR Systems, Applied Biosystems, Thermo Fisher Scientific, Austin, TX). Animals not demonstrating disease or lameness were assigned to 1 of 32 pens to stand overnight (11 or 12 heifers/pen). Each pen was provided long-stem hay and ad libitum access to water through automatic waterers.

The morning after arrival (d 0), calves were weighed, tagged with a pen number, and vaccinated for respiratory and clostridial disease. For clostridial pathogens, Vison 7 Somnus with Spur (Merck Animal Health, Omaha, NE), was used and for respiratory pathogens, Pyramid 5 + Presponse SQ (Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO), a modified-live vaccine against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea types 1 and 2 (BVDI-II), parainflueza 3 (PI3), and bovine respiratory syncytial virus (BRSV). Calves were also treated on d 0 for internal parasites with 10% Fenbendazole (Safe-Guard, Merck Animal Health) and administered enrofloxacin (Baytril 100, Bayer Animal Health, Shawnee Mission, KS). All animals were revaccinated on d 14 with Bovishield Gold 5, an additional modified-live virus against IBR, BVDI-II, BRSV and PI3 (Zoetis, Parsippany, NJ).

Animals were fed once daily at 0700 h using a Roto-Mix feed wagon (model 414-14B, Dodge City, KS). Refusals were collected each day at 0600 h immediately before feeding and those of pens offered the 0.99/100 treatment were used to calculate DMI each day and adjust feed delivery for the remaining treatments as described above. Refusals were targeted at 10% of feed delivery for the 0.99/100 treatment. Individual cattle weights were measured on d 0, at revaccination (d 14), and at conclusion of the study (d 55). A pen scale (Rice Lake Weighing Systems; Rice Lake, WI) was used to measure pen weights on d 27 and 42. After pen weights were measured on d 42, cattle were offered the 0.99/100 treatment for ad libitum intake through

d 55 to equalize differences in gut-fill. Performance data was calculated from d 0 to each weigh period. Samples were collected from ingredients and the total mixed ration for each diet weekly and composited for analysis (Table 2.2) by a commercial laboratory (SDK Laboratories, Hutchinson, KS).

Animals were observed twice daily for signs of morbidity that included overall depression, nasal and/or ocular discharge, and anorexia. Any animal displaying these symptoms was removed from the pen and taken to the hospital facilities. Once restrained in the chute, rectal temperature was measured and a clinical illness score (CIS) were recorded such that a CIS of 1 was a normal healthy animal; 2, slightly ill with mild depression or gauntness; 3, moderately ill demonstrating severe depression/labored breathing/and nasal or ocular discharge; and 4, severly ill and near death showing minimal response to human approach. Animals pulled from the pen with a rectal temperature $\geq$ 40°C and demonstrating a CIS $\geq$ 2 were treated following label instructions. At first morbidity animals received florfenicol and flunixin meglumine (300 and 16.5 mg/mL, respectively; Resflor; Merck Animal Health, De Soto, KS). At second morbidity, ceftiofur (200 mg/mL; Excede; Zoetis, Parsippany, NJ), and at third, oxytetracycline (200 mg/mL; Bio-Mycin 200, Boehringer Ingelheim Vetmedica, Inc., St. Joeseph, MO). On the third treatment, animals were considered chronic and removed from the trial.

Additional Animal Manipulation

Readers are directed to Chapter 3 for procedures involving additional manipulation involving the animals used in this experiment.

Experiment 2. Intake and Digestibility Study

Six ruminally cannulated Jersey crossbred steers (BW = 255 ± 23 kg) were used to determine diet digestibility and characteristics of digestion. The study was designed to be a 4 x 4 Latin Rectangle with 8 animals; however 2 steers were removed from the study and their data was not used due to issues involving the rumen cannulas. Experimental diets were the same as for Exp. 1 (Table 2.1). Feed was mixed daily for Exp. 1 and the amount needed for Exp. 2 removed. Because the feed for Exp. 2 always was removed from the beginning of the wagonload, samples were analyzed separately from those in Exp. 1 (Table 2.2).

Animals were housed in individual stalls (3.7 x 3.7 m) in a fan-cooled barn. Each stall had access to an individual automatic waterer. Animals were fed once daily at 1100 h. Before trial initiation, animals were fed the 0.99/100 treatment from Exp. 1 for 2 wk to determine ad libitum intake to serve as its own control in the design. After the 2-wk adaptation, animals were assigned their treatments and fed in the same fashion as the animals in Exp. 1 based on their initial DMI of the 0.99/100 such that animals assigned the 0.99/100 treatment continued to be fed for ad libitum intake, those on the 1.10/95 were offered 95%, the 1.12/90 offered 90%, and the 1.32 offered 85% of DM consumption in the adaptation period. Refusals for the animals on the 0.99/100 diet were targeted at 10% in the adaptation and during the experiments. The trial consisted of 4 consecutive 15-d periods comprised of 10-d diet adaptation, 4-d fecal sampling, and 1 d for ruminal sampling.

Total mixed ration and ingredient samples were collected on d 10 through 14 and composited for each period for analysis. On d 4 through 14, Cr_2O_3 (10 g) was top-dressed and hand mixed into the ration as a marker to calculate digestibility. On d 11 through 14 refusals were collected and composited for each animal for each period. Also on d 11 through 14, fecal

samples were collected from the rectum of the steers every 8 h with the sampling time increasing by 2 h each day such that every 2 h interval after feeding was represented for 24 h. Refusal and fecal samples were composited for each steer in each period and sent to an independent laboratory for analysis (SDK Laboratories, Hutchinson, KS). Fecal samples (3g) used to determine digestibility were weighed wet into 50-mL crucibles and ashed in a muffle oven at 600°C for 3 h. Chromium concentrations were determined by atomic absorption spectrophotometry after solubilization of Cr_2O_3 in ash following the procedures of Williams et al. (1962). Feed refusals were dried at 105°C in a forced-air oven overnight and then ground to pass through a 1 mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ). They also were ashed at 600°C and chromium concentrations analyzed by atomic absorption following the same procedures of Williams et al. (1962).

On d 15 of each period, 5 locations in the rumen were sampled before feeding, and 50 mL of ruminal fluid was strained through 8 layers of cheesecloth immediately (0-h sample). After straining, 1 mL of ruminal fluid was pipetted into four 2-mL micro-centrifuge tubes each containing 250 μL 25% (wt/vol) *m*-phosphoric acid and then frozen at -20°C. Directly following 0-h sampling, Co-EDTA (0.4 g Co) dissolved in 200 mL water was dosed through the ruminal cannula. Ruminal digesta samples were collected again at 2, 4, 6, 8, 12, 18, and 24 h and an additional 20 mL of strained ruminal fluid collected in 20-mL scintillation vials and frozen to determine concentrations of Co.

Ruminal fluid samples were analyzed for VFA concentrations by GLC and for ammonia according to Broderick and Kang (1980). An indwelling pH monitoring bolus (SmaXtec, Graz, Austria) inserted through the ruminal cannula on initiation of the trial was used to continuously monitor pH over time. Co concentrations were analyzed in ruminal fluid and in the original dose

using atomic absorption spectrophotometry. Liquid passage rate was determined by regressing the natural logarithm of [Co] in the 2-18 h samples against time for each steer in each period using the nonlinear procedure in SAS (ver. 9.4; SAS inst. Inc., Cary, NC); rate was identified as the negative slope of the regression.

Experiment 3. Performance and Health Study

A total of 370 Angus x Brahman heifers (223 ± 19 kg) were assembled from a single source in central Florida and shipped to the Kansas State University Beef Stocker Unit (2,342 km) over a 2-d period from August 11 to 12, 2016 (2 loads each day). The heifers were used to validate results observed in Exp. 1 using the same high-energy limit-fed receiving diets based primarily on Sweet Bran and the use of a novel immunostimulant technology injected intramuscularly at the time of arrival processing (Zelnate, Bayer Animal Health, Shawnee Mission, KS). On arrival, calves were blocked by arrival date, unloaded, and placed in pens to be held over night (11 to 12 animals per pen) where they were allowed access to long-stem hay and water. Personal communication with the cattle owner indicated processing the animals on both d -1 and d 0 could be detrimental to animal health therefore initial processing did not take place on arrival. Because weights were not measured at the time of arrival (d -1), animals were randomly assigned to pens, and pens randomly assigned to 1 of 2 dietary treatments (1.1/95 or 1.32/85) from Exp. 1, and 1 of 2 arrival management protocols where animals did or did not receive Zelnate (Bayer Animal Health, Shawnee Mission, KS) on d 0 in a 2 x 2 factorial arrangement of treatments. There were a total of 8 pens/treatment combination. Pens used in this trial were the same used in Exp. 1.

Animals received the same vaccines and treatment for internal parasites on d-0 processing as those in Exp. 1 with the exception of enrofloxacin. For the first week after arrival, chlortetracycline (350 mg^{-1} hd^{-1} d^{-1}; Aureomycin, Zoetus, Parsippany, NJ) was mixed into the total mixed ration following instructions from the label and personal communication of the owners. Animals were also revaccinated on d 14 with the same vaccine used for revaccination in Exp. 1.

Animals were fed once daily as a percentage of their BW on a DM basis based on observations in Exp. 1 (Table 2.1) such that the 1.10/95 treatment was offered at 2.40% of BW daily and the 1.32/85 treatment offered at 2.2% of BW daily. Treatments diets were fed through d 42, then all animals were switched to the 1.10/95 Mcal NE_g/kg DM diet fed for ad libitum intake for 2 wk to equalize gut-fill. Individual animal weights were measured on d 0, 14, and on conclusion of the trial (d 56). A pen scale (from Exp. 1) was used on d 28 and d 42. Feed delivery was adjusted based on updated cattle weights measured at each weigh period on a pen basis. Ingredient samples were collected every other week and sent to a commercial laboratory for analysis (SDK Laboratories, Hutchinson, KS; Table 2.2). Results from ingredient analyses were used to calculate dietary concentrations of nutrients. Animals were observed twice daily for illness and treated according to the protocol from Exp. 1.

Experiment 4. Performance and Health Study

A total of 400 Angus x Hereford heifers (BW = 205 ± 8 kg) were assembled and shipped from a single source in Chinook, MT to the Kansas State University Beef Stocker Unit (2,022 km) over 7-d period from October 25 to 31, 2016. The heifers were used to determine the effects of 4 intakes using the 1.32/85 Mcal NE_g/kg DM diet from Exp. 1 in a randomized complete

block design with each of 4 loads representing a block. Cattle were managed on arrival using the same medications and treatment assignment protocols as Exp. 1 with the exception of enrofloxacin not being administered. Treatments consisted of the 1.32/85 Mcal NE_g/kg DM diet from Exp. 1 offered at 1.9, 2.2, 2.5, or 2.8% of BW daily. All animals were revaccinated on d 14 using the same protocol as Exp. 1 and 2.

Individual animal weights were measured on arrival, d 0, 14, and on conclusion of the trial (d 49). A pen scale (from Exp. 1) was used to measure weights on d 7, 21 28, and 35 and DM delivery adjusted on a pen basis weekly according to updated cattle weights. On d 35, all animals received a 1.10 Mcal NE_g/kg DM for two weeks to equalize differences in gastro intestinal tract fill. Total mixed ration and ingredient samples were collected every other week and composited for analysis by a commercial laboratory (SDK Laboratories, Hutchinson, KS). Performance was calculated from 0 to each weigh day, and pen was the experimental unit. Animals were observed twice daily for illness and treated according to the protocol from Exp. 1.

Experiment 5. Performance and Health Study

A total of 320 crossbred steers (BW = 254 ± 16 kg) were purchased from a single source and shipped from 2 locations to the Kansas State University Beef Stocker Unit over a 2-d period from February 15 to 16, 2017. Two loads were shipped from Groesbeck, TX (950 km) and 2 loads from Hatch, NM (1,426 km) and used to determine the effects of by-product (Sweet Bran or wet distiller's grains with solubles) and extent of corn processing (whole or dry-rolled corn) in a randomized complete block design with a 2 x 2 factorial arrangement of treatments. All 4 diet combinations were formulated to provide 1.32 Mcal NE_g/kg DM and contain 40% of their respective by-product (Table 2.3). On arrival calves were weighed and assigned by BW to pens,

which were randomly assigned to dietary treatment. There were 10 steers per pen and 8 pens per treatment combination for a total of 32 pens. Pens were the same as described in Exp. 1.

On d 0, all animals were vaccinated against common respiratory and clostridial diseases, treated for internal parasites, and revaccinated on d 14 using the same vaccines and dewormer from Exp. 1. Animals were weighed individually on d -1, 0, 14, and 70. The same pen scale used in the prior experiments was used to measure weights on d 7, 21, 35, 42, 49, 56, and 63. After weighing, diet delivery was adjusted by pen each week such that 2.0% of BW on DM basis was offered for all treatments daily. All pens were fed daily at 0700 h using the same feed wagon described for Exp. 1. Performance was calculated from d -1 to days 14, 28, 42, 56, and 70 and pen was the experimental unit. Ingredients and the total mixed ration were sampled weekly and composited for analysis by a commercial laboratory (SDK Laboratories, Hutchinson, KS; Table 2.4). Animals were observed twice daily for illness and treated according to the protocol from Exp. 1.

Net Energy Calculations

Performance data was used in equations from NASEM (2016) to calculate net energy for maintenance and gain for the experimental diets used in all 4 performance studies. In Exp. 1, because diets were offered at different amounts depending on the diet fed for ad libitum intake, therefore gastrointestinal tract fill would confound differences in performance, net energy was calculated from d 0 to d 41 and from d 0 to d 55. Net energy for the 0.99/100 treatment was averaged for the entire experiment for the pens offered the 0.99/100 treatment and this value used as the theoretical energy value for the diet used in the period designed to equalize differences in gut-fill. The difference in energy intake for the entire 55 d study and the 14 d the

limit-fed treatments were offered the diet designed for ad libitum intakes was used to estimate the net energy values for the diets prior to gut-fill and those values are reported. Exp. 3, 4, and 5 were calculated based on animal performance from the entire trial. Because the diets used in the gastrointestinal tract fill equalization periods in Exp. 3 and 4 were never offered for ad libitum intake prior to the last 14 d making in impossible to estimate accurate energy intake for the diets based on performance. For this reason, net energy was calculated for performance based on the entire trial unlike Exp. 1. Exp. 5 was calculated based on animal performance throughout the entire trial.

Statistical Analysis

Performance data for Exp. 1 were analyzed using MIXED procedure of SAS with the fixed effect of dietary treatment and random effect of block. Orthogonal contrasts were used to evaluate linear, quadratic, and cubic effects. Morbidity, mortality, and chronicity were analyzed in the GLM procedure in SAS.

In Exp. 2, ruminal parameters and diet digestibility were analyzed using the MIXED procedure of SAS. Concentrations and proportions of VFA and ammonia as well as pH were analyzed as repeated measures with dietary treatment as a fixed effect and animal as a random effect. Time served as the repeated term in the model, and animal x period was the subject. The covariance structure was spatial power, which was selected over compound symmetry by better fit statistics of the model. Orthogonal contrasts were used to evaluate linear, quadratic, and cubic effects of dietary treatment on ruminal parameters and diet.

Data from Exp. 3 was analyzed using the MIXED procedure in SAS with the fixed effects of dietary treatment, Zelnate on arrival, and dietary treatment x Zelnate on arrival. Block served as a random effect.

Experiment 4 was analyzed using the MIXED procedure in SAS with intake level as a fixed effect and block as a random effect. Orthogonal contrasts were evaluated using the CONTRAST option for linear, quadratic, and cubic effects.

Experiment 5 was also analyzed using the MIXED procedure in SAS as a 2 x 2 factorial with fixed effects of by-product and extent of corn processing and their interaction. Block served as a random effect.

Net energy calculations for Exp. 1 and 4 were analyzed using the MIXED procedure of SAS with dietary treatment serving as a fixed effect and block as a random effect. Orthogonal contrasts were evaluated for linear, quadratic and cubic effects. Experiments 3 and 5 were analyzed using the MIXED procedure of SAS. Dietary treatment, Zelnate, and their interaction were fixed effects in the model for Exp. 3 and corn processing, by-product and their interaction in Exp. 5. Block served as a random effect.

RESULTS AND DISSCUSSION

Experiment 1. Performance and Health Study

Health data are presented in Table 2.5. The overall percentage of animals initially treated for respiratory disease was 12%, ranging from 11% for the 0.99/100 treatment to 13% for the 1.32/85. There were no differences in animals treated once ($P = 0.99$), twice ($P = 0.86$), or determined chronically ill ($P = 0.86$) from respiratory disease among any of the dietary treatments. In addition, there were no differences in mortality among any of the dietary

treatments ($P = 0.83$). Increasing dietary energy has been shown in some cases to increase sickness early in the feeding period (Lofgreen et al., 1975; Rivera et al., 2005). Often, digestive upsets are blamed for the increase in morbidity. One important difference between our study and those designed to analyze the effects of dietary energy on health is the rate at which they are fed. Here, we use 4 energy levels offered at linearly decreasing amounts with linearly increased dietary energy concentration, whereas Lofgreen et al. (1975) and Rivera et al. (2005) fed diets for ad libitum intake. Another significant difference is the composition of the diets. The treatments in this study were all formulated to contain 40% WCGF on a DM basis, thus the majority of the energy is derived from fiber, not a rapidly fermentable carbohydrate like starch. The combination of limiting the amount being fed, such that animals are less likely to over consume, and using a fibrous by-product as the primary dietary energy source could explain the lack of differences in morbidity among the diets. Results from Exp. 2 (Table 2.7) indicate ruminal pH values are lower when the higher energy treatments were fed but not so low as to cause acidosis. Another trial conducted simultaneously with this trial (Chapter 3) demonstrated that the inflammatory response (as measured by haptoglobin) was not activated differently among dietary energy concentrations. Haptoglobin has been shown to be elevated when experimental acidosis is induced (Enemark, 2002). The overall of percentage of calves initially treated for respiratory disease was less than that observed by Step et al. (2008), where 31.9% of commingled market cattle were initially treated in a 42-d receiving study. One possible reason for the discrepancy could be the use of metaphylaxis on arrival in the present study where as Step et al. (2008) did not. Cole and Hutcheson (1990) also noted morbidity levels much higher than those in our experiment noting that 72.8% of calves were treated for BRD when fed a 12% CP diet and 59.5% when fed a 16% CP diet. Again, in their experiment metaphylaxis was not

used at the time of arrival. Multiple studies have been conducted with the general conclusion that mass medication of highly stressed young cattle on entry into the feedlot decreases morbidity (Lofgreen,1980; Galyean et al., 1995), and this is most likely the reason for the modest incidence of respiratory disease in our study.

Mortality for this trial (3.8%) was greater than usual for the Kansas State University Beef Stocker Unit when considering the history of the cattle before arriving, and it was greater than observed in other work involving auction market derived stressed calves (Step et al., 2008). There could have been several contributing factors increasing mortality. One, dietary CP averaged approximately 18% across the treatments. Fluharty and Loerch (1995) conducted an experiment to analyze the effects of CP concentrations ranging from 12 to 18%, and in this study morbidity increased linearly with increasing CP concentrations. However in a second study, CP concentrations ranged from 11 to 26% CP and no differences in health were observed (Fluharty and Loerch, 1995). The authors speculated after the second trial if increasing CP concentrations truly affected health then there would have been more issues in the second trial. Another possible reason for the increased mortality we observed could have been the Ca:P ratio which averaged 0.73:1; a range of 1:1 to 7:1 recommended by NASEM, (2016). Deviations from the ratio can lead to bone deformation and urinary calculi although symptoms related to these health issues were never observed. Lastly, the typical incidence of persistently infected cattle with BVD (BVD-PI) at this research station when cattle are procured from markets in the southeastern U.S. where these calves originated is approximately 0.33%. In this particular experiment, 9 cattle were removed from the trial for testing positive as BVD-PI. Six of the nine BVD-PI originated from one load, but health parameters did not seem to be related to the BVD-PI incidence within loads. Richeson et al. (2012) commingled PI calves with auction market cattle

and saw the number of cattle needing treated three times more than double (8 vs 17.5%) in a 42-d receiving study. Moreover, the average Ca:P ratio in the diets used in Exp. 2 were similar (0.66:1), and no morbidity or mortality was observed. More simply, the increased mortality could have been the result of failure to detect symptoms and diagnose, thus animals requiring medical intervention were not identified.

Performance results from Exp. 1 are in Table 2.6. In general cattle performed well on all the dietary treatments, which did not influence final ADG ($P = 0.32$). In addition, ADG was not affected by treatment at d 41 ($P = 0.35$) although there were linear decreases in ADG for d 14 ($P < 0.01$) and d 27 ($P = 0.02$) measurements. These results indicate that the compensatory gain demonstrated early by the cattle consuming the higher roughage diet fed ad libitum was diluted by d 41 where ADG was no longer different among treatments and the increased energy in the limit-fed ration was providing more energy for gain. At d 55 after all animals were receiving the 0.99/100 treatment, there were no differences among treatments in ADG.

DMI linearly decreased ($P < 0.01$) with increasing energy by design of the trial. Because ADG was not affected and DMI linearly decreased with increasing energy concentration, there were differences in efficiencies among the treatments with final G:F increasing linearly from 0.15 for the 0.99/100 treatment to 0.19 for 1.32/85 ($P < 0.01$). G:F also increased linearly at d 41 which is in agreement with ADG being similar among all treatments at d 41 with DMI decreasing linearly. Results from weights measured from the cattle earlier in the trial indicate again the differences in gut-fill between the diet fed for ad libitum intake and those limit-fed with G:F on d 14 being 0.31 vs. 0.17 for the 0.99/100 and 1.32/85 treatments, respectively.

The large increases in efficiency in limit-fed rations calculated to produce the same gain as those fed for ad libitum intake are in agreement with results of Schoonmaker et al. (2004)

where limit-fed diets were more efficient in the receiving period, and their results carried over into the finishing phase of the study. In Schoomaker et al. (2004) the limit-fed concentrate ration was 25% more efficient than the high-forage ration which is similar to the 27% increase in efficiency in our trial. These results are also in agreement with Schoonmaker et al. (2003) where limit-fed diets were more efficient. In contrast, Loerch and Fluharty (1998) observed that limit-feeding had no effect on efficiency when compared to diets fed for ad libitum intake. The difference has to do with how long the animals were fed in the 2 experiments. In Loerch and Fluharty (1998), cattle were fed their respective treatments until the average weight in the pen was 372 kg. This took 5 d longer for the limit-fed cattle than it did for the ad libitum-fed cattle. By this time, differences in efficiency calculated by total feed intake were diluted in the limit-fed diets. The cattle being fed for ad libitum intake would have had a higher degree of gut-fill thus reaching the target weight sooner than the limit-fed cattle. This is further explained by NE_g being the same for all the treatments when calculated based on animal performance. If the limit-fed cattle had been placed on the ad libitum diet some time before the ad libitum treatment reached target weight, differences in gut-fill may have been minimized and the limit-fed cattle may not have required extra days on feed to reach target weight.

Net energy calculations based on performance increased linearly with increasing dietary energy. Those values were lower than what was originally formulated most likely because the composition of gain was more lean tissue than fat.

In conclusion, limit-feeding high-energy rations based on fibrous by-products like WCGF does not seem to affect the health of newly-received, stressed cattle. In addition, limit-feeding such diets is a more efficient feeding strategy in the receiving and growing phase as the highest energy limit-fed treatment in this trial was 27% more efficient.

Experiment 2. Intake and Digestibility Study

Results from the intake and digestibility study are in Tables 2.7 and 2.8. These results further explain the health and performance observations from Exp. 1. Intake was linearly decreased again by design with increasing dietary energy ($P < 0.01$), with 3 of the four treatments limit-fed. DMI of the 3 limit-fed diets did not equal that of the results described in Exp. 1, but the animals in this experiment served as their own controls. The calves on the limit-fed rations had a more difficult time consuming 95, 90, and 85% on a DM basis of what they did during adaptation with the 0.99 Mcal NE_g/kg diet. When calves did not consume what they were intended to in the limit-fed treatments, the amount of feed refused was removed from the next day's feed delivery. The feed was added back by offering and additional 0.45 kg/d until the target was reached. After d 10 in each period, feed was held constant for sampling purposes and at times the prescribed level of feed was not achieved. It is possible the increased energy in the limit-fed rations was approaching the steers' capacity for energy intake as similar results of excess energy limiting intake have been reported (Grovum, 1986).

Total tract organic matter digestibility linearly increased with increasing energy and decreasing intake ($P < 0.01$) which can be explained by the increasing energy in the limit-fed rations carried out by the removal of roughage from the diet and also the linear decrease in liquid passage rate observed in the limit-fed diets ($P < 0.01$). Total tract dry matter digestibility for the 0.99/100 treatment was 61.06% compared to 69.87% in the 1.32/85 treatment which represents a 14% increase in dry matter digestibility. The increases in diet digestibility observed in limit-fed rations containing wet corn gluten feed (Sweet Bran) are in contrast to those reported by Montgomery et al. (2004). Those workers reported that digestibility in limit-fed rations was

decreased from 86.8 to 80.3% and reported that some of the difference could have been attributed to the meal-eating behavior of the animals in the study because most of the feed was consumed quickly possibly decreasing mastication and then fermentation as a result of larger particle size. Although in our experiment diet composition was altered by removing feed ingredients that were less digestible such as prairie and alfalfa hays and replacing them with more digestible ingredients like corn. In agreement with the present study are the results of Galyean et al. (1979) and Murphy et al. (1994). Both of these studies reported increased diet digestibility when limit-feeding. Galyean et al. (1979) fed diets at 2.00, 1.67, 1.33, and 1.00 times maintenance and found that dry-matter digestibility decreased from 85.7% in the 1.00 times maintenance treatment to 77.6% for the 2.00 times maintenance treatment. Murphy et al. (1995) reported total tract DM digestibility decreased from 72.38% in diets fed at 80% of *ad libitum* to 68.67% in diets fed *ad libitum*. Because passage rate is a function of intake, limit-fed diets would be more digestible due to decreased passage rate. In the current study, liquid passage rate decreased from 12.60%/hr for the 0.99/100 treatment to 7.31%/hr for the 1.32/85 treatment.

Average ruminal pH (Table 2.7) decreased linearly from 6.07 for the 0.99/100 treatment to 5.65 for the 1.32/85 treatment. Measurements taken at individual times can be found in Figure 2.1. The decrease in pH reported here is most likely due to the increase in starch from corn in the high-energy diets as well as the decreased passage rate observed in the limit-fed rations. In addition to starch fermentation, wet corn gluten feed has been shown to decrease ruminal pH due to the increased digestibility of the ingredient itself, thus increasing VFA and decreasing pH (Huls et al., 2016). Mould et al. (1983) used diets containing 0, 25, 50, 75, or 100% pelleted ground barley fed to sheep to determine effects on ruminal pH and found that it decreased from

6.6 for the 0% barley ration to 5.4 with the 100% ground barley ration as a result of increased starch fermentation. There is also the possibility of decreased mastication and thus salivation in the high concentrate diets limit-fed in the current study. Decreases in saliva flow to the rumen from decreased effective fiber could also decrease its buffering capacity thus decreasing ruminal pH (Allen, 1997).

Concentrations of ammonia are reported in Table 2.7 and Figure 2.2. There were linear ($P = 0.01$) and cubic ($P < 0.01$) effects for ammonia concentrations with increasing energy and decreasing intake. Ammonia concentrations were higher for the 1.10/95 and 1.32/85 diets (14.65 and 15.37 m*M*, respectively) compared to the 0.99/100 and 1.21/90 treatments (10.55 and 13.37 m*M*, respectively. Results for ruminal ammonia are in agreement with Murphy et al. (1994) who found that ammonia concentrations increased linearly with decreasing intake ($P < 0.06$). These workers concluded that ruminal ammonia concentrations were increased in the limit-fed rations due to the increased level of supplement and also because of the effects of intake on liquid dilution rate. They speculated that increasing liquid dilution rate could also be correlated with larger amounts of microorganisms leaving the rumen in the fluid state and thereby not using the ammonia. Moreover, Clark et al. (2007) reported similar responses to ammonia when diets were fed at 80% of ad libitum intakes because the animals consumed feed faster and ammonia release increased accordingly. This could also be a reason for our observations as the limit-fed diets were usually consumed within 4 h of feeding.

VFA concentrations are presented in Tables 2.7 and 2.8 and Figure 2.3. Results from our experiment are similar to others who have limit-fed diets for a common gain by increasing dietary energy (Clark et al., 2007). In our study, total VFA concentrations linearly decreased from 118.6 m*M* for the 0.99/100 treatment to 98.5 m*M* for the 1.32/85 treatment. The decrease

could be explained by increased post ruminal digestion as in Choat et al. (2002). Those workers observed increased post ruminal diet digestibility in limit fed diets, although total ruminal VFA was not affected in their study. Ruminal concentrations of acetate and butyrate decreased, isovalerate increased, while propionate, valerate, and isobutyrate were unaffected by treatment. Proportions of VFA can be found in Table 2.8. Proportions of acetate decreased in accordance with the decreasing roughage in the limit-fed diets decreasing from 62% for the 0.99/100 treatment to 54.5% for the 1.32/100 treatment. Proportions of propionate, isobutyrate, and isovalerate all increased linearly ($P < 0.05$) with increasing dietary energy and decreasing intake. Propionate increasing and acetate decreasing are in agreement with Clark et al. (2007), who saw similar increases as dietary energy concentration increased in the diet as well as numerically increased for the minor VFA.

In summary, results from the digestibility study indicate that limit-feeding higher-energy rations based primarily on wet corn gluten feed (Sweet Bran) leads to greater digestibility, and greater molar proportions of propionate. Although pH decreased with increasing energy density, none of the diets seemed to cause acidosis as diagnosed by visual symptoms or based on ruminal pH. The increases in digestibility and ruminal concentrations of propionate explain the efficiencies from Exp. 1. Additionally, these results show that metabolic disorders related to ruminal acidosis may be minimal when the dietary energy is primarily of fibrous origin rather than starch.

Experiment 3. Performance and Health Study

Experiment 3 was designed to validate the results from Exp. 1 and to study a novel DNA immunostimulant (Zelnate). Results from Exp. 3 are in Table 2.9.

Health data was not statistically analyzed in this study because there were very few instances of morbidity and mortality. Three heifers were removed within the first three weeks of the study due to severe malnutrition. Out of the three, one heifer was treated for respiratory disease and was from the 1.32 Mcal NE_g/kg treatment.

The DNA-immunostimulant or the interaction between dietary treatment and Zelnate did not affect ADG, DMI, or G:F in this trial ($P > 0.10$). Serological testing was not carried out in this trial and may be beneficial in characterizing the effects of dietary energy on the innate immune system, which is thought to be stimulated by Zelnate. Interestingly Zelnate tended to increase intakes as percentage of BW on d 14, 42, and 56 ($P = 0.09$, 0.08, and 0.07, respectively). These results were unexpected and are difficult to explain. Briefly, Zelnate is a liposome containing a section of DNA known as a CpG motif that mimics infection but is not specific to any pathogen. The theory is the liposome surrounded DNA will enter an antigen presenting cell such as dendritic cell where the TLR (toll-like-receptor) 9 receptor is activated. This activation causes the secretion of cytokines that activate the rest of the innate immunes system and prime the adaptive arm of the immune system. Oosthuysen et al. (2016), showed ADG and G:F were lower when an immunostimulant was used on d 0, 14, and 28. Because those workers administered the immunostimulant 3 times vs. 1 time on arrival in the current study, the results are difficult to compare. One reason for the difference in performance results could be that repeated use of the drug could over-stimulate the immune system and decrease intakes.

Performance results from this experiment validate those from Exp. 1. ADG was higher for cattle on the 1.10/95 treatment compared to the 1.32/85 treatment ($P < 0.01$) until d 56 following equalization of gastrointestinal tract fill when no differences in ADG were detected (*P*

= 0.75). In Exp. 3, both diets were fed as set percentages of BW based on Exp. 1. In Exp. 3, cattle intakes were numerically lower than Exp. 1 during the first 14 d after arrival and this could be one reason for there not being a difference in ADG at d 41 in Exp. 1. In this experiment, once the heifers came on to feed the compensatory growth was much greater for the cattle fed the higher roughage diet at 2.40% of their BW compared to 2.20% of BW for the 1.32/85 treatment. However, these differences in ADG were due to gut-fill, which was demonstrated by gains that were the same between d 0 and 56 that were the same between dietary treatments (P = 0.75).

Dry matter intake was less for the 1.32/85 than 1.10/95 as would be expected as one diet was fed a 2.40% of BW and the other at 2.20% of BW. On d 42, DMI was 2.29% of BW for the 1.1/95 treatment and 2.02% of BW for the 1.32/85 treatment, which are less than what was consumed by the heifers at this point in Exp. 1. Most of the difference can be attributed to the lower intakes leading up to d 41. One reason for the lower intakes compared to Exp. 1 may have to do with some cattle procured through auction markets having limited experience eating from a bunk. The level of familiarity is often unknown, but research has shown only one animal in a pen with feeding experience at the bunk can have substantial effects on feeding behavior of newly arrived feedlot cattle and cause intakes to be increased shortly after arrival compared to pens without trainer animals (Loerch and Fluharty, 2000). The cattle in this experiment were gathered and weaned onto the truck immediately prior to being shipped to the Kansas State University Beef Stocker Unit and thus had no prior experience eating from a bunk. By d 56, average intakes were similar to those observed in Exp. 1 for the treatments but the severe depression in intake early in Exp. 3 still affected average intakes at the conclusion of the trial.

Final G:F was affected by dietary treatment/intake level (P = 0.03). At d 55, G:F was 0.17 for the 1.10/95 treatment and 0.19 for the 1.32/85 treatment. These results are in agreement

with Exp. 1 as the same efficiencies for the 2 dietary treatments were observed. Knoblich et al. (2007) reported similar results with the limit-fed rations in their experiment being more efficient than diets fed for ad libitum intakes. The increased efficiencies in the higher energy diet in this experiment is likely due to the same reasons described by Exp. 1 with digestibility and ruminal propionate proportions likely increasing as dietary energy density was increased.

Net energy values based on performance were not affected by Zelnate or the interaction of Zelnate and dietary treatment. However, net energy for maintenance and gain was higher for the higher energy diet, which was expected. Net energy values were lower than what was originally formulated most likely due to the composition of gain being more lean muscle than fat similarly to Exp 1.

Results from this trial validate results observed in Exp. 1. Limit-feeding a ration formulated to supply 1.32 Mcal NE_g/kg DM at 2.20% of BW was more efficient than limit-feeding a higher roughage ration formulated to provide 1.1 Mcal NE_g/kg DM. Furthermore, based on low mortalities these rations seemed not to have adverse effects on health. This is important because at times when hay prices may be higher, or when the capacity to store or remove manure is insufficient, limit-feeding rations based on wet corn gluten feed (Sweet Bran) could be more favorable economically.

Experiment 4. Performance and Health Study

Results from Exp. 4 can be found in Table 10. There were no cases of morbidity or mortality in this trial. This observation is in line with Exp. 1 and 3 that the high-energy diet does not seem to induce health issues. In this trial, because 8 pens were fed at 2.80% of BW daily, our hypothesis stands that a high-energy diet based primarily on Sweet Bran will not precipitate

digestive upsets, at least not within our ability to detect them, and does not lead to increased morbidity.

Average daily gain was linearly increased by treatment on all measurement days ($P < 0.01$). At d 49, after 2 wk of being fed the 1.10 Mcal NE_g/kg DM diet to equalize differences in gut-fill, ADG ranged from 1.21 kg/d for the 1.9% treatment to 1.48 kg/d for the 2.8% BW treatment. Berry et al. (2004) fed 2 diets only altering the energy level and starch concentration and reported that neither energy level nor starch affected ADG. Results from their study are in contrast to the current study as total energy intake increased ADG ($P < 0.01$). However, Berry et al. (2004) fed two diets with different ingredients, which could confound the comparison. Schoonmaker et al. (2003) fed the same high-concentrate diet formulated to provide 1.38 Mcal NE_g/kg at 3 levels to achieve ADG of 0.8 or1.2 kg/d along with ad libitum intake. Those workers reported that ADG was greatest for the ad libitum treatment, lowest for the 0.8 kg/d treatment and the 1.2 kg/d intermediate. In Schoonmaker et al. (2003), final weights were calculated by feeding all treatments at 1.8% of BW for 5 d prior to weighing to minimize differences in gut-fill. In Exp. 4 a gut-fill equalization period was used in which a diet was fed for ad libitum intake to minimize differences in gut-fill. Recent research has shown that limit-feeding prior to weighing could be a better alternative than full feeding because animals are more likely to consume similar amounts of feed when limit-fed than when they are full fed (Watson et al., 2013). Discrepancies in weighing strategies may confound comparisons between trials involving limit-feeding. Nonetheless, results from our study indicate ADG linearly increases when more of the 1.32/85 treatment is fed.

Dry matter intake linearly increased by design with increasing treatment level ($P < 0.01$) for all measurement days. However, during the first 35 d of the trial when treatments were

offered, cattle on the 2.2, 2.5, and 2.8% BW treatments did not consume their allotted amounts of feed. After the gut-fill equalization period, DMI as a percentage of BW was much closer to target. These results may indicate what was described earlier in Exp. 2 and in the research of Grovum (1986). The ad libitum energy intake potential of the calves may have been attained with DMI less than 2.8% of BW daily, particularly in the first 14 d. This would explain the substantial increases in intake once the 1.10 Mcal NE_g/kg DM diet was offered for ad libitum intake as the animals needed a higher DMI to meet their potential for energy intake.

Studying the feed conversion between the 4 intakes used as treatments in this trial was one of the main objectives. We hypothesized that cattle offered the 1.32/85 treatment used in all of the experiments *ad libitum* may show decreases in feed conversion if more adipose tissue was being synthesized than lean muscle growth or frame (Schoonmaker et al., 2003; Rossi et al., 2001). Results from this study show linear increases in G:F with increasing intake level through d 35 ($P < 0.01$). These results, however, are most likely due to differences in gut-fill more than actual differences in growth because there were no differences in G:F at d 49 after the gut-fill equalization period. One source of variability in our trial compared to others could be the duration of the trial. Because Exp. 4 was relatively short in terms of DOF, benefits from compensatory gain over d 35 to 49 could have affected performance at the end of the trial, with more restricted cattle showing more compensatory gain. This could also be part of the reason for better G:F compared to Exp. 1 and Exp. 2.

Net energy for maintenance and gain calculated for the diets based on animal performance decreased linearly ($P = 0.01$) with increasing intake. Cattle that were more restricted in during the trial experienced more compensatory gain during the 14-d gut-fill period. Total BW gain that was used to calculate energy values for the diets could have been largely

affected by the period designed to equalize differences in gastrointestinal fill, which could explain the linear decrease net energy values.

Results from Exp. 4 show that the high-energy treatment from Exp. 1, 2, and 3 can be offered at percentages of BW up to a theoretical ad libitum intake. In this experiment, 2.8% of BW daily was never achieved so cattle were fed in accordance to maximum intake in effort to reach that percentage of BW. Differences in genetics and time of year may have all played an important part in the results of this trial. However, no differences in health were observed, and ADG was increased linearly with intake. This is important because theoretically one diet could be fed at different levels to target a broad spectrum of gains in order to meet certain market goals of the producer.

Experiment 5. Performance and Health Study

Results from Exp. 5 are in Table 2.11. In agreement with all of the trials conducted up to this point, there were no effects of by-product, extent of corn processing, or the interaction of the two on health. Moreover, no animals in this trial were treated for respiratory disease or for any other reason. Under our ability to detect sickness, the diets used in these experiments do not seem to affect health.

Average daily gain was greater in the diets formulated with WCGF vs. WDGS as the by-product source on d 14 ($P = 0.02$), but no differences in ADG were detected following for any of the treatment combinations with ADG on d 70 only ranging from 0.87 kg/d for the WDGS/WC diet to 0.95 kg/d for the Sweet Bran/WDGs diet. Loza et al. (2010) fed feedlot rations that contained either 30% wet corn gluten feed or 30% WDGS and found that ADG was increased in diets containing the WDGS compared to diets containing wet corn gluten feed. These workers

attributed their results to a higher concentration of fat in the WDGS, which would increase dietary energy, and also the possibility of more protein being digested postruminally and being available for energy. In Exp. 5, fat was more concentrated in the WDGS-based diets but these values were expected based on diet formulation. In addition, Loza et al. (2010) conducted their work with finishing cattle fed for ad libitum intakes and we were limit-feeding at 2.0% of BW daily. One of the major causes for the by product effect on d 14 could be the Sweet Bran based diets having a higher DM content than what was formulated and the WDGS diets having a lower DM than what was formulated. This could explain the difference in ADG early in the trial because more feed on a DM basis being delivered to the Sweet Bran treatment pens. Theoretically, ADG would be higher for the diets that were fed at a higher percentage of BW even if the diets were supplying equal amounts of energy on a Mcal NE_g/kg DM basis. These results were validated in Exp. 4 of this report. Extent of corn processing in this experiment did not affect ADG at conclusion of the study ($P = 0.34$) which is in agreement with Siverson et al. (2014) who also saw no differences in performance when whole corn and dry-rolled corn were fed in WDGS-based diets. However these results are in contrast to Chester-Jones et al. (1991) who observed better performance in diets containing whole corn rather than dry-rolled corn.

There was an interaction between by-product and extent of corn processing for DMI for all sampling days ($P \leq 0.01$). These results were not expected as animals were weighed weekly and intake adjusted as a percentage of BW. Of the 2 Sweet Bran diets, the one containing whole corn was approximately 3% wetter than what the diet was formulated to be and the Sweet Bran diet with cracked corn was approximately 1% dryer than originally formulated. This could explain the interaction reported in DMI as a percentage of BW and on an actual kg of DM consumed basis. The cattle consuming the Sweet Bran diets with whole corn were actually fed

less as a percentage of BW than the other Sweet Bran treatments thus confounding results. The 2 diets formulated with the WDGS contained slightly less DM than formulated but they were similar to each other explaining interaction.

Feed conversion was affected by BP ($P = 0.03$) on d 14 but the results are difficult to understand due to the differences in DMI described above and potential differences could be in fill. ADG was affected by BP at d 14, but this affect could be confounded with the discrepancies between expected and analyzed DM content of the diets. If all of the diets were of similar energy concentrations, then G:F may not be affected even though DMI was not the same as seen in Exp. 4 of this report. Results in terms of final G:F are in agreement with those of the experiments leading up to this point if the diets were of similar energy densities. One major difference between this trial and Exp. 1-4 was how differences in gut-fill were minimized to compare weights. In the current study, shrunk weights were taken on arrival and used to calculate performance through the end of the trial. There was never a gastrointestinal tract fill equalization diet fed at the end of the trial as in our previous experiments because gastrointestinal tract fill was expected to be similar among treatments.

Net energy calculations were not affected by the extent of corn processing, the type of by-product, or their interaction. The values were lower than originally calculated similar to results from Exp. 1, 3, and 4. However, energy values were similar in this trial to those observed in Exp. 1, 3, and 4.

IMPLICATIONS

Results from these trials indicate that high-energy diets based on corn by-products can be fed to newly received stocker cattle without negative effects on overall health. In addition, when these

diets are limit-fed, advantages in efficiency are evident. Moreover, feeding the highest energy ration in these trials formulated with cracked or whole corn and Sweet Bran or wet distiller's grains yielded similar performance although more work is needed in this area as there were confounding factors related to DMI. These results are beneficial to the growing cattle sector because gain may be targeted by feeding the same diet and only altering intake. Intake restriction increases in digestibility and efficiency, which may contribute to less total manure production and thus a smaller impact on the environment and reduced costs of removal. More research is warranted addressing the effects of limit-feeding high energy diets based on corn by-products and their effects of performance and carcass characteristic in the finishing phase of production. Nonetheless, the results of these studies introduce a novel programmed feeding protocol based on corn by-products as the primary energy source that is more efficient than high-roughage growing diets and does not negatively affect health.

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Table 2.1 Composition of diets fed in Exp. 1-4

	Diets[1]			
Item	0.99/100	1.10/95	1.21/90	1.32/85
Ingredient, % DM				
Alfalfa	22.50	17.00	12.00	6.50
Prairie hay	22.50	17.00	12.00	6.50
Dry rolled corn	8.57	19.08	28.50	38.82
Wet corn gluten feed[2]	40.00	40.00	40.00	40.00
Supplement[3]	6.43	6.92	7.50	8.18

[1]Treatment diets offered based on DMI of 0.99/100 treatment intake that was offered for ad libitum intake. First number = Mcal NE_g/kg DM. Second number = % of 0.99/100 treatment offered on DM basis.

[2]Cargill Animal Nutrition, Blair, NE.

[3]Supplement pellet was formulated to contain (DM basis) 10% CP, 8.0% Ca, 0.24% P, 5.0% salt, 0.55% potassium, 0.25% magnesium, 1.67% fat, 8.03% ADF, and as 367 mg/kg lasalocid (Bovatec; Zoetis, Parsippany, NJ).

Table 2.2 Analyzed nutrient analysis of diets fed in Exp. 1-4

	Diet (Mcal NE_g/kg DM)			
Item	0.99	1.10	1.21	1.32
Nutrient composition Exp. 1 % of DM				
DM, % as fed	75.5	74.8	74.9	74.3
CP	18.1	18.6	17.9	17.4
Fat	3.5	4.0	4.2	4.1
ADF	20.1	14.2	14.2	10.5
NDF	33.7	29.0	29.6	17.6
Ca	0.7	0.5	0.5	0.4
P	0.7	0.8	0.7	0.7
Nutrient composition Exp. 2, % of DM				
DM, % as fed	75.7	75.1	75.1	75.0
CP	18.6	18.2	18.0	17.3
Fat	3.1	3.3	3.6	3.9
ADF	18.5	16.1	12.5	10.2
NDF	36.3	32.9	26.9	23.1
Ca	0.6	0.6	0.5	0.4
P	0.7	0.7	0.8	0.8
Nutrient composition Exp. 3, % of DM				
DM, % as fed		78.8		78.1
CP		16.9		16.7
Fat		2.9		3.3
ADF		17.3		10.2
NDF		37.3		27.1
Ca		0.5		0.4
P		0.7		0.7
Nutrient composition Exp. 4, % of DM				
DM, % as fed		78.8		73.4
CP		16.6		17.3
Fat		3.3		4.5
ADF		19.0		10.5
NDF		33.6		24.3
Ca		0.8		0.4
P		0.5		0.7

Table 2.3 Composition of diets[1] fed in Exp. 5

	By-product			
	Wet distiller's grains		Sweet Bran	
	Corn processing			
Item	Dry-rolled	Whole	Dry-rolled	Whole
Ingredient, % DM				
Alfalfa	8.00	8.00	6.50	6.50
Prairie hay	8.00	8.00	6.50	6.50
Dry rolled corn	36.50	-	39.50	-
Whole corn	-	36.50	-	39.50
Wet distiller's grains w/ solubles	40.00	40.00	-	-
Sweet Bran	-	-	40.00	40.00
Supplement[2]	7.50	7.50	7.50	7.50

[1]Diets formulated to supply 1.32 Mcal NE_g/kg DM

[2]Supplement pellet was formulated to contain (DM basis) 10% CP, 8.0% Ca, 0.24% P, 5.0% salt, 0.55% potassium, 0.25% magnesium, 1.67% fat, and 8.03% ADF.

Table 2.4 Analyzed nutrient analysis of diets fed in Exp. 5

Item	By-product			
	Wet distiller's grains		Sweet Bran	
	Corn processing			
	Dry-rolled corn	Whole	Dry-rolled corn	Whole
Nutrient composition, % of DM				
DM, % as fed	53.4	54.0	73.1	70.1
CP	16.2	17.3	14.9	14.9
Fat	6.0	5.5	3.7	3.6
ADF	15.3	15.8	12.2	15.3
NDF	28.4	29.1	26.1	32.1
Ca	0.7	1.0	0.8	0.7
P	0.4	0.5	0.6	0.6

Table 2.5 Effects of dietary energy and intake on health (Exp. 1)

	Diet[1]					
Item	0.99/100	1.10/95	1.21/90	1.32/85	SEM[2]	*P*-value
Morbidity, %						
Treated once	11.2	12.6	12.3	12.6	4.6	0.99
Treated twice	3.6	4.8	2.8	4.8	2.9	0.86
Chronic	2.6	3.7	1.8	2.7	2.5	0.86
Mortality, %	4.8	4.4	2.1	4.3	2.1	0.83

[1]Treatment diets offered based on DMI of 0.99/100 treatment intake that was offered for ad libitum intake. First number = Mcal NE_g/kg DM. Second number = % of 0.99/100 treatment offered on DM basis.

[2]Largest SEM among treatments is reported.

Table 2.6 Effects of dietary energy level and intake on performance (Exp. 1)

	Diet[1]					*P*-value		
Item	0.99/100	1.1/95	1.21/90	1.32/85	SEM	Linear	Quadratic	Cubic
No. of pens	8	8	8	8				
No. of animals	90	87	91	86				
BW, kg								
d 0	222.5	223.8	222.4	222.9	2.4	0.96	0.48	0.11
d 55	278.8	280.1	279.7	282.8	3.7	0.30	0.72	0.64
ADG, kg/d								
d 0-14	1.58	1.13	0.98	0.77	0.15	<0.01	0.38	0.57
d 0-27	1.09	1.04	1.00	0.91	0.10	0.02	0.71	0.81
d 0-41	1.11	1.08	1.05	1.05	0.06	0.35	0.81	0.81
d 0-55	1.02	1.02	1.04	1.09	0.05	0.32	0.60	0.96
DMI, kg/d								
d 0-14	5.01	4.69	4.55	4.25	0.29	<0.01	0.93	0.53
d 0-27	5.71	5.26	5.05	4.75	0.25	<0.01	0.56	0.58
d 0-41	6.38	5.85	5.55	5.27	0.23	<0.01	0.38	0.74
d 0-55	6.77	6.27	5.99	5.85	0.22	<0.01	0.24	0.88
Intake as % BW daily								
d 0-14	2.14	2.02	1.98	1.86	0.10	<0.01	0.96	0.45
d 0-27	2.40	2.21	2.14	2.02	0.08	<0.01	0.49	0.48
d 0-41	2.60	2.37	2.27	2.15	0.07	<0.01	0.34	0.57
d 0-55	2.70	2.49	2.39	2.31	0.07	<0.01	0.21	0.72
G:F, kg/kg								
d 0-14	0.316	0.235	0.218	0.175	0.029	<0.01	0.49	0.47
d 0-27	0.191	0.196	0.197	0.190	0.015	0.93	0.54	0.92
d 0-41	0.174	0.185	0.190	0.200	0.010	0.02	0.93	0.74
d 0-55	0.152	0.163	0.174	0.187	0.006	<0.01	0.85	0.99
NE_m, Mcal/kg[2]	1.50	1.63	1.74	1.86	0.04	<0.01	0.92	0.83
NE_g, Mcal/kg[2]	0.90	1.02	1.11	1.22	0.04	<0.01	0.92	0.83

[1]Treatment diets offered based on DMI of 0.99/100 treatment intake that was offered for ad libitum intake. First number = Mcal NE_g/kg DM. Second number = % of 0.99/100 treatment offered on DM basis.

[2]Net energy calculations based on equations from NASEM (2016)

Table 2.7 Effects of energy level and intake on DM digestibility and characteristics of digestion

	Diet					*P*-value		
Item	0.99/100	1.10/95	1.21/90	1.32/85	SEM[1]	Linear	Quadratic	Cubic
Number of observations	6	6	5	6				
DMI, kg/d	9.57	7.53	7.33	7.02	0.80	<0.01	0.08	0.38
Ruminal								
pH[2]	6.07	5.93	5.77	5.65	0.15	<0.01	0.90	0.90
Ammonia, m*M*	10.6	14.7	13.4	15.4	1.27	<0.01	0.16	0.01
VFA total, m*M*	118.9	103.2	103.9	98.5	6.75	<0.01	0.24	0.27
Acetate, m*M*	73.3	61.3	59.6	53.1	3.44	<0.01	0.25	0.17
Propionate, m*M*	26.0	24.8	26.0	27.7	2.05	0.20	0.19	0.70
Butyrate, m*M*	15.0	13.0	13.7	12.5	1.16	0.05	0.63	0.24
Isobutyrate, m*M*	0.9	0.7	0.8	0.8	0.06	0.72	0.11	0.33
Isovalerate, m*M*	1.2	1.1	1.6	2.0	0.19	<0.01	0.06	0.32
Valerate, m*M*	2.4	2.3	2.3	2.5	0.29	0.93	0.37	0.92
Digestibility, %DM	61.1	62.5	64.6	69.9	2.04	<0.01	0.33	0.77
Liquid passage rate, %/hr[3]	12.6	8.1	8.5	7.3	0.73	<0.01	0.02	0.03

[1] Largest value among treatments is reported.
[2] Average of values collected at 0, 2, 4, 6, 8, 12, 18, and 24 h after feeding.
[3] Calculated values from samples collected at 2, 4, 6, 8, 12, and 18 h after feeding.

Table 2.8 Effects of energy level and intake on ruminal VFA profiles (Exp. 2)

	Diet[1]					*P-value*		
Item	0.99/100	1.10/95	1.21/90	1.32/85	SEM[2]	Linear	Quadratic	Cubic
No. of observations	6	6	5	6				
VFA, mol/100 mol								
Acetate[3]	62.0	60.2	57.9	54.5	0.80	<0.01	0.11	0.74
Propionate[3]	21.6	23.6	24.9	27.7	0.80	<0.01	0.48	0.41
Butyrate[3]	12.6	12.3	12.8	12.6	0.56	0.83	0.95	0.42
Isobutyrate[3]	0.73	0.75	0.78	0.86	0.05	0.03	0.43	0.82
Isovalerate[3]	1.06	1.11	1.56	2.07	0.20	<0.01	0.13	0.63
Valerate[3]	1.98	2.05	2.06	2.32	0.17	0.06	0.44	0.59

[1]Treatment diets offered based on DMI of 0.99/100 treatment intake that was offered for ad libitum intakes. First number = Mcal NE_g/kg DM. Second number = % of 0.99/100 treatment offered on DM basis.

[2]Largest value among treatments reported.

[3]Average of values collected at 0, 2, 4, 6, 8, 12, 18, and 24 h after feeding expressed as a percentage of total VFA.

Table 2.9 Effects of Zelnate administered on arrival and energy level and intake on performance (Exp. 3)

Item	Zelnate[1]							
	NO		YES					
	Diet[2]					*P*-value		
	1.10	1.32	1.10	1.32	SEM	Zelnate	Diet	Zelnate x Diet
No. of pens	8	8	8	8				
No. of animals	93	91	93	93				
BW, kg								
d 0	216.1	228.8	221.5	226.0	12.0	0.82	0.14	0.47
d 56	274.2	289.3	280.1	283.2	11.7	0.99	0.17	0.35
ADG, kg/d								
d 0-14	0.16	-0.09	0.24	-0.11	0.21	0.72	<0.01	0.56
d 0-28	0.54	0.45	0.60	0.39	0.07	0.94	<0.01	0.25
d 0-42	0.75	0.67	0.81	0.62	0.05	0.97	<0.01	0.21
d 0-56	1.04	1.08	1.05	1.02	0.03	0.41	0.75	0.26
DMI, kg/d								
d 0-14	3.84	3.36	4.19	3.50	0.21	0.18	<0.01	0.56
d 0-28	4.62	4.20	4.90	4.28	0.25	0.26	<0.01	0.54
d 0-42	5.26	4.86	5.52	4.86	0.26	0.40	<0.01	0.43
d 0-56	6.00	5.72	6.27	5.68	0.26	0.50	0.01	0.36
DMI, % of BW daily								
d 0-14	1.77	1.47	1.88	1.55	0.05	0.09	<0.01	0.76
d 0-28	2.06	1.78	2.13	1.85	0.04	0.08	<0.01	0.96
d 0-42	2.27	2.00	2.31	2.04	0.03	0.11	<0.01	0.89
d 0-56	2.45	2.21	2.50	2.23	0.02	0.07	<0.01	0.46
G:F, kg:kg								
d 0-14	0.041	-0.033	0.062	-0.036	0.059	0.73	<0.01	0.65
d 0-28	0.119	0.110	0.122	0.091	0.021	0.51	0.11	0.37
d 0-42	0.144	0.141	0.148	0.127	0.014	0.62	0.26	0.37
d 0-56	0.174	0.190	0.168	0.181	0.010	0.24	0.03	0.80
NE_m, Mcal/kg[3]	1.63	1.60	1.79	1.74	0.03	<0.01	0.37	0.65
NE_g, Mcal/kg[3]	1.02	0.99	1.16	1.11	0.03	<0.01	0.37	0.65

[1]Bayer Animal Health, Shawnee Mission, Kansas

[2]1.1 = 1.1 Mcal NE_g/kg DM offered at 2.40% BW daily. 1.32 = 1.32 Mcal NE_g/kg DM offered at 2.20% of BW daily.

[3]Net energy calculations based on equations from NASEM (2016)

Table 2.10 Effects of amount of feed offered as a percentage of BW on performance (Exp. 4)

	Feed offered, % of BW daily[1]					*P*-value		
Item	1.9	2.2	2.5	2.8	SEM	Linear	Quadratic	Cubic
No. of pens	8	8	8	8				
No. of animals	100	99	100	101				
BW, kg								
d 0	212.4	211.5	212.5	211.5	4.56	0.57	0.88	0.16
d 49	271.4	275.4	281.6	284.1	6.53	<0.01	0.68	0.46
ADG, kg/d								
d 0-14	0.51	0.86	0.98	1.17	0.09	<0.01	0.35	0.42
d 0-28	0.79	0.97	1.20	1.41	0.07	<0.01	0.73	0.72
d 0-35	0.89	1.07	1.23	1.46	0.06	<0.01	0.64	0.77
d 0-49	1.21	1.30	1.41	1.48	0.05	<0.01	0.71	0.81
DMI, kg/d								
d 0-14	3.92	4.31	4.56	4.50	0.20	<0.01	0.07	0.79
d 0-28	4.14	4.70	5.17	5.48	0.10	<0.01	0.13	0.84
d 0-35	4.23	4.83	5.40	5.78	0.09	<0.01	0.12	0.67
d 0-49	5.51	5.98	6.45	6.79	0.08	<0.01	0.39	0.63
DMI, % BW daily								
d 0-14	1.80	1.96	2.06	2.02	0.12	<0.01	0.06	0.82
d 0-28	1.85	2.09	2.26	2.38	0.07	<0.01	0.09	0.95
d 0-35	1.85	2.10	2.31	2.44	0.06	<0.01	0.09	0.81
d 0-49	2.28	2.46	2.62	2.74	0.05	<0.01	0.38	0.87
G:F, kg/kg								
d 0-14	0.131	0.199	0.219	0.265	0.023	<0.01	0.56	0.38
d 0-28	0.191	0.205	0.233	0.259	0.014	<0.01	0.57	0.73
d 0-35	0.210	0.220	0.229	0.254	0.012	<0.01	0.47	0.70
d 0-49	0.219	0.218	0.218	0.218	0.006	0.98	0.94	0.94
NE_m, Mcal/kg[2]	1.87	1.83	1.80	1.78	0.05	0.01	0.61	0.87
NE_g, Mcal/kg[2]	1.22	1.19	1.17	1.15	0.04	0.01	0.61	0.87

[1]Diet was formulated to contain 1.32 Mcal NE_g/kg DM and offered at 1.9, 2.2, 2.5, or 2.8% of BW daily. Animals were weighed weekly and feed delivery adjusted accordingly.

[2]Net energy calculations based on equations from NASEM (2016)

Table 2.11 Effects of by-product and corn processing in limit-fed diets[1] on performance (Exp. 5)

	By-product[2]							
	Sweet Bran		Wet distiller's grains					
	Corn processing[3]					*P*-value		
Item	Dry-rolled	Whole	Dry-rolled	Whole	SEM	B	P	B x P
No. of pens	8	8	8	8				
No. of animals	80	79	79	79				
BW, kg								
d -1	253.0	254.4	253.1	253.6	8.8	0.62	0.14	0.50
d 70	320.5	319.4	318.2	315.7	11.6	0.36	0.57	0.83
ADG, kg/d								
d 0-14	0.78	0.69	0.56	0.54	0.14	0.02	0.47	0.60
d 0-28	1.02	0.92	0.94	0.86	0.07	0.26	0.15	0.85
d 0-42	0.83	0.85	0.79	0.76	0.06	0.15	0.96	0.69
d 0-56	0.92	0.86	0.86	0.83	0.05	0.24	0.27	0.66
d 0-70	0.95	0.92	0.92	0.87	0.06	0.34	0.34	0.93
DMI, kg/d								
d 0-14	4.99	4.84	4.82	4.89	0.13	0.12	0.25	<0.01
d 0-28	5.21	5.05	5.08	5.11	0.12	0.35	0.09	0.01
d 0-42	5.40	5.21	5.25	5.28	0.14	0.21	0.02	<0.01
d 0-56	5.66	5.48	5.45	5.53	0.15	0.06	0.17	<0.01
d 0-70	5.77	5.57	5.57	5.63	0.16	0.09	0.10	<0.01
DMI, % of BW daily								
d 0-14	1.93	1.87	1.88	1.90	0.02	0.44	0.14	<0.01
d 0-28	1.95	1.89	1.91	1.92	0.02	0.78	0.07	<0.01
d 0-42	2.00	1.91	1.94	1.96	0.01	0.80	<0.01	<0.01
d 0-56	2.03	1.96	1.97	1.99	0.02	0.08	0.04	<0.01
d 0-70	2.01	1.94	1.95	1.98	0.02	0.04	<0.01	<0.01
G:F, kg:kg								
d 0-14	0.16	0.14	0.12	0.11	0.03	0.03	0.55	0.80
d 0-28	0.20	0.18	0.18	0.17	0.02	0.32	0.19	0.92
d 0-42	0.16	0.16	0.15	0.15	0.01	0.18	0.84	0.43
d 0-56	0.16	0.16	0.16	0.15	0.01	0.34	0.30	0.97
d 0-70	0.16	0.16	0.16	0.15	0.01	0.46	0.38	0.51
NE_m, Mcal/kg[4]	1.87	1.89	1.89	1.82	0.05	0.54	0.58	0.24
NE_g, Mcal/kg[4]	1.23	1.25	1.24	1.19	0.04	0.54	0.58	0.24

[1]Diets formulated to supply 1.32 Mcal NE_g/kg DM.

[2]B= By-product, Sweet Bran (Cargill, Blair, NE), wet distiller's grains plus solubles (Wamego, KS)

[3]P = extent of corn processing, DRC stands for dry rolled corn, WC stands for whole corn

[4]Net energy calculations based on equations from NASEM (2016)

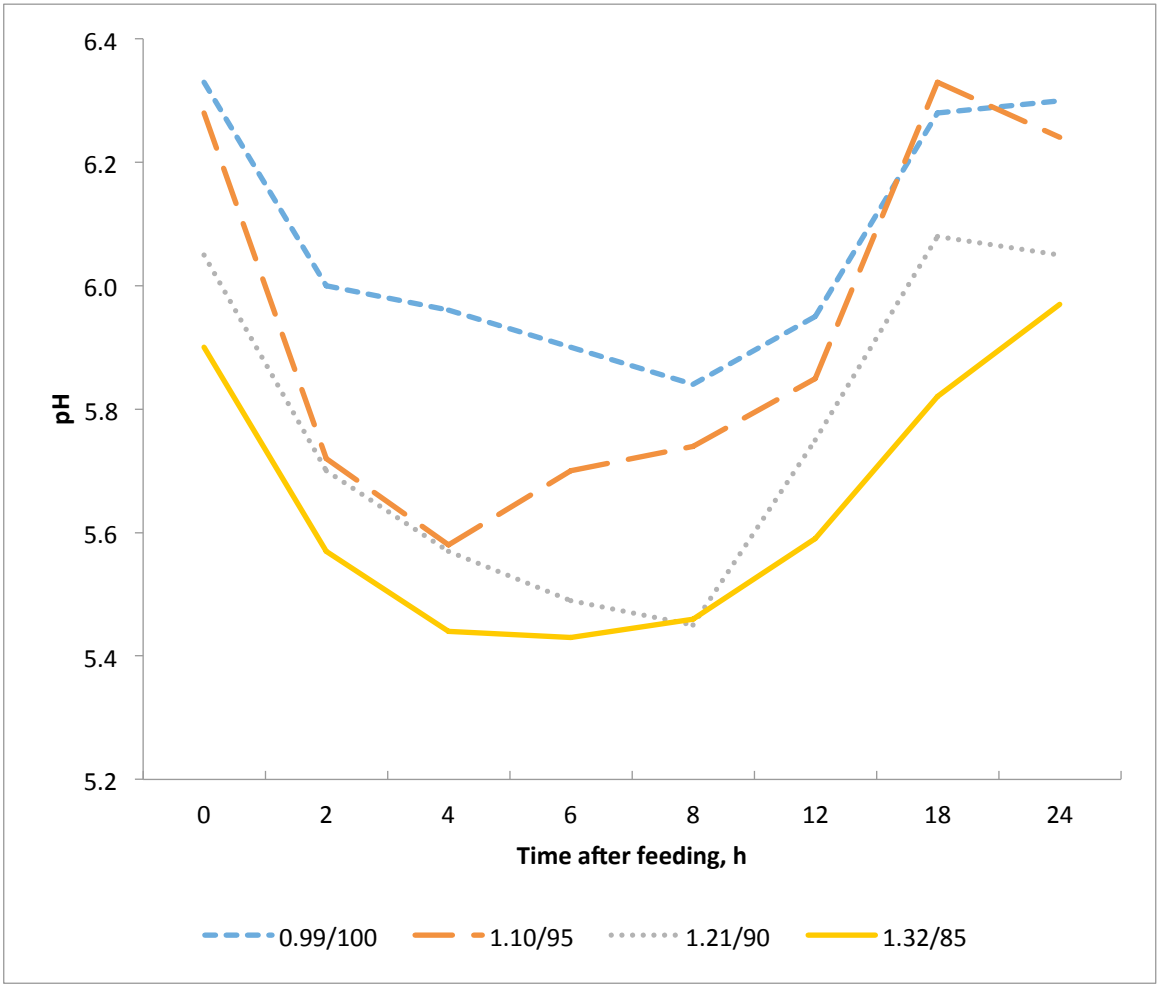

Figure 2.1 Effects of energy level and intake on ruminal pH measured over 24 h. 0.99/100 = 0.99 Mcal NE_g/kg DM offered for ad libitum intake (100%). 1.10/95 = 1.1 Mcal NE_g/kg DM diet limit-fed at 95% of 0.99/100. 1.21/90 = 1.21 Mcal NE_g/kg DM diet limit-fed at 90% of 0.99/100 diet. 1.32/85 = 1.32 Mcal NE_g/kg DM diet limit-fed at 85% of 0.99/100 diet. Diet effect ($P < 0.01$), hour effect ($P < 0.0001$), diet x hour effect ($P = 0.93$). Measurements taken using indwelling pH monitoring bolus (smaXtec, Graz, Austria).

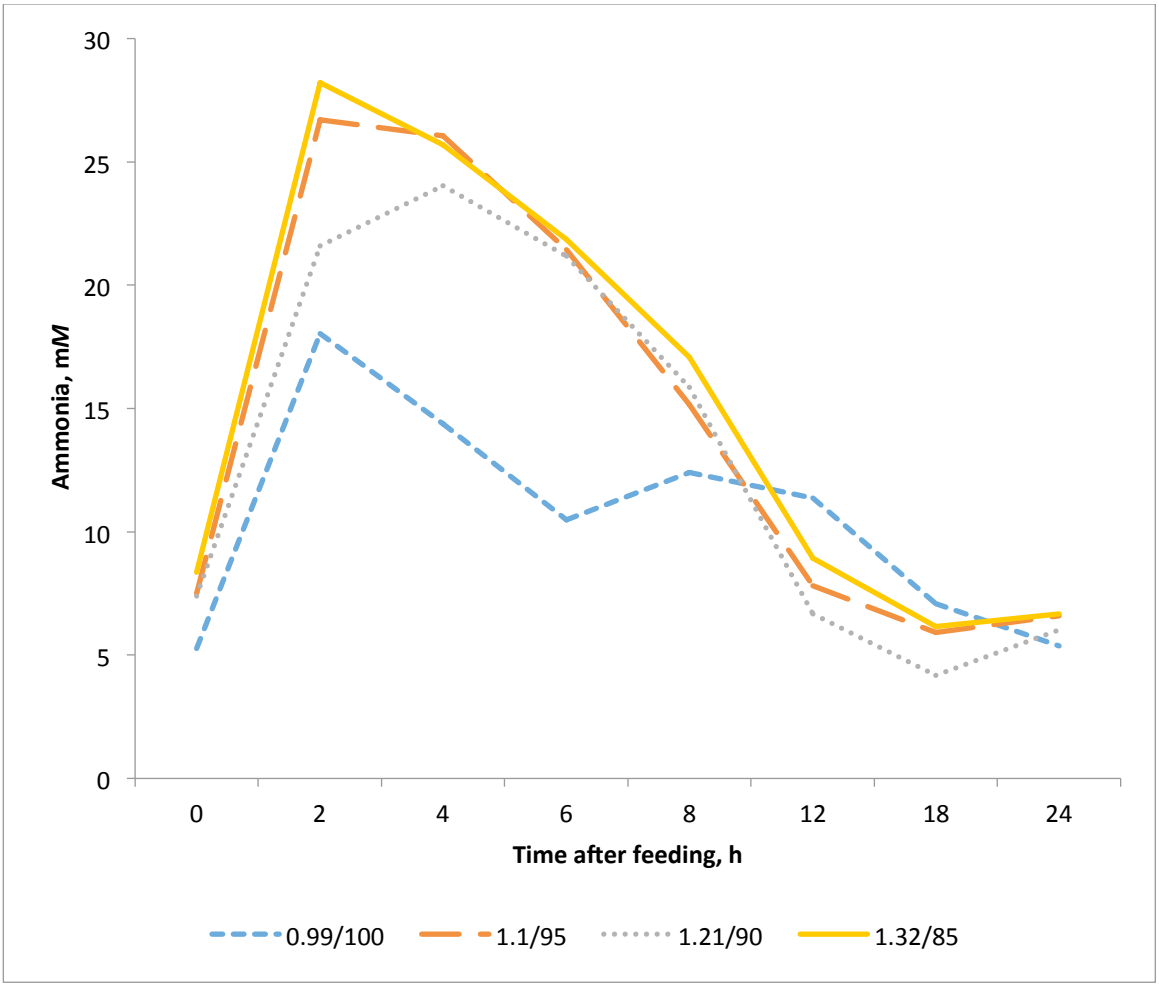

Figure 2.2 Effects of energy level and intake on ruminal ammonia measured over 24 h. 0.99/100 = 0.99 Mcal NE_g/kg DM offered *ad libitum* (100%). 1.1/95= 1.1 Mcal NE_g/kg DM diet limit-fed at 95% of 0.99/100. 1.21/90 = 1.21 Mcal NE_g/kg DM diet limit-fed at 90% of 0.99/100 diet. 1.32/85 = 1.32 Mcal NE_g/kg DM diet limit-fed at 85% of 0.99/100 diet. Diet effect ($P < 0.0001$), Hour ($P < 0.0001$), Diet x Hour ($P = 0.0002$).

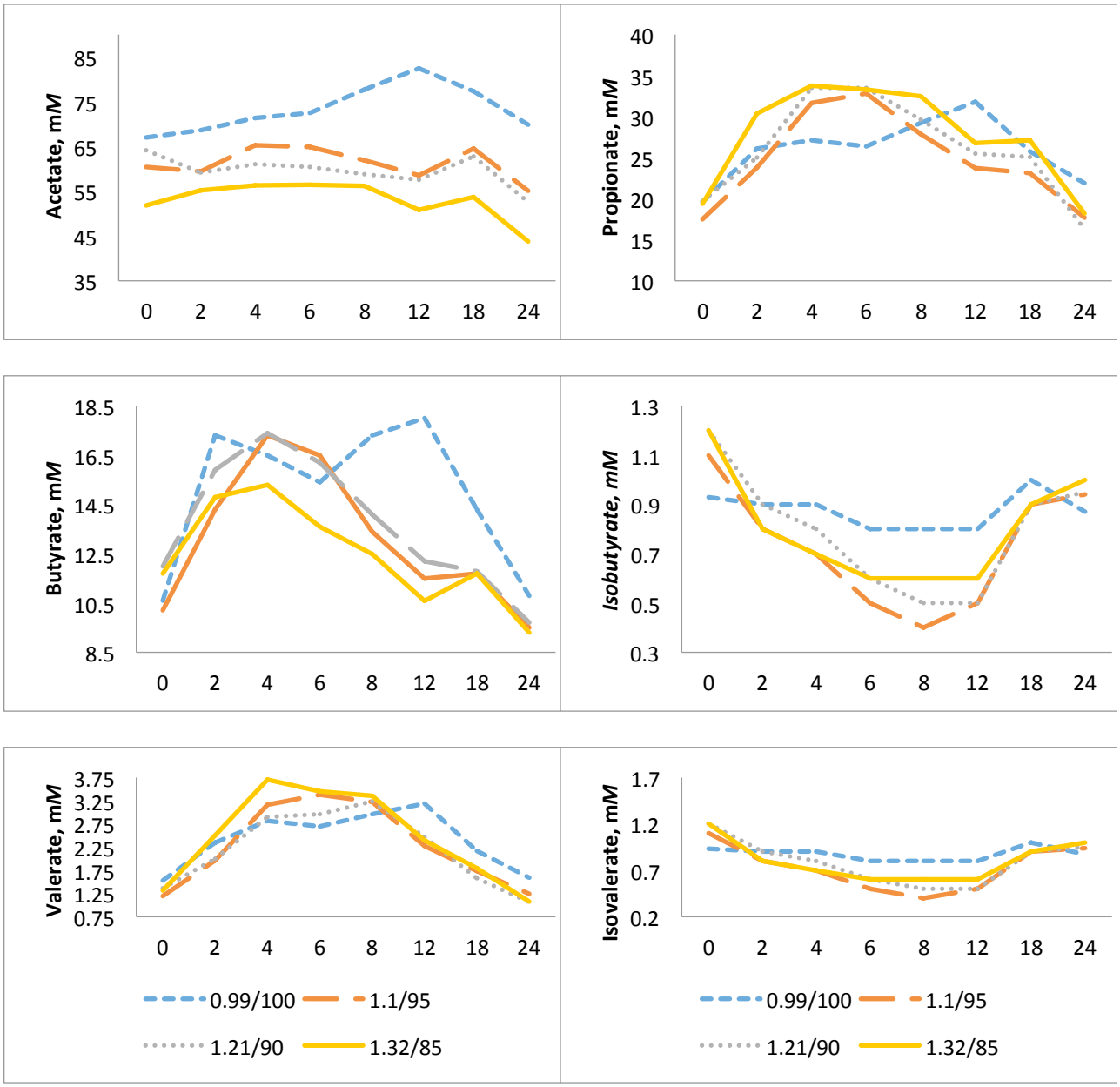

Figure 2.3 Effects of dietary energy and intake level on ruminal VFA concentrations over 24 h. 0.99/100 = 0.99 Mcal NE_g/kg DM offered for ad libitum intake (100%). 1.1/95 = 1.1 Mcal NE_g/kg DM diet limit-fed at 95% of 0.99/100. 1.21/90 = 1.21 Mcal NE_g/kg DM diet limit-fed at 90% of 0.99/100 diet. 1.32/85 = 1.32 Mcal NE_g/kg DM diet limit-fed at 85% of 0.99/100 diet.. For acetate, diet effect ($P < 0.0001$) hour ($P < 0.03$) hour x diet ($P = 0.84$). For propionate, diet effect ($P < 0.23$) hour ($P < 0.0001$) diet x hour ($P = 0.05$). For butyrate, diet effect ($P = 0.14$), hour ($P < 0.0001$), diet x hour ($P = 0.01$). For isobutyrate, diet effect ($P = 0.29$), hour ($P < 0.0001$), diet x hour ($P = 0.09$). For valerate diet effect ($P = 0.84$), hour effect ($P < 0.0001$), diet x hour effect ($P = 0.77$). For isovalerate, diet effect ($P < 0.0001$), hour effect ($P < 0.0001$), hour x diet ($P < 0.28$).

Chapter 3 - Effects of dietary energy level and intake of corn by-product based diets on newly received growing cattle: II. Antibody production, acute phase protein response, stress, and immunocompetency of healthy and morbid animals

INTRODUCTION

Newly received stocker cattle exposed to stress of marketing typically have low DMI on arrival to feeding facilities (Hutchinson and Cole, 1986). One strategy to mitigate the risk of dietary deficiencies from low intakes is to increase the concentration of dietary energy. Increasing dietary energy in receiving diets has been correlated in some research to increased morbidity (Lofgreen et al., 1975, Rivera et al., 2005). It is often thought the increase in disease could be associated with metabolic disorders initiated by excessive carbohydrate fermentation from starch because cereal grains are often used to increase dietary energy.

Restricting intake of high-energy diets for receiving and growing cattle to target specific gains has been shown to be a more efficient way of growing cattle (Schoonmaker et al., 2003), and limiting the amount of carbohydrate available for fermentation could also help to decrease metabolic disorders. Limit-feeding high-energy diets based primarily on fibrous by-products such as wet corn gluten feed (Sweet Bran; Cargill Animal Nutrition, NE) as the energy source (40% of the diet on DM basis) and not on cereal grains has not been studied to our knowledge in pen based experiments involving growing cattle. Therefore, there is little information of how

limit-feeding diets of this nature could affect the animal's immune system and stress level early in the feeding period.

The objectives of this experiment were to: 1) monitor immune function through the use of serological titer analysis, 2) characterize stress by measuring concentrations of glucocorticoid metabolite in feces, and 3) index the acute phase protein response using haptoglobin, across a broad range of dietary energy concentrations and intakes. Moreover, the trial was designed to identify differences in the serological and inflammatory parameters between healthy and morbid animals under the different dietary conditions.

MATERIALS AND METHODS

All procedures involving the use of animals were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC # 3745).

Arrival Management and Design

Readers are directed to the Materials and Methods section of Chapter 2 under Experiment 1 for arrival management and study design details.

Blood sampling and analysis

Thirty-two animals from each dietary treatment (4 from each pen) were randomly selected after arrival (d-1) and bled via a tail vein to serve as a subset for analysis of antibody production toward vaccines and the acute phase protein haptoglobin (**Hp**). Animals were bled on d 0, 14, and 27 using venipuncture with an 18 gauge-bleeding needle. Blood was collected in glass 10 mL serum separator tubes tubes (Monoject Blood Collection Tubes; Sherwood Medical,

St. Louis, MO) and allowed to clot for 30 to 45 min in an upright position following collection. After the sample had clotted, the tubes were centrifuged at room temperature for 15 min at 2000 x *g*. Immediately after being centrifuged, approximately 4 mL clear serum was pipetted from the top of the tubes with a transfer pipette and transferred to 5 mL glass tubes (Monoject Blood Collection Tubes; Sherwood Medical, St. Louis, MO) to be frozen for analysis. Tubes containing serum were shipped to the Kansas State University Veterinary Diagnostic Laboratory and analyzed for antibody titers for bovine viral diarrhea I and II (BVDI, BVDII**)** and infectious bovine rhinotracheitis (IBR) as well as Hp. Viral neutralizing antibody titers were determined using the American Association of Veterinary Laboratory Diagnosticians approved test procedure that is a varied serum-constant virus method and uses standardized specific viral strains as the indicator viruses. Haptoglobin concentrations were measured using a colorimetric assay (Smith et al., 1998)

Healthy and morbid animal blood analysis

Animals removed from the pen according to the protocol above for illness were also bled via a tail vein and the blood sample handled identically to the samples taken from the subset of cattle. In addition, a pre-determined random order of animals from each pen was generated on d 0 that served as a means to select a healthy control animal from each pen to obtain a blood sample following the same protocol and to use for pairwise comparisons. Animals that became morbid were permanently removed from the list of healthy candidates and therefore could never serve as a "healthy" animal for comparison.

Fecal cortisol metabolite analysis and sampling

Two randomly selected animals from each pen (16/dietary treatment) were used to determine fecal cortisol metabolite as a means of quantifying stress levels. Fecal grab samples were obtained from the rectum of each of the selected animals on d 0 and 14 processing. Samples were labeled by individual animal identification number and immediately frozen at -20 °C for analysis. All fecal samples were shipped to the Kansas State University Veterinary Diagnostic Laboratory to determine fecal cortisol metabolite concentrations by enzyme immunoassay following the procedures of University of Veterinary Medicine, Vienna, Austria,. (2010).

Statistical Analysis

Serological and fecal cortisol data were analyzed as a split-plot design in the MIXED procedure of SAS (ver. 9.4; SAS inst. Inc., Cary, NC), where sampling day, dietary treatment, and the interaction of sampling day x dietary treatment were fixed effects. The natural logarithm of titer levels for BVDI-II and IBR, and concentrations of haptoglobin were analyzed as the response variable. Contrast statements were used to detect linear, quadratic, and cubic effects of dietary treatment, sampling day, and their interactions when significant ($\alpha = 0.05$). All values were back-transformed to normal scale and reported as such with accompanying back-transformed standard errors.

Data from the comparisons of healthy and sick animals was also transformed to the natural log scale and analyzed in the MIXED procedure of SAS. In this analysis, health status and the interaction of health status x dietary treatment were also used as a fixed effect to test differences between healthy (H), animals pulled once (M1), animal pulled twice (M2), and

animals pulled three times (M3). When the fixed effects above were significant, orthogonal contrasts were used to detect linear, quadratic, and cubic effects of the fixed effects.

RESULTS AND DISCUSSION

Blood analysis

Results from the analysis conducted on samples from the subset of cattle are in Table 3.1. There were no effects of dietary treatment on titer levels for BVD-I ($P = 0.89$), BVD-II ($P = 0.92$), IBR ($P = 0.62$), or haptoglobin concentrations ($P = 0.64$). There were also no dietary treatment x sampling day interactions for BVD-I ($P = 0.89$), BVD-II ($P = 0.92$), IBR ($P = 0.62$), or haptoglobin ($P = 0.94$). In this trial dietary energy intake was not meant to be different among treatments as all animals were theoretically programmed to gain weight similary. The performance data from Chapter 2 (Exp. 1) validates this as ADG was not affected by treatment ($P = 0.72$).

Pahlavani (2000) reviewed a large number of studies and determined the effects of caloric restriction on several aspects of the immune system. Results predominately favored a heightened innate and adaptive response in the face of pathogens and immunostimulants particularly increased IL-2 production. Pahlavani (2000) speculated the effects of caloric restriction could be due to altered transcription of IL-2, which aids in the maturation and activation of naive T-cells to become TH1 cells. Therefore increased IL-2 could modulate adaptive immunity. Lymphocytes of the TH1 phenotype are central in the control and elimination of viruses.

In partial agreement with Pahlavani (2000), Perkins et al. (2001) indicates that restriction of feed to 60% of maintenance had little effect on leukocytes or adhesion molecules in Holstein

cows, but there were some instances of up-regulation. Whitney et al. (2006) observed that the febrile response was increased in steers on a high-forage diet compared to one of high concentrate and serum IgG was also decreased by the high concentrate diet after a Bovine Respiratory Syncytial Virus challenge. However, morbidity was not affected by dietary treatment. These authors speculated that the IgG produced in the calves fed the high concentrate diet could have been more effective at clearing the infection, and thus a more potent immune response was not necessary.

It is difficult to compare our results directly with these studies because our treatment altered dietary energy concentration, but total energy intakes were similar. Because our diets were not formulated to produce a state of energy deficiency, we did not expect differences in immune function due to dietary treatment. We hypothesized that, if differences were detected in immune function, it would be due to increased morbidity, which is often times associated with decreased intakes and performance; that was not the case for any of those parameters. More research is warranted addressing the effects of how energy is delivered (programmed vs. full-fed) and its effects on the immune system of growing cattle.

We also hypothesized that if the high-energy diets that were limit-fed were increasing the incidence or severity of sub acute ruminal acidosis (SARA), then there could be detectable differences in inflammation as measured by the acute phase protein response. Cattle experiencing acidotic conditions have ruminal epithelia that are more susceptible to damage caused by lipopolysaccharide and this damage could result in increased acute phase protein concentrations as endotoxin translocate into the bloodstream (Enemark et al., 2002). Dietary treatment did not affect haptoglobin concentrations in our study, which suggests no effects on the incidence of ruminal acidosis; research shows marked increases in acute phase proteins

following SARA (Gozho et al., 2005). Moreover, in Chapter 2 (Exp. 1), pH was measured in a digestibility study. Although pH was lower for cattle fed the high-energy diets, it was not suggestive of being a cause of metabolic disorders, similar to other work involving Wet corn gluten feed (Montgomery et al., 2004).

Immunological parameters responded quadratically to sampling day ($P < 0.01$), except for BVD-II titers where only a linear effect was detected ($P < 0.01$; Table 3.1). For haptoglobin, concentrations were lowest on d 0, peaked at d 14, and were similar to base line levels by d 27. These results differ slightly from Berry et al. (2004) where peak levels of haptoglobin were realized on d 7 and returned to arrival levels by d 14. One discrepancy between our study and Berry et al. (2004) could be that we did not sample on d 7 and levels could have been higher than on d 14. The initial increase between d 0 and d 14 is most likely an effect of vaccination as Arthington et al. (2013) reported an acute phase protein response for 2 wk following vaccination against common respiratory and clostridial pathogens.

Titers for BVD-I and IBR viruses responded quadratically to sampling day. All animals were vaccinated on d 0 against both viruses and again on d 14. These results are a prime example of adaptive immunity as the immune system was primed and sensitized after d 0 vaccination and re-exposure on d 14 incited a much more robust response. This would explain the increase in titers between d 0 and d 14 and the large magnitude of increase between d 14 and d 27. More research is warranted which addresses the effects of programmed feeding on humoral immunity to vaccines.

The BVD-II titer response for sampling day was linear ($P < 0.0001$) and titer level numbers appeared in this study to be less than those for BVD I. The lower titers for BVD type 2 compared to type 1 are in agreement with Fulton et al. (1997) and could be explained by lower

immunomodulation from the type 2 antigen as evidence of antigen diversity between the two types.

Immuno-characterization of healthy and morbid animals

There were no interactions detected ($P = 0.83$) between dietary treatment and health status for haptoglobin concentrations (Figure 3.1). In addition, there were no interactions detected ($P = 0.28$) between dietary treatment and health status for titer levels. In contrast, health status had profound effects on haptoglobin concentrations, BVD-I, and IBR titers (Figures 3.2, 3.3, and 3.4; respectively).

For haptoglobin, concentrations in serum were increased ($P < 0.05$) with increasing morbidity. These results are in agreement with multiple studies involving Hp concentrations between healthy and sick livestock (Berry et al., 2004; El Deeb, 2016; Humblet et al., 2004) and would be expected if the animals being tested were truly suffering from infection. Titers for BVD-I and IBR titers were higher in healthy animals than in morbid animals ($P < 0.05$), but health status did not affect BVD II titers (Figure 3.5). There are several possible reasons for the lower titers in morbid animals. One, the calves were from typical sale barn marketing protocols and arrived at the Kansas State University Beef Stocker Unit with little to no medical history. We do not know how many of the animals arrived already infected with a variety of different diseases. The effects of initial sickness could have hindered the body's immune system responses to the vaccines and also led to failure of the immune system to protect them from the sickness they were suffering from originally. Martin and Bohac (1985) observed increased morbidity was correlated with decreased titers. In either case, a lower titer level was observed for the morbid animals. Furthermore, the calves were transported for approximately 12 h before

arriving at the receiving facilities and research has shown shipping could decrease immune response (Blecha et al., 1984). This theory would further advocate research into the field of delayed vaccination where vaccines are not administered on arrival but at some time later, when stress levels could have subsided and the immune system can mount a robust response to vaccines as well as to field pathogens.

Research which involves delayed vaccination has shown positive results for antibody titers against vaccines (Richeson et al., 2009). However, more research investigating the nutritional effects of such protocols should be conducted. Our results indicate immune function was depressed for morbid compared to healthy animals, but dietary treatment had no effect on these parameters.

Although no statistical differences were observed for BVD-II titers among morbidity groups, it should be noted that numerical patterns were similar between BVD-II titers and titers for BVD-I and IBR titers. It is possible that the immune system's low affinity for the BVD-II antigen could be responsible for the very large standard errors associated with this specific analysis.

Fecal cortisol

Fecal cortisol was unaffected by dietary treatment ($P = 0.18$) and there were no dietary treatment x sampling day interactions ($P = 0.22$) however sampling day did affect fecal cortisol ($P < 0.05$; Table 3.2). The results in regard to energy concentration are similar to those observed by Tolleson et al. (2013) where a low (9% CP, 58% TDN) and moderate (14% CP, 60% TDN) diet was fed and no differences in fecal cortisol were observed. Major differences between this trial and ours include how diets were fed. The current study used 4 diets however only one was

fed for ad libitum intakes compared to all of them being fed for ad libitum intakes in Tolleson et al. (2013). Like Tolleson et al. (2013), we too used fecal cortisol as an index of overall stress. Fecal cortisol concentrations have been shown to increase following stressful events such as transportation (Morrow et al., 2002; Chen et al., 2015).

Results from this trial disagree with results from Bourguet et al. (2011) in terms of feed deprivation and stress. Their study showed increased levels of plasma cortisol in Holstein heifers when feed was deprived for 30 h. Heifers in the current study were fed every morning at approximately 0700 and the limit-fed rations were typically consumed within 3 h, which translates on most days to feed deprivation of 21 h. Further, cattle become quickly acclimated to stressors, and fecal cortisol can decline accordingly as reported by Andrade et al. (2001). These workers found that when cattle were restrained in a chute for 10 min repeatedly for 19 d, fecal cortisol gradually declined. Fecal cortisol samples were taken on d 0 and 14, which could explain the day effect detected in the current study. On d 0, fecal cortisol concentrations were much higher when compared to d 14 most likely as a result of stress from shipping and initial processing and acclimation by d 14. Measuring plasma cortisol concentrations may have been a more accurate or useful analysis to analyze the effects of diet deprivation however sampling can often confound results. Results from this trial indicate that limit-feeding and the increased energy in the limit-fed diets did not induce stress as measured by fecal cortisol.

IMPLICATIONS

Limit-feeding wet corn gluten feed based rations formulated to contain up to 1.32 Mcal NE_g/kg DM does not affect stress or immune function in healthy or sick animals when compared to lower-energy high-roughage diets fed for ad libitum intake. This is important because limit-

feeding to program gain is a efficient strategy for growing cattle and the higher energy in this case did not cause health issues or modulation of the immune system. Additionally, haptoglobin was increased and titer levels for BVD-1 and IBR decreased in morbid animals compared to healthy pen mates. More research is warranted addressing the effects of programmed feeding systems based on by-products on the immune system and the subsequent effects on performance. Results from this study also indicate more research could be valuable that addresses titer levels before and after vaccination as well as haptoglobin concentrations on arrival as prognostics for morbidity in calves subjected to marketing stress

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Table 3.1 Effects of intake and energy level on haptoglobin and titer levels over time

Item	Diet[1]				SEM[2]	*P*-value[3]		
	0.99/100	1.10/95	1.21/90	1.32/85		Diet	Day	Diet x Day
No. of pens	8	8	8	8				
No. of animals	29	32	29	29				
Haptoglobin,mg/dL[c]						0.26	<0.01	0.64
d 0	15.2	13.3	25.8	13.8	6.8			
d 14	35.8	19.3	32.5	27.2	9.5			
d 27	22.1	19.8	21.5	19.6	5.9			
IBR, titer level[b,d]						0.62	<0.01	0.94
d 0	0.3	1.0	0.5	0.3	0.6			
d 14	11.6	16.6	8.2	10.7	5.1			
d 27	15.7	19.1	17.3	14.4	5.9			
BVDI, titer level[b,c]								
d 0	1.7	2.9	1.2	1.6	1.1	0.89	<0.01	0.99
d 14	48.6	51.7	46.5	40.8	20.1			
d 27	286.2	303.1	284.7	257.9	121.6			
BVDII, titer level[b]						0.92	<0.01	0.99
d 0	3.0	2.8	1.9	2.4	2.8			
d 14	20.6	18.4	12.5	24.4	15.0			
d 27	55.7	75.4	45.3	68.4	44.5			

[1]Treatment diets offered based on DMI of 0.99/100 treatment intake that was offered for ad libitum intake. First number = Mcal NE_g/kg DM. Second number = % of 0.99/100 treatment offered on DM basis.

[2]Largest value between treatments is reported.

[3]Fixed effects of dietary treatment, day, and dietary treatment x day interaction.

[a]Linear effect of day ($P < 0.01$).

[b]Linear effect of day ($P < 0.0001$).

[c]Quadratic effect of day ($P < 0.01$).

[d]Quadratic effect of day ($P < 0.0001$).

Table 3.2 Effects of intake and energy level on fecal cortisol over time

Item	Diet[1]				SEM[2]	*P*-value[3]		
	0.99/100	1.10/95	1.21/90	1.32/85		Diet	Day	Diet x Day
No. of pens	8	8	8	8				
No. of animals	16	15	16	15				
Fecal cortisol, ng/g						0.23	<0.01	0.21
d 0	45.2	72.0	52.6	37.4	7.5			
d 14	16.2	13.7	17.7	11.6	9.6			

[1]Treatment diets offered based on DMI of 0.99/100 treatment intake that was offered for ad libitum intake. First number = Mcal NE_g/kg DM. Second number = % of 0.99/100 treatment offered on DM basis.
[2]Largest value between treatments is reported.
[3]Fixed effects of diet, day, and diet x day

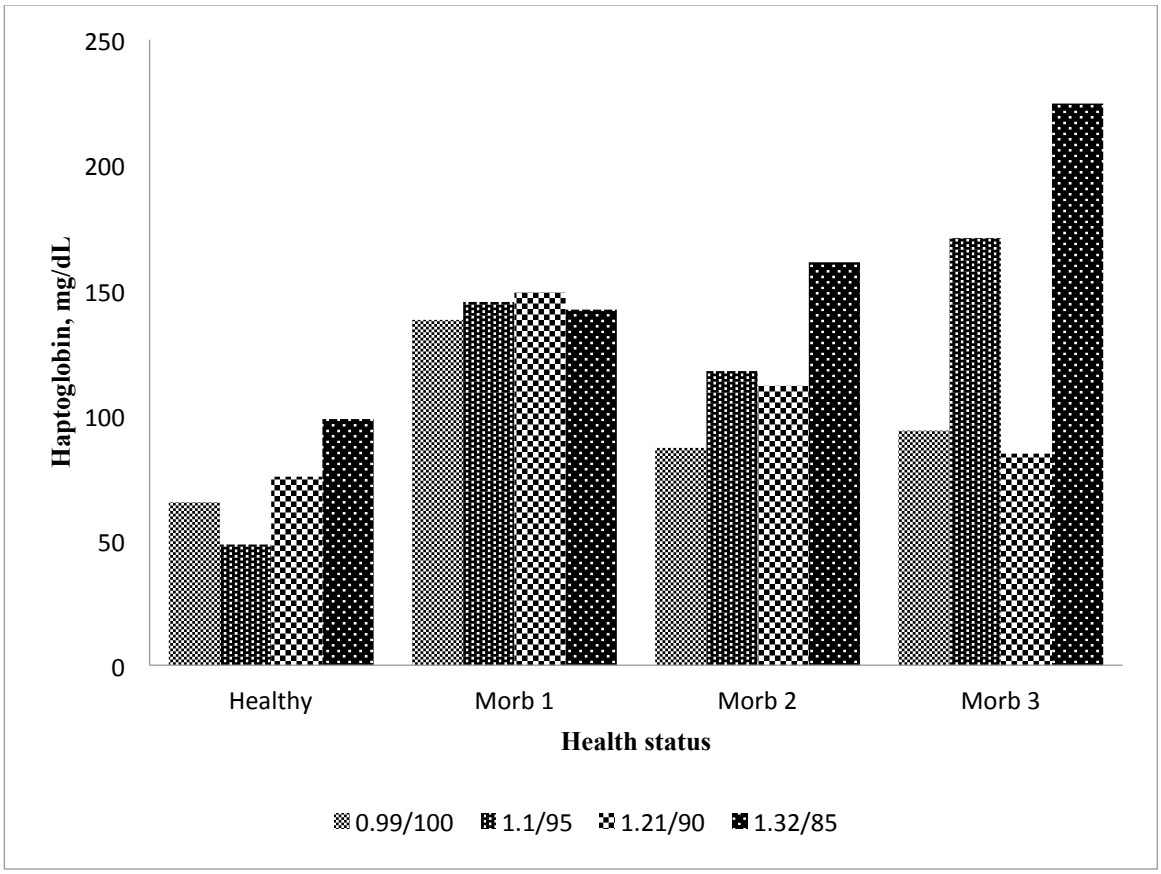

Figure 3.1 Effects of dietary energy level and intake on haptoglobin concentrations in healthy and morbid animals. 0.99/100 = 0.99 Mcal NE_g/kg DM offered ad libitum intake (100%). 1.10/95= 1.1 Mcal NE_g/kg DM diet limit-fed at 95% of 0.99/100. 1.21/90 = 1.21 Mcal NE_g/kg DM diet limit-fed at 90% of 0.99/100 diet. 1.32/85= 1.32 Mcal NE_g/kg DM diet limit-fed at 85% of 0.99/100 diet.. Healthy = healthy pen mate pulled with sick animal for pairwise comparisons (SE = 24). Morb 1 = first pull for illness (SE = 28). Morb 2 = second pull for illness (SE = 50). Morb 3 = third pull for illness (SE = 84). Health status effect ($P < 0.0001$), Diet effect ($P = 0.28$), Diet x Health Status effect ($P < 0.83$).

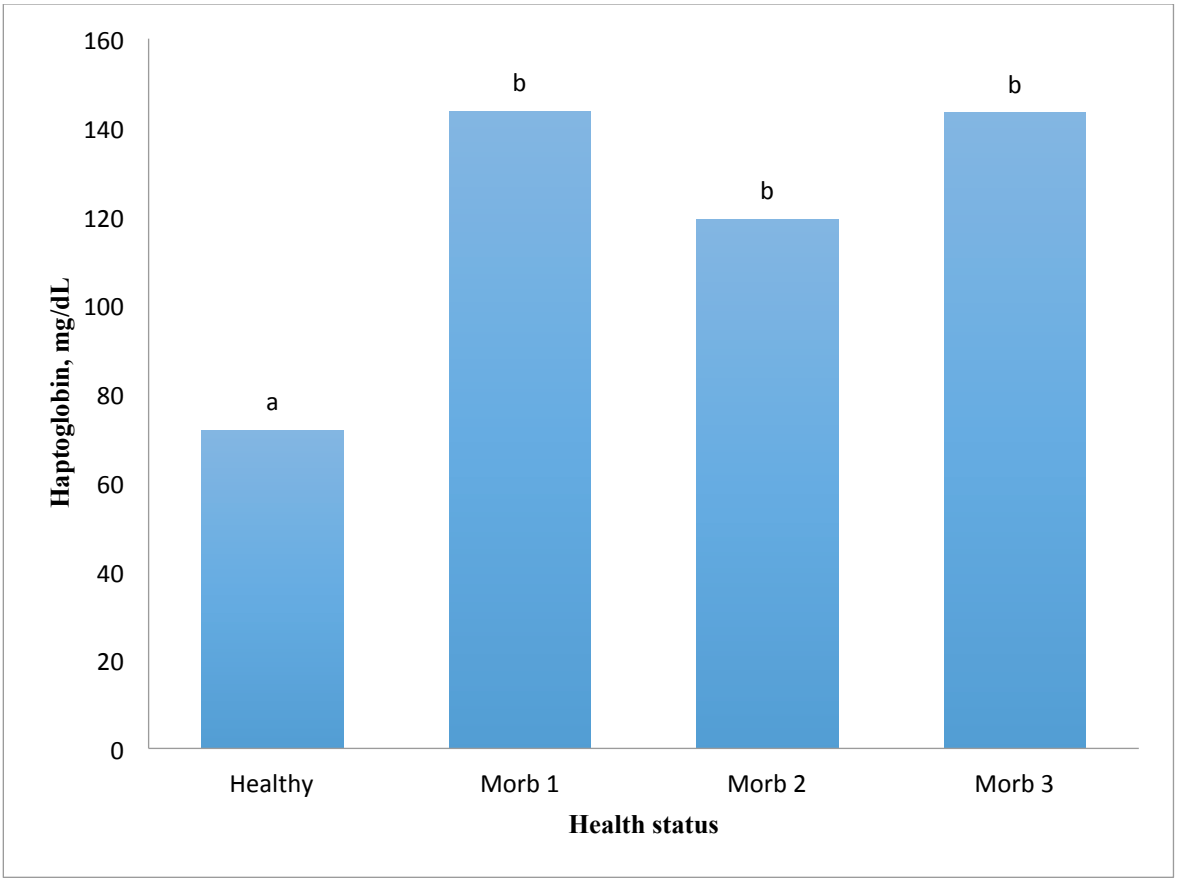

Figure 3.2 Effects of health status on haptoglobin concentrations. Healthy = healthy pen mate pulled with sick animal for pairwise comparisons (SE = 11). Morb 1 = first pull for illness (SE = 13). Morb 2 = second pull for illness (SE = 23). Morb 3 = third pull for illness (SE = 31). [a,b]Unlike superscripts above bars in chart differ ($P < 0.05$)

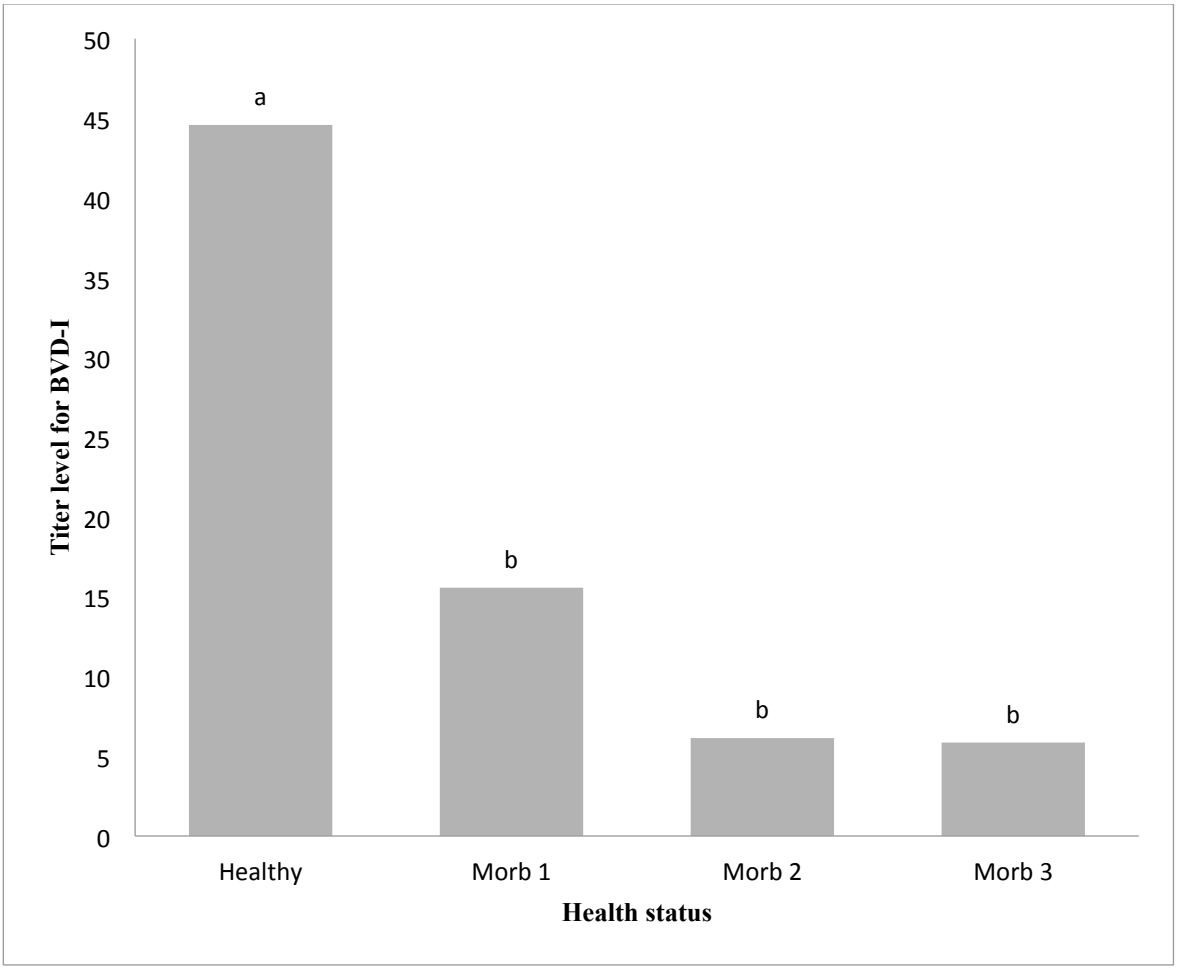

Figure 3.3 Effects of health status on antibody titers for BVD-I. Healthy = healthy pen mate pulled with sick animal for pairwise comparisons (SE = 16.56). Morb 1 = first pull for illness (SE = 6.57). Morb 2 = second pull for illness (SE = 4.50). Morb 3 = third pull for illness (SE = 5.76). [a,b]Unlike superscripts above bars in chart differ ($P < 0.05$)

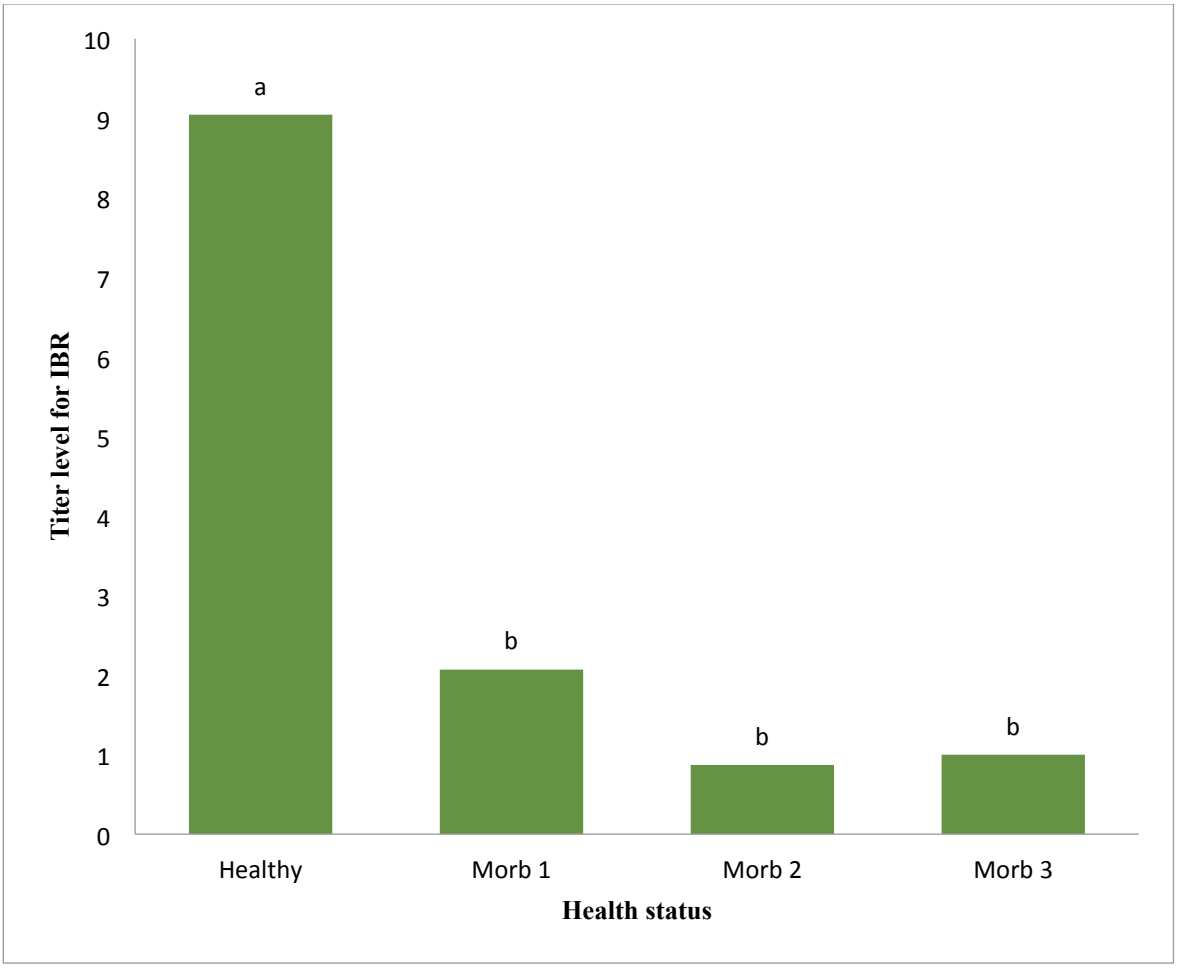

Figure 3.4 Effects of health status on antibody titers for IBR. Healthy = healthy pen mate pulled with sick animal for pairwise comparisons (SE = 2.64). Morb 1 = first pull for illness (SE = 0.88). Morb 2 = second pull for illness (SE = 0.85). Morb 3 = third pull for illness (SE = 1.21). [a,b]Unlike superscripts above bars in chart differ ($P < 0.05$)

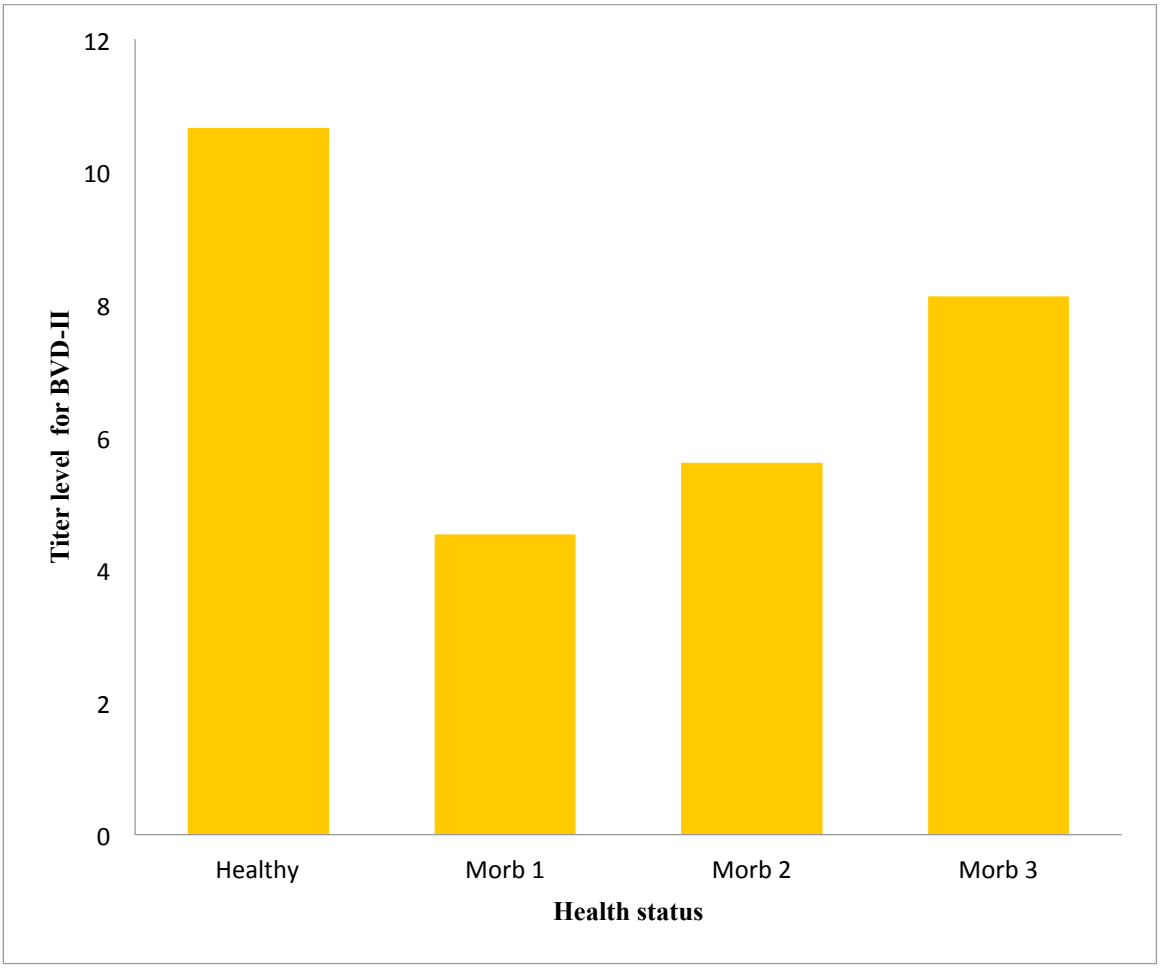

Figure 3.5 Effects of health status on antibody titers for BVD-II. Healthy = healthy pen mate pulled with sick animal for pairwise comparisons (SE = 5.98). Morb 1 = first pull for illness (SE = 5.98). Morb 2 = second pull for illness (SE = 4.93). Morb 3 = third pull for illness (SE = 8.69).