Effects of limit feeding cold stressed growing calves in the morning versus the evening, as well as bunk line sharing on performance

by

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B.S., Oklahoma State University, 2018

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry College of Agriculture

> KANSAS STATE UNIVERSITY Manhattan, Kansas

> > 2021

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Abstract

Two growth performance studies and two digestibility trials were conducted to evaluate limit feeding cold stressed growing calves in the morning versus the evening, as well as bunk line sharing and a study to evaluate the effects of limit feeding corn grain containing an alphaamylase gene to growing cattle. In the first trial, there was one diet offered at 2.0% of body weight on a DM basis. Diets were formulated to provide 1.32 Mcal NEg/kg DM. The experiment consisted of one treatment fed in the morning (AM), one in the evening (PM), one fed half of their feed in the morning and half in the evening (AM/PM), and two additional groups of cattle (Shuttle AM, Shuttle PM) that were fed in the same pen yet were rotated twice daily utilizing an adjacent holding pen. ADG for calves fed in the evening and/or assigned to share a bunk line was not significantly different (P>0.9) compared to calves fed in the morning and not rotated daily. Feed efficiency was not improved in calves fed in the evening nor shuffled between pens (P>0.98). A digestibility trial was conducted concurrently to the first trial using 6 cannulated Holstein steers receiving the same treatments, less the bunkline sharing. Ruminal pH did not differ between treatments (P > 0.35), and ruminal VFA concentrations of propionate and valerate were higher for PM fed calves while concentrations of isobutyrate and isovalerate were lower for PM fed calves (P<0.03). Altering time of feed delivery during times of cold stress and cattle housing management changes digestibility characteristics of the rumen but does not result in any cattle growth performance advantages.

In the second experiment, there were a total of four diets offered at 2.2% of body weight on a dry matter (DM) basis. The four diets consisted of two varieties of corn (Enogen Feed Corn (EFC) vs. negative isoline control corn (CON)) with two different methods of corn processing (dry-rolled corn (DRC) vs. whole-shelled corn (WC)) and were formulated to provide 1.32 Mcal NEg/kg DM. ADG and final BW were not significantly different for calves fed EFC (P > 0.56). Feed efficiency was not different for calves fed EFC (P > 0.94), or for calves fed DRC (P>0.26). A digestibility trial was conducted concurrently using 8 cannulated beef steers fed the same 4 diets as the second experiment. Ruminal pH was not affected by corn hybrid (P > 0.34). EFC fed cattle produced greater concentrations of ammonia and isovalerate (P<.02), as well as having greater digestion of ADF and NDF (P<0.03). Feeding corn grain containing an alpha-amylase gene alters digestion characteristics of the rumen but does not result in improved cattle performance.

Key Words: Enogen Feed Corn, growing cattle, evening feeding, bunkline sharing

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Acknowledgements

I can't thank all who helped me through my graduate career enough. First and foremost, Dr. Dale Blasi opened up his program and resources to me and gave me the opportunity to pursue a master's at Kansas State University. Dr. A.J. Tarpoff is and continues to be a wealth of knowledge on cattle health. I appreciate him answering my endless questions about how I should manage my cattle at home and perform necropsies. Dr. Sean Montgomery formulated ideal diets for both of my trials, which I reference often when trying to decide what to feed cattle for the most cost effective growth. Dr. Chris Vahl helped with my experimental design and analysis, ensuring that our approach was statistically solid from the beginning to the end. Dr. Evan Titgemeyer was instrumental in helping me to complete my lab analysis, as well as interpret the results, for which I cannot thank him enough for.

Mr.'s Bill Hollenbeck and Ross Wahl kept the KSU Stocker Unit running smoothly, allowing me to perform both of my trials there. Marissa Glaser trained me on how to perform digestibility sampling. I'll forever be indebted for her patience. The Stocker Unit undergrads trudged through the mud with me to move cattle in the winter, which I appreciated more than they realized. In addition to what I learned through research, I gained tremendous knowledge through the classes I took. To that I must thank Dr.'s Nagaraja, Olson, Drouillard and Thomson.

And finally, I wouldn't have been successful if it weren't for the love and support of my friends and family. First and foremost, my amazing fiancé, Ashley Hartman, kept me motivated in addition to helping me with statistical analysis. My support system ranged from my parents in Maryland to my best friends in Texas, Oklahoma, California, Kansas and Nebraska. Without their support, I wouldn't be where I am today.

Chapter 1 - Review of Literature

Introduction

In 2018, the national calf crop for the United States was 36.4 million head. These calves were born from a national cow herd consisting of 31,765,700 animals. Of these, 51.74% of the cow herd is located in northern states (NCBA, 2020). Beef cattle are located in many climatic regions and are exposed to naturally occurring climatic conditions, unless kept within intensive production systems. Even within these intensive beef production systems, such as confinement barns or feedlots with shelters, stress factors such as crowding, dust, mud or gaseous contaminants may be generated and negate animal performance. These stress factors maybe otherwise improved by the protection and modulation from climatic stressors. Aside from the vast differences in the regions where cattle are kept, millions of animals are transported to different areas throughout the year for grazing, backgrounding and finishing. This transportation can result in animals experiencing vast shifts in environmental conditions throughout different stages of their lives. Cattle are very adaptable animals, capable of surviving in environments varying from extreme heat to subzero temperatures. Understanding how livestock adjust metabolic rates and behavior to thermoregulate themselves can help producers better manage cattle in adverse conditions. Utilization of feedstuffs for maintaining core body temperature is a main tool employed by cattle. A great deal of maintenance heat is derived from the heat of fermentation of feedstuffs. It is also beneficial for producers to understand how cattle are adversely affected by temperature, humidity, wind, precipitation and mud in order to make management decisions regarding animal housing.

Basal Metabolism

Basal metabolism (HeE) is the chemical change that occurs in the cells of an animal that is in a state of fasting and resting. This means using sufficient energy to sustain vital cellular activity, respiration and circulation. In order to measure H_eE , the animal must be in a postabsorptive state, resting yet conscious, calm and without stress, and in a thermoneutral environment. Determining when a ruminant is in a postabsorptive state can prove difficult. The amount of time allowed for the animal to fast is important. The HeE derived from minimal heat output may appear to plateau, but it will continue to decline as fasting continues. Food passage rate through the digestive tract is important to base the fasting time on. Commonly, utilizing the respiratory quotient (RQ) to indicate when fat catabolism begins is a good benchmark to use. H_eE is generally established when there is no thermal stress (no sweating, shivering, panting, or gular response) on the animal, and is thus where heat production has reached a minimal level. Therefore, the acceptable range of temperatures may vary. Christopherson et al., 1979 and Webster *et al.*, 1969 provide supportive data indicating that prior cold exposure in growing calves results in a shift in resting thermoneutral heat production. This point is also supported by data from sheep, suggesting an influence of temperature the sheep where exposed to prior to metabolic measurement on resting metabolic rate (Slee, 1968; Webster et al., 1969). Further data from several studies of both individual cows and groups shows a consistent increase in resting metabolic rate correlated to a decrease in average prior exposure outside temperature measured the month prior to the measurements of metabolic rate (Young, 1975a). An animal's surface area is an important variable in estimating its rate of heat loss. Surface area of the animal is positively correlated to production of heat. Environment, activity, prior plane of nutrition, age, rate of feed intake, disease and infection, species, breed, sex, type and extent of pelage, and others are all

possible routes by with energy transfer can occur in livestock, thus influencing H_eE . Animals acclimated to the cold generally produce more heat at temperatures greater than those at which the acclimation took place (Young, 1975a, b). This information is useful in evaluating alternatives for extending efficiency of energy expenditures for maintenance of animals, and weight gain in climates where cold stress may force producers to invest in shelter versus feed for fuel. H_eE is also modified by behavioral components of animals, interplayed with managerial systems and the stage of the reproductive cycle.

Animal- Environment Interactions

The environment in which livestock live is complicated by a variety of factors encompassing both psychological and physical aspects of the animal's surroundings. Thermal environment strongly influences livestock with a primary effect resulting from air temperature. Humidity, solar radiation, wind speed and precipitation also alter the thermal environment. Effective ambient temperature (EAT) can be used to describe the impact of various climatic events, combined with their impact on the thermal environment. By altering feed intake, metabolism, and heat loss, animals adjust within limits for deviation in EAT. This in turn shifts the partition of dietary energy by the animal resulting in an altered energetic efficiency. This can require dietary adjustments in nutrient-to-energy balances.

Thermal Balance

Core temperature is maintained in homeothermic animals by balancing the heat gained from metabolism, against that gained or lost to the environment. Collaborative effects of behavioral, morphological and physiological thermoregulatory mechanisms work to maintain heat balance (Monteith, 1974; Robertshaw, 1974). Hypothermia is caused by too rapid of a rate in heat loss. Hyperthermia results from too slow of a loss of heat. Neither of these can be endured for long periods of time. Heat is continuously lost by animals through their body surface by radiation, convection and conduction, as well as through evaporative heat from the skin surface and respiratory tract. The total rate of heat loss depends on the demand by the environment in which the animal lives and the resistance to heat flow of the skin, tissue, and its pelage.

EAT is an index in terms of heat demanded by the environment and is useful in evaluating the total thermal environment's impact on the animal. Several factors are considered in this index. Thermal radiation absorbed by livestock has two main sources: solar radiation and terrestrial or long-wave radiation. The overall impact of thermal radiation depends on the net difference between the total solar and long-wave radiation received and the long-wave radiation derived by the animal. Factors that can influence the total impact of thermal radiation can include ground cover, surface characteristics of the animal, clouds, interior surfaces of housing, insulation, shades and nearby structures and other animals. Animals in sunlight can receive a net gain of heat from thermal radiation. Effectively, this can increase EAT by 3 to 5°C. This can be beneficial in the winter and detrimental in the summer.

The moisture content of the air also influences the heat balance of an animal. This is particularly critical in warm and hot environments where homeothermy relies on evaporative heat loss. A higher ambient vapor pressure results in a lower vapor-pressure gradient from the skin or respiratory tract to the air. This results in less evaporation. Increases in ambient vapor pressures often times has less of an impact on the heat balance of livestock that depend more on panting than sweating to dissipate heat during times of stress. Rate of convective and evaporative heat exchange are affected by air movement. However, the reduction in skin temperature due to vasoconstriction reducing the animal-environmental temperature gradient moderates the

magnitude of this effect. The rise in the rate of heat loss or gain per unit of increase in air velocity is highest at low air velocities. This is due to relatively little air movement being needed for disruption of the boundary layer of still air surrounding the body. An increase above 6 km/h of air velocity leads to little increase in convective heat transfer. Rate of conductive heat transfer from an animal is determined by the temperature and nature of contact surfaces such as the floor. Despite accounting for a small portion of total heat exchange, it can be significant for some species.

Behavioral changes in posture by animals may occur, as well as its orientation to specific environmental factors, such as area of contact with a warm or cool floor, orientation to drafts and winds as well as adjustment to radiation sources and sinks.

A final consideration is that animals are occasionally exposed to inclement weather. Combining wind, low temperature, rain or wet snow can negatively affect an animal's heat balance. Furthermore, water can accumulate in an animal's pelage, replacing still air, which effectively reduces external insulation. Rain can also flatten pelage, effectively lessening its depth and overall insulative value. Cold rain or snow work to increase conductive heat loss, in addition to the cooling of the animal by evaporative heat loss when it is later dried.

Analyzing the relationship between livestock and their thermal environment begins with the thermoneutral zone (TNZ). The TNZ can be defined as the range of effective ambient temperatures within which the heat from normal maintenance and productive functions of the animal in nonstressful situations, offsets the heat loss to the environment without requiring an increase in rate of metabolic heat production (Mount, 1974). It should be noted that changes in the TNZ may occur due to acclimation by livestock to hot or cold environments. For example, a cow's TNZ can be adjusted downward as much as 15°C as a result of acclimation to the cold

during the winter. At temperatures just below the optimum TNZ, there is a cool zone where livestock will initiate mechanisms to conserve body heat. These can be subtle shifts such as changes in hair, postural adjustments and vasoconstriction of peripheral blood vessels. Various behavioral and insulative responses to cold stress have maximal effectiveness at the lower boundary of the TNZ. This point is called the lower critical temperature (LCT). Below this point, rate of metabolic heat production must be increased to maintain homeothermy.

Generally speaking, the initial response of cold stressed animals is more reliant on increasing metabolic heat production. Adaptive responses through morphological and physiological change result from long-term exposure to cold. An example of this is increased insulation, which is an added impediment to heat flow in livestock. Insulation encompasses tissue lining (fat, skin), external insulation (hair coat and wool) as well as the insulative value of the air around the animal. These insulative barriers are additive and are important for determining LCT and the amount of heat loss that occurs below LCT. The limits of an animal's thermal zones shift as its insulation changes. Thermal insulation and thermoneutral heat production can be evaluated and utilized to predict LCT (Blaxter, 1962; Monteith, 1974; Webster et al., 1970). Yet actual LCT may vary in accordance with breed type, age, pen and housing conditions, nutrition, lactational state, time after feeding, history of thermal acclimation, behavior, and hair or wool coat. Large ruminants on high feeding levels have LCT values much lower than other livestock species. This is a result of the large quantities of heat produced as a result of digestion and metabolism to support high levels of production. They also benefit from small surface area to mass ratios, as well as significant quantities of insulative tissue. For these animals, the influences of a cold thermal environment are often times negated through season acclimation as well as digestive and metabolic adjustments to the environment.

Environment nutrition interactions

Intake energy (IE) is the combustible energy ingested by the animal each day. IE is determined from the combustible energy density of the feed, its opportunity for ingestion, and the appetite of the animal. Feedstuffs are never completely digested or absorbed. The fraction that is nonabsorbed is voided as feces and its remaining combustible energy is labeled as fecal energy (FE). Digestible energy (DE) is thus calculated as IE-FE. Metabolizable energy (ME) is the intake energy minus energy losses in feces, urine (UE) and the gaseous products of digestion (GE). The metabolizable energy intake is what is available to an animal for maintenance requirements and productive functions. Metabolizable energy is utilized and oxidized to complete maintenance functions. The first of these is basal metabolism which is represented by the energy from heat involved in maintaining the body integrity as a result of the vital life processes. The second maintenance function is voluntary activity and the obtainment of nutrients, including the muscular activity needed to seek and obtain feed, the activities of digestion, absorption, conversion of food into metabolizable forms, and the formation and excrement of waste products. A final maintenance function is to combat outer stressors in relation to an abrupt and direct imposition of stress or stresses on the animal.

Animals are constantly faced with a multitude and magnitude to stressors in which they must adapt both physiologically and behaviorally (Stott, 1981). Stressors, such as exposure to cold environments, are known to raise the rate of oxidation of feed or body energy to generate heat. The ME oxidized to satisfy maintenance requirements is released from the animal as heat, and ultimately dissipated to the environment. Once maintenance requirements are met, ME for production is available. When IE exceeds immediate needs, energy is retained as lipids, glycogen, and/or protein. When IE falls below immediate needs, these reserves are mobilized.

During times of cold stress, the need to generate additional body heat through shivering or other cold stimulated thermogenic processes can be lessened by the heat generated from maintenance and productive processes. Physiological and behavioral adjustments that arise from external stressors of the animals can affect the intake of energy and its partition within the animal, the quantity of energy available for production, the extent of productivity, and the overall efficiency of feed utilization.

Digestibility and Metabolizability

Digestibility and metabolizability are natural measures of nutrient value or energy designated to feeds and based on both the chemical and physical nature of the feed itself as well as the animal consuming the feed (NRC, 1981). Separate from any effects of the environment on plant growth and the quality or composition of animal feed by itself, there is substantial evidence (Sharma and Kehar, 1961) that indicates the animal's environment directly influences the digestion and metabolic functions of animals. Though the nature and extent of the physiological changes in livestock are not fully understood, the potential consequences to applied animal nutrition are critical. Within temperate climatic zones, the capability of animals to digest roughages decreases with colder ambient temperatures and increases with warmer temperatures. With that being said, severe heat stress such as found in the tropics, can depress and animal's ability to digest feed (Bhattacharya and Hussain, 1974). Fuller, 1965 and Graham, 1965 both expressed hesitation in accepting the notion that the nutrient value of a feedstuff could be altered by the environment to which the animal is exposed. As with sudden shifts in the thermal environment, there are transient changes in rate of passage of digesta and the volume of the gastrointestinal tract. Conversely, there are sufficient evidence to bias short term estimates of apparent digestibility (Degen and Young, 1980; Graham, 1965). Thus, hesitation should

accompany interpreting feedstuff digestibility results acquired during periods of constant hot or cold stress outside of the normal thermal environment.

While observed changes in feed digestibility can be accredited to appetite changes resulting from exposure to hot or cold environments, similar shifts in digestibility cannot solely be attributed to feed intake. The effects are also seen when feed intake is equalized, restricted, and controlled (Christopherson, 1976; Kennedy et al., 1977; Lippke, 1975). Most of this data was observed in trials conducted with ruminants consuming roughages. In similar studies where sheep received concentrate diets, digestibility values are seldom influenced by ambient temperature. Studies of potential direct thermal effects on microbial populations or gastrointestinal tract temperature have not shown any significance of the routes of action (Cunningham et al., 1964; Warren et al., 1974). Rate of passage for digesta in ruminants in relation to diet digestibility is clearly apparent (Balch, 1950; Blaxter et al., 1956; Mertens and Ely, 1979). Recent studies have shown that ambient temperate may influence feed digestibility by affecting the fill of the gastrointestinal tract and the rate of digesta passage. During heat stress, rumen motility of livestock decreases (Attebery and Johnson, 1969), and there is a concurrent rise in the retention time of digesta that should enhance digestibility (Warren et al., 1974). Nearly opposite responses have been shown for cold-exposed cattle and sheep, meaning an increase in reticulorumen motility and rumination activity, increase in the rate of digesta passage (less retention time), and a decrease in the apparent digestibility of feedstuffs (Buckebusch and Marguet, 1964; Christopherson, 1976; Gonyou et al., 1979; Kennedy et al., 1977; Westra and Christopherson, 1976).

Miller *et al.* (1974) reported that reduced passage rate is seen in cattle with damaged thyroid glands, and that this function could be restored by feeding thyroprotein. This was also

supported by reduced rate of digesta passage in thyroidectomized sheep, which was restored by thyroid therapy (Kennedy et al., 1977). Cold temperatures increase thyroid activity and warm temperatures reduce thyroid activity (de Andrade et al., 1977; Gale, 1973; Johnson, 1976). Exposure to varying ambient temperatures result in shifts in thyroid activity in livestock, which is a result of both changes in rate of digesta passage and gut motility. This can result in changes in diet digestibility. Dry matter and DE value of a feedstuff are affected by thermal environment which subsequently alters its ME value. Losses of urinary energy as well as combustible gases from fermentation by microbes alter the ME value of a feedstuff. These loses, similar to fecal loss, are affected by the animal's environment. Cold exposure often leads to higher nitrogen output and urinary energy as a result of increased tissue protein breakdown to provide substrate for thermogenesis (Blaxter and Wainman, 1961; Graham et al., 1959). This would result in lower calculated ME value. Kennedy and Milligan, 1978 observed a reduction in methane production in cold stressed sheep, which is likely explained by decreased microbial activity. This would slightly affect the ME value of the diet consumed during times of cold stress. This improvement is unimportant in relation to the reduced digestibility seen during exposure to cold temperatures.

Feed intake effects on performance

Level of voluntary feed intake and the use of the ME ingested is affected by environmental conditions. It is often difficult to systematically relate from existing knowledge how environmental fluctuations change nutrient requirements in animals. This is due to much of the research regarding shifts in feed intake due to changes in climatic conditions, such as relative humidity, rate of air movement and temperature, being conducted only in controlled situations within laboratories, with usually only one variable being altered. Drastic alterations in feed intake at low and high temperatures is often seen in laboratory experiments, however applying this knowledge to industry practice is often difficult due to climatic conditions being considerably more variable than laboratory environments.

Feeding trials to simulate farm conditions were used to estimate the change in feed consumption as affected by temperature in feedlot cattle. Seventy percent digestible diets were used. Intake was increased in a near linear fashion as temperature decreased from 10°C to -10°C. Further drop in temperature resulted in high variation among animals, potentially explained by possible prior acclimation. In some animals, intake was decreased at very low EAT due to behavior patterns, like standing to shiver instead of eating. Another example of this is shown by average daily gain of steers decreasing by 70 percent in mid-winter Canada conditions when the temperature reached -17°C. In this study, ME intake per unit of gain was 140 percent higher than March through November values (Milligan and Christison, 1974). Test ADG and feed per unit of gain had correlations of -0.85 to 0.74 with ambient dry-bulb temperature. This reduction in ADG can be explained by both intake and efficiency of utilization of ME for gain are lowered.

When temperatures rise above 25°C or drop below -10°C, the type of ration and level of temperature drastically affect intake. Within these temperatures, digestibility of the diet is more important than ambient temperature. Though we generally focus on temperature as the environmental variable most associated with feed intake, space per animal, lot surface and their interaction effects are also important to consider for intake (Elam, 1971; McDowell and Hernandez-Urdaneta, 1975).

Acclimatization vs acute stresses to maintenance energy requirements

Maintenance energy requirements of cattle are influenced by thermal environment in two ways. The first is due to acclimatization as a result of extended exposure to a thermal environment. The second results from animal exposure to an acute cold or heat stress that requires an immediate increase in heat generation in order to maintain homeothermy. Acclimatization comes about due to metabolic and hormonal functional changes that develop as a result of continued exposure. These changes are more correlated with seasonal changes in the thermal environment, instead of with short-term or daily deviations. While mean monthly temperatures are a more acceptable basis to adjust for metabolic acclimatization, acute heat and cold stress responses resulting from exposure to extreme temperatures requires a much more rapid response. Therefore, beef cattle diets should be adjusted according to daily weather fluctuations. These adjustments should be made with consideration to avoid possible disruptions of rumen microbial functions. Adjustment of maintenance energy requirements for metabolic acclimatization is necessary due to the resting or basal metabolism of livestock being to an extent dependent upon the thermal environment the animal is fully or partially acclimatized. Lower basal metabolic rates are seen in animals adapted to warm environments, whereas cold acclimated cattle have elevated basal metabolic rates. Continuous changes in the adaptation of an animal occur in correlation to the variable seasonal conditions of the natural environment. It is highly likely that cattle never become fully adapted to the extremes in regions where there are significant seasonal fluctuations in ambient temperature.

When cattle are exposed to a thermal environment that is below their lower critical temperature (LCT), they often require adjustments to their diet to help counter the acute cold stress. Cattle tend to have a lower LCT than other species of livestock and domestic animals. Generally speaking, direct cold stress is not a practical nutritional problem in cattle except for in extremely cold areas and winters. Moisture and wind can also lead to chilling on young animals with poor resistance to the cold. Beef cattle are overall very cold hardy due to their large size, relatively large amounts of heat from fermentation, and metabolic processes (heat increments) as

well as their effective thermal insulation. While the immediate increase in energy required during sever cold stress, such as during a blizzard or winter storm, animal survival can prove to be more of a challenge. Severity of the challenge is dependent upon both an animal's prior acclimatization, as well as the environmental conditions in which they face. Cattle that are adapted to the cold cannot only have an increased metabolic rate but are also more capable at increasing their rate of metabolic heat production (summit metabolism) in order to prevent hypothermia when severely challenged with cold stress. This adaptation can allow for survival in situations where nonacclimatized cattle succumb. Immediate utilization of every substrates from tissue reserves or the diet is required to support increased heat production during cold stress. Animal productivity can be maintained through nutritional adjustments.

While it is clear that environmental stressors have direct effects on the energy requirements of cattle, there is less certainty as to the amounts of non-energy components of diets. There is some evidence that cattle exposed to the cold may have increased Vitamin A requirements (Hidiroglou and Lessard, 1971; Jones *et al.*, 1962). Ames *et al.*, (1980) suggested that the percentage of protein in the diet can be decreased during winter months without affecting the growth rate of feedlot cattle. This decrease in diet protein should be in proportion to increased feed intake so that the absolute level of protein consumed in maintained. All diet adjustments should be in consideration of economics, and the possibility that excess of some diet components is fed. Excess protein fed to cattle is catabolized and utilized as an energy source. Increasing the amount of roughage in a diet will result in slight increases in animal heat production due to the heat from fermentation (Mader et al., 1999). For cattle that are restricted fed in cold climates, an increase in diet roughage may be advantageous for maintaining animal temperature. This is not necessarily feasible in ad libitum fed cattle, as substituting concentrates for roughages can limit the overall amount of energy intake, subsequently reducing animal productivity.

Effects of wind and precipitation

McCarrick and Drennan (1972b) investigated the growth response to providing of a roof over 9-month-old Friesian steers or a windbreak for outwintered cattle during the winter versus cattle housed indoors. They found no difference in live-weight gain despite above average precipitation and below average temperatures. Distress as indicated by shivering and a humped posture was occasionally seen in animals on unsheltered sawdust pads during times of heavy rainfall. Overall cattle suffered remarkably little from occasional cold weather in these experiments (McCarrick and Drennan, 1972a). Similar results were observed when comparing growth of Friesian weanlings outwintered in grass fields and fed silage compared to livestock kept indoors (Walshe, 1966; Gleeson and Walshe, 1967 and 1968). Similar growth rates are also exhibited in 8-month-old Hereford x Shorthorn and Friesian weanlings wintered outdoors (Harte, 1967 and 1968), as well as when wintered in roofless cubicles instead of indoor cubicles (McCarrick and Drennan, 1972b). Sykes and Slee (1969) derived from climate chamber trials that closely shorn sheep developed acclimatization to acute cold after exposure for only a 2-week period to moderate cold (8°C). This process of acclimatization occurred regardless of restricted food intake, and loss of body weight but was additionally altered by plane of nutrition. This acclimatization to cold may explain the absence of a response in live weight gain as a result of housing or lack thereof. This corresponds with development of longer and more dense coats of hair by cattle wintered outdoors in comparison to their housed counterparts. It also explains why outwintered cattle do not exhibit compensatory gain when turned out to grass following times of cold stress (Gleeson and Walshe, 1967 and 1968; McCarrick and Drennan, 1972a).

Effects of mud

Mud exaggerates problems facing beef cattle feeders. Bond et al. (1970) investigated the effects of mud on daily gains and feed conversion efficiency of beef cattle. They reported that mud reduced daily gains 25 to 37 percent while increasing feed required per pound of gain by 20 to 33 percent. Several mechanisms were hypothesized to explain these results. First, extra energy may be needed for standing more often, as the animal is uncomfortable lying in the mud. Second, heat loss from the animal due to the mud laden hide having a reduced insulative value and increased evaporative heat loss is greater. Third, the cattle may have a decreased feed intake because they are reluctant to venture out in the mud to the feedbunks. Finally, it is possible that extra energy is required for the animal to pull its feet out of the mud. The effect of mud on cattle is important to effectively design economical feedlots (Riskowski and DeShazer, 1975). In a working feedlot, there is a hard soil-organic interface layer under the organic layer which resists penetration of a hoof. Therefore, an animal will only sink as deep as the organic layer (Mielke et al., 1974). The force required to pull a hoof out of mud is a combination of three forces: force of friction within the mud along with the surface of the hoof, a constant suction force created by the resistance of mud to flow into the void left by the moving hoof, and a small force due to the accumulation of mud on the hoof. Riskowski and DeShazer (1975), concluded that the work required for beef cattle to navigate through a 20 cm depth or less of mud (63 cal per step) is not a prominent contributing factor in the decreased performance of beef cattle. This was supported by Bond et al. (1970), who calculated energy losses from beef cattle in feedlot mud experiments and saw no significant differences in heat and work losses of cattle in muddy lots versus those in concrete lots. They concluded that the detrimental effect of mud was a decreased energy intake.

Thurston (1894) reported that human muscles have the capability to exert an average force of 77 N/cm² of muscle. Assuming muscles of cattle are similar in these capabilities, beef animals should have more than ample strength to pull their hoofs out of mud in all but the most severe conditions. Furthermore, an ox can sustain a work output of 315 W for a 10-hour period. Assuming beef animals have similar attributes, they would have to take over 72 steps per min for a period of 10-hour in the 20 cm mud before exceeding their energy output capabilities. This all reinforces that beef cattle have the ability to walk through the mud with a low expenditure of energy. Muddy feedlots appear to lower the desire of cattle to eat, possibly due to reluctance to subject themselves to the extra force required to walk to the feed bunk. Yet, decreased feed intake and increase of work for walking in muddy feedlots probably are not the total answer to decreased cattle performance. Rain and the accompanying wet resting areas lead to damp hides which have a high potential for heat loss. A kilogram of water requires about 555 kcal of heat for evaporation (Riskowski and DeShazer, 1975). Bond et al. (1970) showed that beef cattle in a muddy lot stood an average of 2 percent more time than the animals in a concrete surfaced lot. Bond et al. (1970) reported that cattle require 2 kcal per day per kg to stand. This would be an energy loss of 18 kcal per day for a 450 kg animal because of extra standing in mud. Therefore, extra standing does not appear to be a main contributing factor to decreased cattle performance in muddy lots. Overall, an increase in depth of mud increased the energy and the maximum force required to pull a hoof out of the mud and increased moisture content of a soil generally decreased the energy and force required to pull a hoof out of the mud.

Handling and daily movement effects on behavior and growth

Exercising receiving cattle is thought to be a management technique to mitigate receiving stress in cattle, as well as aiding consumer interest regarding cattle welfare (Lyles and Calvo-

Lorenzo, 2014). Care must be taken to ensure exercising calves does not increase stress on the pulmonary system, as cattle are equipped with unique lungs that are small in relation to body size with minimal reserve, no collateral ventilation and with interlobular interdependence (Robinson et al., 1983). Benefits of exercise programs can include enabling a positive human-animal association between stockperson and calf through repeated neutral or positive exchanges during exercise sessions. Exercise programs increased calf familiarization to their new environment and created additional opportunities for visual evaluation of calf health (Daigle et al., 2018). Woolsoncroft et al. (2018), reported that calves exercised 529 meters for 3 days per week had a 6 percent increase in feed efficiency compared to calves that were not exercised. However, more of these calves tended to require a second treatment for bovine respiratory disease. This was similar to Daigle et al. (2018), who found that percentage of calves treated for respiratory disease was greater in exercised calves versus none exercised calves. Gain-to-feed ratio and mortality rate were similar among treatments, and ADG was decreased for exercised calves. Daigle et al. (2017) suggested shorter durations of programmed exercise may result in improved production responses as calves often become tired during exercise sessions lasting more than 20 minutes. Behavior of calves exercised regularly also changes, as exercising leads to the ratio of the pen lying and resting simultaneously to increase while subsequently decreasing the number of animals feeding, drinking, ruminating, and walking. Moving cattle can decrease feed intake by roughly 1 kg/d for up to 48 hours after the act. It is also observed that moving cattle in the morning decreases the number of animals resting in the afternoon and increases the number of animals panting throughout the day (Mader, 2005). Enrolling cattle in exercise regimens increases the monetary investment (e.g., yard maintenance; labor costs; and medical costs for humans, cattle, and equids) in the cattle, the working equids used to execute the exercise

protocols, and the personnel to conduct the exercise. The risks to cattle welfare by exercising calves tends to outweigh the benefits (Daigle et al., 2018).

Limit feeding twice a day vs. once a day in cold environments

Feed costs make up a large proportion of total production costs, and changing feeding management can lead to changes in feeding behavior that may be associated with differences in cattle performance. Changes in feeding behavior and feed intake influence the production of cattle (González et al., 2012). Some research suggests that more efficient calves, measured using residual feed intake, consume reduced meals at a slower rate, while spending less overall time at the bunk each day in comparison to less efficient calves (Montanholi et al., 2010; Schwartzkopf-Genswein et al., 2002). Feeding behavior and feed intake (time per day or per feeding event, number of feeding events, and per feeding event or amount of feed consumed per day) are considered to be controlled by ruminal fill in addition to metabolic feedback mechanisms (Rines, 1971; Fisher et al., 2003).

Limit-feeding has the potential to improve growth efficiency (Galyean, 1999), possibly through enhancements in digestion (Murphy et al., 1994a; Murphy et al., 1994b). Furthermore, research has indicated that feeding calves in the evening yields superior growth performance in cold climates (Bergen et al., 2008; Holt and Pritchard, 2005; Small et al., 2004). This is potentially due to heightened heat production, resulting from digestion of feedstuffs that helps to sustain body temperature. Not as much is known about how feed delivery in the evening influences feeding activity. Providing split feed consumption with 1/2 of the diet consumed during the day and 1/2 at night is potentially another approach that influences nutrient utilization by allowing for a more constant supply of dietary substrate during the day, thus reducing the potential for digestive disturbance (González et al., 2012). These authors determined that limit-

feeding a forage based growing diet in the nighttime as compared to limit-feeding in the daytime improved growth performance (F:G and ADG) and increases feeding activity (number of visits and meals per day). These results could be due to an increased heat generation to help maintain body temperature, and thus reduce maintenance energy requirements (Prezotto et al, 2017). Improvements are seen in ADG and G:F when comparing evening versus daytime fed cattle in cold environments (Kennedy et al., 2004; Small et al., 2004; Holt and Pritchard, 2005; Bergen et al., 2008). Heat production as a result of having feed access during night hours could come from a variety of factors such as heat increment of feeding (Emmans, 1994), heat of fermentation (Ferrell, 1988), or increased physical activity (van Milgen et al., 1998; van den Borne et al., 2007). Cattle tend to eat more during the day than during the night (Fraser and Broom, 1997). Thus, altering the time when feed is available may lead to changes in eating patterns (increased visits and meals per day) between day and night fed cattle. These changes support the idea that increased activity by night fed cattle is associated with increased heat production, which potentially contributes to improvements in ADG and G:F (Prezotto et al, 2017).

Receiving diets

Concentrates as an energy source

Traditional cattle diets are composed of roughages and concentrates. With the introduction of the ethanol industry, modern cattle diets are made up of cereal grains and their byproducts which tend to be energy dense. Nonetheless, it is important to still have a roughage source in the diet, as it provides "scratch factor". This stimulates the motility of the rumen, increases the production of saliva which acts as a buffer to ruminal contents and helps to raise rumen pH, subsequently reducing the incidence of acidosis. Traditional receiving diets consist of at least 30 percent roughage for yearling and light-weight cattle. (Samuelson et al., 2016).

Inclusion of concentrates in ruminant diets supply an abundant source of energy. The price per unit of energy in grain versus forage-based diets is much lower, allowing for more cost-effective gain of cattle. Due to potential nutrient deficiencies upon arrival and low feed intake, lightweight and stressed calves exhibit high requirements for energy that are at times difficult to provide.

Corn, sorghum, wheat, barley, and oats are the predominant grains fed to cattle. In order of starch content: wheat has the most (77%), followed by corn and sorghum (72%), while barley and oats exhibit the least (58%). Ruminal digestibility differs amongst grain with wheat and oats exhibiting the highest percentage (94%), followed by barley (93%), corn (73%), and sorghum (66%) (Huntington, 1997). Starch granules from grain are very digestible by ruminal bacteria. For this reason, receiving cattle diets generally are formulated to contain 50-75 percent concentrates (Lofgreen et al., 1980; Samuelson et al., 2016). Processing of grain increases the surface area of the grain which allows for more ruminal microbes to attach, increasing the overall digestibility. It also gelatinizes the starch in the grain and disrupts the granular structure.

The most common grain used in both growing and finishing cattle diets in the U.S. is corn. (Samuelson et al., 2016). To supply 95 percent of the demand for corn feed grain, United States farmers plant ninety million acres annually (USDA-ERS, 2018). Due to being readily available, processed in a variety of ways, and containing a relatively consistent starch content, corn is the most economically feasible grain source for cattle diets. Its more consistent starch content in comparison to other grains, also provides that cattle fed corn are less at risk of digestive upsets (Herrera-Saldana et al., 1990).

Dietary characteristics of corn grain

The high energy content of corn grain is due to its 72 percent starch content. The three components of the corn kernel are the pericarp, the germ, and the endosperm. The majority of the

starch found in corn is located in the endosperm. This is composed of the aleurone and subaleurone layers as well as the floury endosperm. Very little cellular structure is found within the floury endosperm, but instead the highest density of starch granules is present here. These starch granules are the most susceptible to enzymatic hydrolysis. Contrastingly, the pericarp and germ contain little to no starch but instead are responsible for water uptake (Kotarski et al., 1992). Starch is a glucan comprised of amylopectin and amylose. Most cereal starches contain 70-80 percent amylopectin and 20-30 percent amylose. Hydrogen bonding holds these together within starch granules (Rooney and Pflugfelder, 1986). Waxy grains, such as corn, are more readily digested than nonwaxy grains due to their starches being high in amylopectin (Kotarski et al., 1992). Three phases are required for the digestion of starch as energy (Owens et al., 1986; Harmon et al., 2004; Huntington, 1997). Starch breakdown of amylopectin and amylose to produce linear oligosaccharides and dextrins is initiated by alpha-amylase in the lumen of the duodenum, which is secreted from acinar cells in the pancreas. The second phase occurs in the brush border membrane and yield glucose from brush border carbohydrates. The final phase occurs when the glucose is transported out of the intestine and into the blood stream (Huntington et al., 2006).

In a review focusing on high-concentrate diets fed to growing cattle, Harmon et al. (2004) reported that roughly 77 percent of starch ingested by the animal is digested in the rumen. Ruminal fermentation results in the production of three major volatile fatty acids (VFA) which are acetate, propionate, and butyrate. Large propionate proportions that provide energy to the animal result from diets heavy in fermentable grain starch (Martin et al., 1999; Orskov, 1986). Propionic acid, acting as a major hydrogen-sink product, also lowers levels of methane synthesis in the rumen. Increases in ruminal outflow of microbial protein which provide amino acids to the

host animal result from energy from starch fermentation in the rumen (Huntington, 1997; Rowe et al., 1999). A microbial biofilm covers the surface of feed particles (McAllister et al., 1994), and is produced by starch digesting microbes such as *Bacteroides ruminicola, Butyrivibrio fibrisolvens*, and *Streptococcus bovis*, which readily attach to carbohydrates (Cotta, 1992). The ruminal microbial population is extremely susceptible to fermentation, and quickly responds to carbohydrates. A rapid increase in VFA production can decrease ruminal pH, and in tandem with a reduction in ruminal motility can result in digestive upsets, such as acidosis. A decrease in ruminal pH leading to acidosis can result from lactate accumulation in the rumen. Fiber digestion is depressed with low ruminal pH which result from high-starch diets. The mechanisms for this reduction are the inactivation of fiber digesting microbes due to lower pH environments, decreased acetate:propionate ratio, and decreased ruminal motility (Orskov, 1986). Inclusion of byproducts which contain highly digestible fiber instead of starch in the diet can help to avoid digestive upsets.

Between 18 to 42 percent of dietary starch from sorghum and corn grains in cattle diets reaches the small intestine for digestion (Owens et al., 1986). Starch passage into the large intestine is the least efficient area of digestion (Harmon and McLeod, 2001; Mayes and Orskov, 1974; Owens et al., 1986). Ruminal conversion of starch to glucose is only 64 percent as energetically efficient compared with conversion to glucose in the small intestine (Huntington et al., 2006). The capacity for small intestinal digestion is limited in comparison to the ability of the rumen to ferment starch, despite the small intestine being able to provide more energy compared to ruminal fermentation (Owens et al., 1986; Huntington, 1997; Huntington et al., 2006). Secretion of pancreatic amylase limits the digestion capacity of the small intestine (Owens et al., 1986; Kreikemeier et al., 1991; Mayes and Orskov, 1974). Increasing concentrate levels in the

diet prompts an increase in pancreatic amylase secretions in steers (Clary et al., 1969; Van Hellen et al., 1978). Additionally, newborn animals exhibit low levels of amylase secretions which increase with age (Siddons, 1968). Limits to starch digestion in the small intestine can be explained by feeding highly palatable diets, with an increased degree of grain processing which results in rapid passage rate in the small intestine. The amount of starch that reaches the small intestine from the rumen is decreased by increased processing and accompanied increases in digestibility. Compared to feeding less concentrate in the diet, diets containing 72 percent corn increase ruminal digestion, and decrease intestinal digestion (Galyean et al., 1979). Nutritionists tend to focus on increasing starch fermentation in the rumen in order to increase overall digestion and energy efficiency since much is unknown regarding the limitations and aspects of small intestinal starch digestion (Brake and Swanson, 2018). Nonetheless, in the small intestinal digestion in cattle there is the potential for significant improvements in the energy efficiency of starch due to greater net energy production with post-ruminal glucose absorption.

Grain processing

Digestibility of grain is improved with increased degree of processing. The mechanism behind this is increasing the surface area for ruminal microbes to attach to in order to begin the breakdown of feed particles. The most common processing methods used in receiving cattle diets in the U.S. is steam flaking (65.2%) followed by dry rolling (30.4%) (Samuelson et al, 2016). However, processing is not required for whole corn to be digested as fully as processed corn by growing cattle (Gorocica-Guenfil and Loerch, 2005; Kaiser, 1999). Feeding whole corn to receiving and growing cattle does not result in any negative effects on productivity or rumen health (Reinhardt et al., 1998). The pericarp of corn kernels can efficiently be broken down during mastication by younger cattle. Processing corn may not be required to optimize digestion

as a majority of kernels are able to be broken during the intake and mastication of whole-shelled corn when fed to cows (Beauchemin et al., 1994). Whole-shelled corn has even been reported to out-perform dry-rolled corn (Chester-Jones et al., 1991). Increased retention time of whole corn grain versus rolled corn grain can result in a greater metabolizable energy value (Owens et al., 1997).

Syngenta Enogen[®] Feed Corn

The Syngenta Corporation is a global company who strives to advance sustainable agriculture through crop protection, seeds, seed treatments, and improved genetics, while working closely with small growers across the world. After being established in 2000, Syngenta soon launched their Enogen corn hybrids, which were designed specifically for ethanol production. These corn hybrids contain a bacterial transgene that produces an alpha-amylase enzyme in the grain, therefore eliminating the need to add liquid alpha-amylase. This enzyme is responsible for breaking down corn starch into fermentable sugar, which reduces the viscosity of the corn mash during the ethanol process. This new innovation provided the opportunity for ethanol plants to increase yield, production efficiency, and flexibility, while reducing production costs associated with the use of a liquid enzyme, natural gas, water, electricity, and maintenance chemicals. Ethanol from corn has been proven to reduce greenhouse gas emissions by up to 60%, water use by 7.7%, electricity use by 1.8%, and natural gas use by 8.9% (Urbanchuk et al., 2009). Only 15% of the total corn ground in ethanol plants is required to be Enogen grain in order to reap the benefits of this alpha-amylase enzyme (Syngenta, 2019a). Acidic environments, moisture, and high temperatures activate the enzyme. Enogen corn allows growers to earn a bushel premium by supplying their local ethanol plants with the enzyme. Bushel premiums for growers generated \$28.5 million in 2018 (Syngenta, 2019b). Syngenta announced Enogen Feed

corn (EFC) for cattle in 2013 (Greikspoor, 2018). EFC improves the digestibility of the corn by breaking down starch to fermentable sugars to provide the animal with energy. This is done through the action of a thermo-tolerant alpha-amylase enzyme, which was isolated from gene fragments of three different organisms. Salivation and mastication activate the enzyme during consumption by the animal and rumen fermentation. An increase in the supply of amylase should enhance intestinal starch digestibility and the absorption of glucose, as amylase is typically thought to be the limiting factor in small intestinal starch digestion (Owens et al., 1986; Kreikemeier et al., 1991). Efficiency for producers can be maximized by maximizing starch digestion in cattle.

EFC hybrids offer strong agronomic characteristics and improved genetics in the field (Urbanchuk et al., 2009). Unlike other hybrids designed specifically for silage use, Enogen corn can be utilized for either grain or chopped for silage. No-tillage operations with lower nitrogen and insecticide administration, yet greater amounts of potassium chloride (K₂O), phosphorus pentoxide (P₂O₅), and herbicide are recommended by Syngenta management for EFC (Urbanchuk et al., 2009). Reductions in adjusted agronomic practices and other inputs help to offset recommended increases for fertilizer and herbicide. Such characteristics make EFC hybrids beneficial for producers that grow their own silage or grain. Growers store EFC in separate bins/silos in addition to planting buffer strips of control corn around EFC fields in accordance with commercial agreements that the Syngenta Stewardship program establishes. EFC is prevented from entering into the human food supply through these identity preservation measures. Due to its rapid breakdown of starch in the grain, the corn is not suitable for food processing, as it could have negative effects on food performance and quality i.e. crumbly corn chips and corn tortillas.

Feed by-products

As a result of ethanol production in the U.S., 41.3 million metric tons (mmt) of corn gluten meal, corn gluten feed, and corn distillers grains were produced in 2018 (RFA, 2019a). Production of ethanol aids the United States in reducing its dependence on foreign oil imports, lower greenhouse gas emissions, as well as lower fuel prices (RFA, 2019b). The rise in demand for ethanol production has corresponded to increases in corn prices. This results in corn byproducts being economically acceptable energy and protein sources for beef cattle. Energy in the form of digestible fiber and fat in distillers grains compared to highly fermentable starch decrease the risk of digestive upsets in cattle. By-product feeds such as wet distillers exhibit 97 to 147 percent the energy value of corn. This concentration of nutrients results as a result of starch removal during the dry milling process. Dry milling is the most common ethanol distillation method in the U.S. due to is cost and flexibility in the quality and type of grain that can be utilized (Stock et al., 2000).

Ethanol production through dry milling starts with the grinding of grain to form a meal which is combined with water to form a mash. Starch is then broken down into fermentable sugars by enzymes. With the addition of yeast, sugars are converted into alcohol. After the process of fermentation is completed, distillation of the mash is used to separate the alcohol from the feed particles. The remaining feed slurry is termed stillage. Removal of the coarse grain particles allows for sale of either wet distillers grains (WDG) or after a drying process, dried distillers gains (DDG). Dried distillers grains with solubles (DDGS) can be produced by evaporating the remaining thin grain particles from the stillage to produce condensed distillers solubles which after drying can be added to DDG. These solubles can also be added to WDG to produce wet distillers grains with solubles (WDGS) (Stock et al., 2000). Condensing of nutrients through distillation raises the crude protein (CP) content from 9% in the original corn grain to 27% in whole stillage (Stock et al., 2000). Most feed yards use a form of corn by-products as the primary source of CP in receiving diets. 58% of yards utilize wet distillers grains, and some consulting nutritionists prescribe CP levels as high as 18% to compensate low feed intake of new calves (Samuelson et al., 2016). Distillers by-products are also higher in fat content than corn, as not all oil is removed through the dry-milling process. Fiber, fat, sulfur and phosphorus are nearly 3 times as high compared to the original corn, due to condensing through distillation (Klopfenstein et al., 2008). Problems for growing and finishing cattle can result from high S levels exceeding the maxim tolerable level of 0.4% in cattle diets (NRC, 2000; Sarturi et al., 2013). In research on yearling and calf finishing trials comparing diets with up to 40% wet distillers byproducts vs. cattle fed a 79% dry-rolled corn diet, net energy was increased 47% and 29% respectively, when wet distillers byproducts average 169% and 128% the value of corn (Larson et al., 1993). The dried form of distillers by-products and corn gluten feed have a higher energy value than the wet form (Ham et al., 1994).

Wet milling is accomplished by first separating corn grain into its basic component so starch, fiber, and protein through a process called steeping. Ethanol is produced through the fermentation of isolate starch using a similar process to dry milling or is converted into fructose for corn sweeteners and corn syrups. Corn germ meal is then made by separating the corn germ and extracting the oil from the slurry. What remains is bran and steep liquor. After removal of water from the bran, distillers solubles and steep liquor are evaporated to produce corn gluten feed, which can be sold as wet or dry (Stock et al., 2000).

Limit feeding corn by products

Effects of limit feeding

Limit feeding is a useful feeding management strategy that can be used to maintain energy intake while having low total feed intake. It is also useful in mitigating the risk of selfinflicted potential digestion-related health issues, by restricting the animal's ability to overconsume energy dense diets. This can also be referred to as programmed feeding utilizing high energy diets. This approach involves programming energy in diets with the intention of providing less total feed than what the animal is capable of consuming in terms of energy or gastro-intestinal fill. This management approach is often used to achieve specific gains during the growing phase or early in the feeding period. Increases in efficiency are often seen due to less total feed being required to achieve comparable gains.

A trial involving four diets was conducted to observe the effects of energy source, and concentration on the growing period performance of Angus x Simmetal steers (Schoonmaker, 2004). The treatments consisted of a positive control silage diet fed *ad libitum*, a forage based diet fed *ad libitum* (ALF), A 50% concentrate diet fed *ad libitum* (ALC), and 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). The study also evaluated two different weaning protocols in which the silage fed cattle were weaned at 204 days while the ALC, LFC, and ALF diets were weaned at 119 days of age. At the end of the growing phase, limit-fed calves had the highest G:F ratios, however exhibited the lowest BW amongst treatments. Marbling scores as well as quality grade distribution were not different by any of the treatments indicating that physiological maturity was accelerated by limit-feeding. Due to more rapid deposition of adipose tissue limit-fed calves reached physical maturity at lighter BW compared to more traditionally fed calves. Results of this study can be

mostly explained by Schoonmaker et al. (2003), in which circulating insulin was monitored in limit-fed cattle programmed to gain 1.2kg/d. Insulin was increased by limit-fed diets high in concentrates. This in turn increases uptake of glucose by adipocytes in tissues. Propionate, a predominant VFA produced during starch fermentation and an important precursor for gluconeogenesis by the liver, was increased by the limit fed diet. This would in theory accelerate gluconeogenesis and thus insulin production. This study also showed that limit-fed calves were more efficient than *ad libitum* fed calves. Schoonmaker et al. (2004) also demonstrated that carcass characteristics may differ at the end of the growing phase, but carcasses are equalized after all calves were fed a common finishing diet. Hypertrophy of adipocytes illustrated more of an effect on lipid deposition than hyperplasia did. Thus, hypertrophy was more influenced by level of energy intake than energy source.

The work by Schoonmaker et al. (2003, 2004) demonstrates that limit-feeding high concentrate diets to program gain can potentially extend the growth curve of calves by preparing them to experience compensatory growth after the growing phase. Through restriction of intake on high-energy diets, it is possible to achieve satisfactory gains without the risk of over-fleshing cattle that can occur with free choice access to diets. Due to a lack of available total energy in the forage diets to satisfy growth requirements, the same effect was not seen with limit feeding the forage-based diets. High energy limit-fed calves in both trials were more efficient in the growing phase and this effect was still realized through the finishing phase. Knoblich et al. (1997) indicated similar results while utilizing the same diet at different levels of intake to program gain. After a target weight was achieved in the growing phase, the cattle were transitioned to an ad libitum diet. Due to some calves reaching the target weight sooner and thus being put on the ad libitum diet sooner, the effects on efficiency seen in Schoonmaker et al. (2003, 2004) may

have been diluted. Knoblich et al. (1997) completed an economic analysis that showed that offering diets *ad libitum* during the growing phase was less economical compared to limit-feeding systems.

Due to concerns with cattle health, many nutritionists do not recommend high-energy diets for newly received calves, or for growing calf rations. In work done by Lofgreen et al. (1975, 1980, 1981), a slight correlation between dietary energy and respiratory sickness is shown, however this trend is not repeatable amongst trials. Additionally, Lofgreen et al. (1975) demonstrated that stressed calves often prefer higher energy diets. Furthermore, Fluharty and Loerch (1996) demonstrated that diets containing between 70 and 85% concentrate result in equal performance with minimal effects on health. Conclusively, high-energy diets are beneficial to newly received cattle (Spore et al., 2018).

Further research does not explicitly tie increased dietary energy in the receiving and growing phases with negative effects on health, but instead shows increases in performance. Greater efficiency in the receiving and growing phases, as well as improved efficiency throughout the finishing period as a result of carry-over effects have been shown as a result of limit-feeding high-energy rations. Over consumption of diets lead to health issues and metabolic disorders, stemming from excessive non-structural carbohydrate fermentation. These issues can potentially be avoided through diets formulated to contain corn by-products that provide high levels of energy without excessive amounts of starch.

Utilizing corn byproducts in limit feeding protocols

Due to their generally high amounts of protein and energy, corn by-products have been the focus of recent research regarding limit-feeding. Two experiments were used to evaluate the use of diets containing varying levels of wet corn gluten feed and alfalfa (Montomery et al.,

2003). In the first experiment, three diets containing 40% wet corn gluten feed (WCGF), steam flake corn, and either 0, 10, or 20% ground alfalfa (0AH, 10AH, and 20AH, respectively) were offered at 1.8% of BW on a dry matter basis once daily. A control diet of steam-flake corn and 20% alfalfa was used as a control. ADG and gain efficiency decreased linearly with increasing amounts of alfalfa in the diets containing WCGF. It was concluded that higher levels of alfalfa in the diet decreased NE values of the diet from the dilution of energy. Additionally, increased alfalfa in the diet increased DMI. A second 3 x 3 factorial experiment was conducted in which diets consisted of either 10, 20, or 30% steam-flaked corn and 0, 40, or 68% WCGF fed at 1.6% of BW daily. Results illustrated that ADG and feed efficiency were decreased with increasing alfalfa or WCGF with the exception of a WCFG x Alfalfa interaction detected in the 40% WCFG 30% alfalfa diet. A similar ADG was seen with this diet to those consuming 30% alfalfa and 0% WCFG or 20% alfalfa 0% WCGF. Higher efficiency of gain was seen because DMI was decreased with decreasing roughage from the alfalfa. In summary, some of the energy from steam-flaked corn can be replaced with WCGF in diets containing alfalfa.

Digestion of the by-product and intake's effects on digestion was analyzed by Montgomery et al. (2004) to explain the increased performance due to WCGF. WCFG inclusion increased ruminal pH as well as total VFA concentration, presumably due to the replacement of steam flaked corn. When the same diets were fed ad libitum and compared to limit-fed, total tract organic matter digestion was decreased as a result of limit feeding. These results contradicted those found by others who saw increased total tract organic matter digestion through limit feeding (Galyean et al., 1979; Murphy et al., 1994a). The difference was hypothesized to be a result of the meal eating behavior of the limit-feed diets producing an unstable ruminal environment (Montgomery et al., 2004). Limit-feeding at 1.6% of BW daily regardless of the

diet led to rapid consumption, thus increasing passage rate and presumably decreasing digestion. Increased levels of digestion as a result of limit-feeding has been highlighted as an advantage of the practice, but the amount of starch in the diets can still lead to digestive upsets in the face of meal-eating behavior.

Another limit-feeding trial by Felix et al. (2011) analyzed the effects of corn and corn distiller's grains fed at two intakes in the growing phase and the performance throughout the finishing period. Diets were fed to either obtain 0.9 kg/d or 1.4 kg/d ADG. Inclusion of corn compared to distillers grains in the growing phase increased DMI, ADG, and G:F. Furthermore, lower programmed diets decreased DMI and ADG but did not affect feed efficiency. An energy source x intake was reported for DMI and it was speculated that this was due to adverse effects of increased dietary N and sulfur concentrations of the DDGS, both of which have been shown to have negative digestive effects (Gunn et al., 2009; Gould et al., 1997). ADG and G:F were increased in the finishing phase as a result of limit-feeding, whereas energy source had no effect. Compensatory growth in the finishing phased can be used to explain these carry-over effects of limit feeding in the growing phase (Hicks et al, 1990). In conclusion, intake and energy source did not affect calf performance in regards to DMI, ADG or G:F.

Conclusions

Newly received and growing calves face a variety of challenges. In addition to stressors such as weaning, commingling, and shipping, fluctuations in the environment can substantially hinder a calf's ability to adapt to new surroundings and cost effectively grow. Through sound nutritional and health management, immunological and environmental challenges can be mitigated. Limit-feeding of growing calves can be utilized to provide much needed nutrients for growth and maintenance while reducing feed waste and fecal output.

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Chapter 2 - Effects of limit feeding cold stressed growing calves in the morning versus the evening, as well as bunk line sharing on performance

Introduction

Previous work (Reinhardt and Brandt; 1994, Prezotto et al., 2017) has shown that shifting time of feed delivery from morning to evening hours for cold stressed growing calves can result in greater feed efficiency. The suspected mode of action behind this is shifting the heat of fermentation from daytime hours to nighttime hours, when temperatures tend to be lower. It is hypothesized that this heat helps to maintain body temperature and thus, the animal uses less of the energy from digestion to meet maintenance requirements. It is not known to what extent feed efficiency can be improved when limit feeding cold stressed growing calves later in the day. New infrastructure (feedbunks and concrete aprons) for cattle feeding can be cost prohibitive to facility expansion. Bunk line sharing is a management strategy where two groups of cattle eat from one bunk and are rotated daily to a holding pen to allow the other group to eat. This maximizes infrastructure utilization, as limit fed cattle tend to consume their allocated feed within a few hours of delivery. Furthermore, work has been done that shows moderate routine exercise conducted in a low stress manner can potentially improve feed efficiency during the receiving period (Woolsoncroft et al., 2018).

Materials and Methods

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

Experiment 1. Performance Study

A total of 383 English crossbred steers (BW = $289 \text{ kgs} \pm 122 \text{ kgs}$) were purchased from Oklahoma, Texas, and Idaho and then shipped 417-1897 km on 5 trucks to the Kansas State University Beef Stocker Unit on January 4-5, 2019. The steers were used in a complete randomized design with a 1 x 5 factorial arrangement of treatments to examine the effects of limit feeding cold stressed growing calves at different times of the day in a 77-d growing study. The experiment consisted of one treatment fed in the morning (AM), one in the evening (PM), one fed half of their feed in the morning and half in the evening (50/50), and two additional treatments (Shuttle AM, Shuttle PM) that were fed in the same pen yet were rotated twice daily utilizing an adjacent holding pen. This scheme provided half of the calves were fed in the morning and half were fed in the evening. These treatments doubled the use of the pen and bunk line. The five treatments all received a diet formulated to provide 1.32 Mcal NEg/kg DM and all were limit fed 2.0% of their body weight in dry matter intake. Diets contained 25.5% corn, 7.5% supplement, 27% corn silage and 40% wet corn gluten feed (Sweet Bran) on a DM basis. Nine days post arrival, in which the calves were fed the experimental diet, all animals were individually weighted using a hydraulic squeeze chute on load cells (Silencer, Moly Manufacturing Inc., Lorraine, KS) and given an individual visual identification ear tag and a radio-frequency identification (RFID) button tag. Four steers on the lower end of the weight spectrum and thirteen steers on the higher end of the weight spectrum were removed from the research population. The remaining 360 steers were stratified by individual weight, sorted into 4 weight blocks, and then each weight block was randomly divided into 10 groups (40 groups total). Each group was randomly assigned to one of 40 different pens (32 feeding and 8 holding pens). Each pen was then randomly assigned to one of five treatments. This resulted in 40 groups consisting of 9 steers each. Feeding pens were soil surfaced and of equal size (9.1 x 15.2 m) with concrete bunks measuring 9.1-m in length attached to a 3.6-m apron. Holding pens were soil surfaced and of equal size (12.2 x 15.2 m) with ad libitum access to automatic water tanks (Lil' Spring 3000; Miraco Waterers, Oswego, IL).

The morning after initial processing (d 0), calves were weighed, and ear tagged with a pen number. All calves had previously been vaccinated prior to shipment for viral (BoviShield Gold; Zoetis Animal Health, Parsippany, NJ) and clostridial diseases (Vision 8 Somnus; Merck Animal Health, Madison, NJ). All steers were tagged with a 3- axial sensory accelerometer ear tag (Allflex Livestock Intelligence, Madison, WI) that continuously recorded rumination and activity in 2 h increments throughout the study (min/d). On d 64 and 65, calves were topically treated for external parasites with Vetrimec containing 5mg Ivermectin/mL (VetOne, Boise, Idaho).

Steers were fed their respective diets once daily at approximately 0800 for the morning fed steers and 1600 for the evening fed steers using a Roto-Mix feed wagon (model 414-14B). Feed delivery was increased weekly in accordance with pen weights to ensure restricted intake of 2.0% of BW on a DM basis. Individual animal weights were measured on d-1 (9 days post arrival), d 0 (initial processing), d 64/65 (blood collection) and d 77 (final weights). Blood samples were obtained individually from 3 steers per group and frozen for later analysis of glucose and insulin. Pen weights were collected on day 0, 21, 28, 35, 56, 63, 70, and 77. Individual ingredient samples were collected weekly and composited for analysis, and total mixed ration samples from each treatment were collected weekly and analyzed individually (dry matter [DM], crude protein [N × 6.25; AOAC International, 1997], acid detergent fiber (ADF; Van Soest et al., 1991), neutral detergent fiber (NDF; Van Soest et al., 1991), starch (Richards et

al., 1995), calcium [Bowers and Rains, 1988], and phosphorus [AOAC International, 1997]; Table 2.1) by a commercial laboratory (SDK Laboratories, Hutchinson, KS).

Animals were observed each day for signs of morbidity, such as depression, decreased appetite, and nasal or ocular discharge. Steers showing any of these signs were removed from the pen and moved to the treatment area. Once restrained in the chute, rectal temperature was measured, and a clinical illness score (CIS) was assigned. CIS was assessed on a scale of 1 to 4: 1 = normal and healthy; 2 = slightly ill with mild depression/gauntness; 3= moderately ill with severe depression/ labored breathing/ ocular or nasal discharge; and 4 = severely ill to the point of death with little responses to human approach. Animals with a rectal temperature > 39.9°C and a CIS > 1 were treated. Treatment protocol consisted of; first treatment Draxxin (100 mg of tulathromycin/mL; Zoetis Animal Health, Parsippany, NJ); second treatment, Norfenicol (300 mg/mL florfenicol; Norbrook Labs, Newry, Ireland); third treatment, animals were considered chronic and removed from the research population.

Weather data including daily temperature highs and lows, precipitation, and wind speed were collected using a Davis Vantage Pro 2, model 6153 (Davis Instruments, Hayward, CA).

Experiment 2. Intake and Digestibility Study

Six ruminally cannulated Holstein steers (BW = 435 kgs \pm 66 kgs) were used in a 1 x 3 one-way treatment structure to determine diet digestibly and digestion characteristics. Data from one steer in third period was removed due to issues with digestive health. Experimental diet and treatments were the same as in EXP. 1 (Table 2.1), less the treatments involving moving animals from holding pens to feeding pens. The study consisted of three consecutive 15-d periods consisting of a 10-d diet adaptation, 4-d fecal collection, and 1 d for ruminal fluid sampling. As the load of feed was mixed daily for Exp. 1, the amount needed for Exp. 2 was removed from the beginning of each load and feed samples were analyzed independently from those in Exp. 1 Table (2.1).

Animals were housed in individual stalls (3.7 x 3.7 m) in a fan-cooled barn at the Kansas State University Beef Cattle Research Center (BCRC). Each stall had access to an individual automatic waterer. Animals were fed once daily in accordance with the treatments in Exp. 1. Diets were fed at 2.0% of BW on a DM basis. Total mixed ration (TMR) samples were collected on d 10 through 14 and composited for each period for analysis. TMR and weekly individual ingredient samples from Exp. 1 overlapping with the sampling week were sent to an independent laboratory (SDK Laboratories, Hutchinson, KS) for nutrient analysis (DM, CP, ADF, NDF, calcium, phosphorus, and starch). On d 4 through 14, chromium oxide (Cr2O3) (10 g) was topdressed and hand mixed into each animal's diet as a marker to calculate digestibility. Refusals were collected on d 11 through 15 and composited for each animal for each period. Fecal samples were also collected on d 11 through 14, taken from the rectum of the steers every 8 h with the sampling time increasing by 2 h each day so that every 2-h interval after feeding was represented. Fecal samples were stored frozen (-20°C) for later analysis. Refusal and fecal samples were composited for each steer in each period and analyzed following the same procedures as Exp. 1. Refusal samples were dried at 55°C, air equilibrated, and ground through a 1-mm screen using a Wiley mill. Fecal samples were dried at 105°C and ground through a 1-mm screen using a Wiley mill. Fecal and refusal samples were weighed (0.5g) into 50-mL crucibles and ashed in a muffle oven at 600°C for 4 hours. Chromium concentrations were determined by atomic absorption spectrophotometry according to the procedures of Williams et al. (1962).

On d 15 of each period, ruminal fluid samples were collected from 4 different locations in the rumen at 0, 2, 4, 6, 8, 12, 18, and 24 h after feeding and pooled within sampling time. Rumen samples were analyzed for pH with a portable pH meter (Orion Model 230A (Beverly, MA)) and strained through 8 layers of cheesecloth. Strained rumen fluid was pipetted into four 2mL micro-centrifuge tubes containing 0.25 mL of m-phosphoric acid and then frozen at -20°C for later analysis of VFA concentrations by gas liquid chromatography (GLC) and ammonia (Broderick and Kang, 1980). Additionally, 20 mL of strained rumen fluid was collected and frozen at -20°C for later analysis of Co concentration to determine liquid passage rate. Co concentrations were analyzed in the ruminal fluid and in the original dose by atomic absorption spectrophotometry.

Statistical Analysis

Performance data including DMI, ADG, F:G, G:F, total weight gain was analyzed using the GLIMIX procedure of SAS (v9.4, SAS Institute Inc., Cary, NC). Feeding method was included as a fixed effect and day zero body weight was included as a covariate. Least squared means were then generated for treatment comparisons. Experimental unit was pen. Activity and rumination were analyzed using the MIXED procedure of SAS. Feeding method, hour and feeding method x hour were included as fixed effects in the model. Least square means were then generated for comparisons of treatment, hour, and treatment x hour. Experimental unit was pen. Ruminal pH was analyzed using the GLIMIX procedure of SAS. Fixed effects include treatment, time, and treatment x time. Animal was treated as a random effect in the model and period was included as a repeated measure in the model. Kenward-Roger adjustment was made on denominator degrees of freedom. Least Square Means were generated for all fixed effects comparisons. VFA's and ammonia were analyzed using the MIXED procedure of SAS. Fixed effects included treatment, period, hour, and treatment x hour. Repeated effects in the model were hour within animal x period. Animal was included as a random effect in the model. Least Square Means were generated for all fixed effects comparisons. Fixed effects included period and treatment. Animal was included as a random measure. Least Squared Means were generated for treatment comparisons. Tract digestibility data was analyzed using the MIXED procedure of SAS. Fixed effects included period and treatment. Animal were generated for treatment. Animal was treated as a random effect. Least Squared Means were generated for treatment comparisons. Results were considered significant if a P value was equal to or less than 0.05. A tendency was observed if a P value was equal to or less than 0.10.

Results and Discussion

Experiment 1. Performance Study

Low morbidity and low mortality were observed in this experiment (Table 2.2). One animal was treated for lameness, 5 for conjunctivitis, and 7 for respiratory illness. Three steers died due to respiratory illness, and one was removed from the research population due to lameness.

Performance results from Exp. 1 are presented in table 2.3. Over the entire 77-d trial, DMI of the calves did not differ among treatments (P>0.77). Average daily gain (ADG) and offtest weights were not significantly different (P>0.95) among treatments over the entire 77-day trial. However, at day 35, ADG and body weight were significantly less (P<0.01) for both the AM and Shuttle AM treatments. Gain:feed (G:F) was not significantly different (P>0.98) between treatments on day 77. At the mid-point of the study (d 35), greater ADG and equal DMI resulted in significantly greater feed:gain (F:G) and G:F (P<0.03) for AM/PM, PM and Shuttle PM treatments.

Daily activity and rumination were significantly different amongst several treatments and can be seen in table 2.5. Average daily activity was significantly greater for the AM/PM treatment than it was for all other treatments (P<0.04). Activity within the pen for cattle fed twice was greater than that of the shuttle treatments (P<0.0001). There was no significant difference in AM and PM daily activity (P<0.68), or between the Shuttle AM and Shuttle PM (P<0.14). AM cattle had significantly less activity than the AM Shuttle treatment (P<0.02). There was no difference in the activity of the AM and Shuttle PM treatments (P<0.38). Similarly, PM had significantly less activity than the Shuttle AM cattle (P<0.006), and equal activity to Shuttle PM cattle.

Rumination was significantly less for the AM/PM treatment versus the PM cattle (P<0.0002) and tended to be less than the AM cattle (P<0.08). Between the Shuttle treatments and the split feeding AM/PM treatment, rumination did not differ (P<0.64). AM cattle ruminated significantly less than PM cattle (P<0.05). AM cattle had no significant difference in rumination compared to both shuttle treatments (P<0.79). PM fed cattle had significantly greater rumination than both shuttle treatments (P<0.02).

Wet corn gluten feed was utilized as a protein and fermentable fiber energy source. Previous research has shown that replacing dietary corn with corn by-products that are high in fermentable fiber instead of starch can decrease the risk of acidosis in cattle and has the potential to increase animal performance (Krehbiel et al., 1995; Owens et al., 1997; Corrigan et al., 2009; Bremer et al., 2011). The early improvement (d 35) seen in efficiency may coincide with colder temperatures and greater precipitation during the beginning half of the trial (Table 2.4). As weather conditions became more favorable, calves with less growth were able to compensate during the second half of the study, resulting in no overall significant differences in cattle efficiency (P<0.98). These mid-study results are supported by Prezotto et al., 2017, who found limit-feeding in the nighttime as compared to limit-feeding in the daytime improves growth performance and increases feeding activity (number of visits and meals per d). They hypothesized this could be because of increased heat production to help maintain body temperature and thus reduce maintenance energy requirements.

Significantly greater daily activity for AM/PM over other treatments resulted from the animals being stimulated by feed delivery and coming to the feedbunks to eat twice a day vs once for all other treatments. Shuttle cattle spent much of their time inactive in the pen once their meal was consumed, as they received most of their activity during their twice a day rotation. The significantly less activity seen by the AM cattle verses the AM Shuttle treatment is expected as a result of moving cattle from their pens. This is similar to behavioral data from Prezotto et al., 2017 who observed limit fed calves fed twice per day had a more balanced DMI, time and visits over the daytime and nighttime limit fed calves. Additionally, calves limit fed in the daytime and nighttime consumed the majority of feed, spent the majority of time, and had the majority of visits during the daytime and nighttime, respectively.

Feeding cattle once a day versus twice can increase rumination, as the cattle spend more time stationary, digesting the diet and less time coming to the bunk. Feeding twice a day results in similar amounts of rumination as seen with shuttle feeding, and this corresponds with the time spent active instead of lying down and ruminating the diet. AM cattle ruminating significantly

less than PM cattle could be in correspondence with bovine's natural tendency to be active during the day and ruminate during night hours, especially during cold seasons, which for the PM treatment would be closer to the time of feeding. This may be supported by AM cattle having no significant difference in rumination compared to both shuttle treatments. Cattle naturally ruminate during night hours and providing their diet closer to this time results in greater rumination (Gregorini et al., 2006). This cannot be manipulated by even forced movement as seen with the shuttle treatments, as PM fed cattle still had significantly greater rumination than both shuttle treatments. The greatest amount of time spent ruminating is achieved when the diet is delivered in the evening hours, when cattle are not necessarily less active, but follow their natural instinct to ruminate during night hours.

Experiment 2. Intake and Digestibility Study

Effects of AM vs PM feeding on digestibility and ruminal characteristics can be found in table 2.6. DMI across treatments did not differ, nor did ruminal pH. Ruminal pH follows expected trends of decreasing at time of feeding and then rising again over time as seen in Figure 2.1. Ammonia production did not differ across treatments and followed expected trends, as seen in figure 2.2, of increasing in amount within the rumen following feeding and then decreasing overtime as it is taken up and utilized. Total VFA and acetate production did not differ among treatments (P<0.73). PM fed cattle produced more propionate than other treatments (P<0.03), which may correspond with greater time spent ruminating. Greater time spent ruminating may also explain the increased amount of valerate and decreased amounts of isobutyrate and isovalerate produced (P<0.03) for PM cattle. These differences in amounts of different VFA's while no difference in total VFA production led to significant differences in the percentages of VFA's as seen in table 2.7. PM fed cattle had the greatest percentages of propionate and valerate

produced, while having the lowest percentage of acetate, butyrate, isobutyrate, and isovalerate. No differences in digestibility of DM, NDF, or starch were seen, and only a tendency for greater ADF digestion by AM/PM cattle (P<0.08).

Implications

Despite changes in daily activity, the time spent ruminating and the VFA profile, no significant differences in overall performance results were observed in cold stressed growing steers, regardless of what time of day they were fed or whether they shared a bunk line and were moved to and from a holding pen daily. Significant decreases in d 35 AM and Shuttle AM calf efficiency were seen, which corresponds to colder environmental conditions during the first half of the study. As weather became more favorable in following weeks, stunted calves were able to compensate. Calf performance in similar cold environments may change drastically if cattle were fed ad-libitum diets verses being restricted. More work in this area is needed to better understand managing growing cattle cost effectively during periods of cold stress.

Many other management considerations must be taken into account when choosing whether or not to limit feed growing cattle once versus twice a day and share pens between multiple groups of cattle, especially in cold environments. Greater amount of labor is needed to feed twice a day, as well as move cattle in and out of a pen twice a day. The cost of labor may be justified when comparing it to the infrastructure needed for each group of cattle to have their own bunk space that is not shared. Labor may also be more available or willing to feed and care for cattle during certain parts of the day than others.

Winter conditions also must be considered when discussing these management practices. Equipment hours may be increased from time spent maintaining diesel engines to feed during morning hours versus warmer temperatures later in the day. Pen and alley conditions must be

taken into account when moving cattle. Incidence of cattle lameness may increase if moving when muddy pens and alleys are frozen during morning hours versus evenings or compared to not moving them at all. Muddy conditions are known to discourage cattle from standing up and coming to the bunk to eat (Riskowski and DeShazer, 1975). Limit feeding may counter this by increasing appetite and training cattle to come to the bunk when stimulated by equipment noise, presence, and the delivery of feed.

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| Ingredient, % of DM ¹ | |
|---|-----------------------------|
| Yellow #2 corn grain, dry rolled | 25.5 |
| Corn Silage | 27.0 |
| Wet Corn Gluten Feed ² | 40.0 |
| Supplement ³ | 7.5 |
| Nutrient composition, % of DM | |
| Exp. 1 | |
| Dry matter, % | 50.5 |
| Crude protein | 14.2 |
| Neutral detergent fiber | 16.5 |
| Acid detergent fiber | 13.2 |
| Calcium | 0.75 |
| Phosphorus | 0.60 |
| Exp. 2 | |
| Dry matter, % | 51.9 |
| Crude protein | 14.1 |
| Neutral detergent fiber | 27.9 |
| Acid detergent fiber | 12.2 |
| Starch | 30.8 |
| Calcium | 0.66 |
| Phosphorus | 0.58 |
| Distance former lated to contain 1.05 Most NEw /las | DM and 1 22 Maal Naa/Ira DM |

Table 2.1 Ingredient and nutrient composition of diets (Exp. 1 and 2)

¹ Diets were formulated to contain 1.95 Mcal NEm/kg DM and 1.32 Mcal Neg/kg DM.

²Sweet Bran, Cargill Animal Nutrition, Blair, NE.
³Supplement pellet was formulated to contain (DM basis) 11.09% crude protein, 8.50% calcium, 0.42% phosphorus, 5.50% salt, 0.80% potassium, 0.57% magnesium, 1.70% fat, 11.04% acid detergent fiber, and 331 mg/kg lasalocid (Bovatec; Zoetis, Parsippany, NJ).

| Disease Diagnosis | Treatment ¹ |
|-----------------------------------|------------------------|
| Lameness | AM/PM |
| Lameness | Shuttle AM |
| Conjunctivitis | Shuttle PM |
| Conjunctivitis | Shuttle AM |
| Conjunctivitis | Shuttle AM |
| Conjunctivitis | Shuttle AM |
| Conjunctivitis | Shuttle PM |
| Bovine respiratory disease, death | PM |
| Bovine respiratory disease, death | AM/PM |
| Bovine respiratory disease, death | PM |
| Bovine respiratory disease | Shuttle PM |
| Bovine respiratory disease | PM |
| Bovine respiratory disease | AM |
| Bovine respiratory disease | AM/PM |

Table 2.2 Effects of Morning vs Evening Feeding and Activity on Health (Exp. 1)

¹AM/PM: Half of diet delivered in morning and half of diet delivered in evening AM: Full diet delivered in the morning

PM: Full diet delivered in the evening

Shuttle AM: Full diet delivered in morning; steers held in holding pens overnight Shuttle PM: Full diet delivered in evening; steers held in holding pen throughout day

| | | r | Treatment ¹ | L | | _ | |
|-------------------|------|------------|------------------------|------------|-------|------------------|---------|
| Item | AM | AM Shuttle | PM | PM Shuttle | AM/PM | SEM ² | P-value |
| No. of groups | 8 | 8 | 8 | 8 | 8 | | |
| No. of steers | 72 | 72 | 70 | 72 | 70 | | |
| Body weight, kg | | | | | | | |
| d 0 | 268 | 281 | 279 | 295 | 285 | 10.9 | 0.47 |
| d 21 | 295 | 295 | 302 | 299 | 304 | 2.23 | 0.02 |
| d 28 | 308 | 310 | 311 | 308 | 315 | 1.93 | 0.09 |
| d 35 | 306 | 307 | 318 | 316 | 317 | 3.02 | 0.01 |
| d 56 | 347 | 349 | 350 | 349 | 352 | 3.46 | 0.83 |
| d 63 | 361 | 366 | 366 | 364 | 362 | 3.37 | 0.71 |
| d 70 | 367 | 368 | 370 | 371 | 369 | 3.88 | 0.94 |
| d 77 | 372 | 375 | 375 | 377 | 373 | 4.66 | 0.95 |
| | | | | | | | |
| ADG, kg/d | | | | | | | |
| d 0-21 | 0.67 | 0.65 | 0.96 | 0.85 | 1.08 | 0.11 | 0.02 |
| d 0-28 | 0.95 | 1.02 | 1.05 | 0.96 | 1.20 | 0.07 | 0.09 |
| d 0-35 | 0.71 | 0.73 | 1.04 | 0.98 | 1.03 | 0.09 | 0.01 |
| d 0-56 | 1.17 | 1.22 | 1.22 | 1.21 | 1.27 | 0.06 | 0.83 |
| d 0-63 | 1.26 | 1.35 | 1.34 | 1.31 | 1.28 | 0.05 | 0.71 |
| d 0-70 | 1.22 | 1.24 | 1.26 | 1.28 | 1.26 | 0.06 | 0.94 |
| d 0-77 | 1.18 | 1.22 | 1.22 | 1.24 | 1.19 | 0.13 | 0.95 |
| | | | | | | | |
| Average DMI, kg/d | | | | | | | |
| d 0-21 | 5.26 | 5.38 | 5.46 | 5.31 | 5.48 | 0.13 | 0.68 |
| d 0-28 | 5.30 | 5.47 | 5.51 | 5.41 | 5.55 | 0.14 | 0.71 |
| d 0-35 | 5.35 | 5.54 | 5.56 | 5.49 | 5.61 | 0.15 | 0.78 |
| d 0-56 | 5.70 | 5.93 | 5.93 | 5.91 | 5.98 | 0.17 | 0.77 |

Table 2.3 Effects of limit feeding cold stressed growing calves in the morning versus the evening, as well as bunk line sharing on performance (Exp 1)

| d 0-63 | 5.91 | 6.15 | 6.15 | 6.13 | 6.21 | 0.18 | 0.77 |
|------------|-------|------|------|------|------|------|------|
| d 0-70 | 6.04 | 6.30 | 6.28 | 6.28 | 6.35 | 0.18 | 0.77 |
| d 0-77 | 6.18 | 6.45 | 6.41 | 6.42 | 6.50 | 0.19 | 0.77 |
| | | | | | | | |
| F:G, kg/kg | | | | | | | |
| d 0-21 | 31.34 | 9.92 | 5.55 | 9.66 | 6.13 | 12.8 | 0.56 |
| d 0-28 | 6.74 | 5.48 | 5.43 | 5.93 | 4.80 | 0.75 | 0.43 |
| d 0-35 | 8.75 | 8.81 | 5.52 | 5.90 | 5.67 | 1.01 | 0.03 |
| d 0-56 | 5.20 | 4.98 | 4.98 | 5.01 | 4.75 | 0.32 | 0.90 |
| d 0-63 | 4.83 | 4.61 | 4.68 | 4.73 | 4.90 | 0.24 | 0.90 |
| d 0-70 | 5.06 | 5.12 | 5.08 | 4.97 | 5.09 | 0.27 | 0.99 |
| d 0-77 | 5.36 | 5.31 | 5.51 | 5.22 | 5.50 | 0.35 | 0.96 |
| | | | | | | | |
| G:F, kg/kg | | | | | | | |
| d 0-21 | 0.13 | 0.12 | 0.18 | 0.16 | 0.20 | 0.02 | 0.06 |
| d 0-28 | 0.18 | 0.19 | 0.19 | 0.18 | 0.21 | 0.02 | 0.39 |
| d 0-35 | 0.13 | 0.13 | 0.19 | 0.18 | 0.18 | 0.02 | 0.02 |
| d 0-56 | 0.21 | 0.20 | 0.21 | 0.20 | 0.21 | 0.01 | 0.99 |
| d 0-63 | 0.21 | 0.22 | 0.22 | 0.21 | 0.21 | 0.01 | 0.94 |
| d 0-70 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.01 | 0.99 |
| d 0-77 | 0.19 | 0.19 | 0.19 | 0.19 | 0.18 | 0.01 | 0.98 |
| | | | | | | | |

¹AM/PM: Half of diet delivered in morning and half of diet delivered in evening AM: Full diet delivered in the morning

PM: Full diet delivered in the evening

Shuttle AM: Full diet delivered in morning; steers held in holding pens overnight

Shuttle PM: Full diet delivered in evening; steers held in holding pen throughout day

²Largest SEM reported

| Item | Avg. High Temperature, °C ¹ | Avg. Low Temperature, °C ¹ | Total Precipitation, cm ¹ |
|---------|--|---------------------------------------|--------------------------------------|
| d 0-7 | -0.28 | -6.28 | 2.54 |
| d 8-14 | 3.94 | -8.72 | 0.18 |
| d 15-21 | 7.50 | -5.72 | 0.03 |
| d 22-28 | -2.39 | -8.56 | 0.28 |
| d 29-35 | 0.83 | -8.11 | 0.08 |
| d 36-42 | 1.33 | -6.72 | 1.42 |
| d 43-49 | -5.00 | -12.4 | 0.03 |
| d 50-56 | 3.06 | -4.67 | 0.48 |
| d 57-63 | 13.1 | 2.00 | 1.75 |
| d 64-70 | 15.2 | 4.94 | 0.81 |
| d 71-77 | 12.9 | 3.00 | 1.73 |

Table 2.4 Weather Trends at KSU Beef Stocker Unit

¹Measured using a Davis Vantage Pro 2, model 6153 (Davis Instruments, Hayward, CA).

| Item | AM | AM Shuttle | PM | PM Shuttle | AM/PM | SEM | P-value |
|---|--------------------|--------------------|-------------------|--------------------|-------------------|------|---------|
| Average Daily Rumination, min/d ¹ | 32.4 ^{ac} | 31.4 ^{ac} | 33.9 ^b | 32.2 ^{ac} | 31.1° | 0.52 | 0.002 |
| Average Daily Activity, min/d ¹ | 25.7ª | 26.2 ^{bc} | 25.6ª | 25.9 ^{ac} | 26.6 ^d | 0.16 | <0.0001 |

Table 2.5 Activity and Rumination

¹Measured using 3-axial accelerometer ear tags (Allflex Livestock Intelligence, Madison, WI). Rumination measures, on average, total time in minutes per day spent ruminating. Activity measures, on average, total time in minutes per day of general movement or activity. ^{a,b,c,d} Values within a row without a common superscript differ (P < 0.05)

| Item | AM | PM | AM/PM | SEM | P-value |
|------------------------|--------------------|-------------------|-------------------|------|---------|
| Number of observations | 6 | 6 | 5 | | |
| DMI, kg/d | 9.67 | 9.60 | 9.48 | 0.35 | 0.47 |
| Ruminal ¹ | | | | | |
| pН | 6.08 | 5.91 | 6.04 | 0.21 | 0.35 |
| Ammonia, mM | 7.81 | 6.92 | 6.34 | 1.23 | 0.14 |
| Total VFA, mM | 90.1 | 92.6 | 93.1 | 7.03 | 0.73 |
| Acetate, mM | 45.2 | 44.6 | 47.3 | 2.6 | 0.13 |
| Propionate, mM | 23.2 ^a | 27.8 ^b | 23.5ª | 3.1 | 0.03 |
| Butyrate, mM | 15.2 | 12.9 | 14.9 | 1.7 | 0.18 |
| Isobutyrate, mM | 1.03 ^a | 0.91 ^b | 1.13 ^a | 0.09 | < 0.001 |
| Valerate, mM | 3.04 ^a | 4.15 ^b | 2.90ª | 0.54 | 0.02 |
| Isovalerate, mM | 2.42 ^{ab} | 2.18 ^b | 2.97ª | 0.32 | 0.03 |
| Digestibility, % | | | | | |
| DM | 0.82 | 0.83 | 0.84 | 0.01 | 0.38 |
| NDF | 0.67 | 0.70 | 0.73 | 0.02 | 0.16 |
| ADF | 0.61 | 0.64 | 0.68 | 0.02 | 0.08 |
| Starch | 0.97 | 0.96 | 0.98 | 0.01 | 0.43 |

 Table 2.6 Effects of AM vs PM feeding on digestibility and ruminal characteristics (Exp. 2)

¹Average of values collected at 0, 2, 4, 6, 8, 12, 18, and 24 h after feeding. ^{a,b} Values within a row without a common superscript differ (P < 0.05)

| Item | AM | PM | AM/PM | SEM | P-value |
|--------------------------------------|----------------------|-------------------|---------------------|------|----------|
| Number of observations | 6 | 6 | 5 | | |
| Ruminal VFA, % of total ¹ | | | | | |
| Acetate | 51.9 | 50.0 | 52.1 | 2.25 | 0.06 |
| Propionate | 25.0 ^a | 29.0 ^b | 24.7^{a} | 1.68 | < 0.0001 |
| Butyrate | 16.0 ^a | 13.5 ^b | 15.5 ^a | 0.86 | 0.02 |
| Isobutyrate | 1.26 ^a | 1.09 ^b | 1.28 ^a | 0.06 | 0.02 |
| Valerate | 3.11 ^a | 3.99 ^b | 2.96 ^a | 0.34 | < 0.0001 |
| Isovalerate | 2.77^{ab} | 2.43 ^b | 3.25 ^a | 0.23 | 0.03 |

 Table 2.7 Effects of AM vs PM feeding on ruminal VFA profile (Exp. 2)

¹Average of values collected at 0, 2, 4, 6, 8, 12, 18, and 24 h after feeding, expressed as a percentage of total runnial VFA concentration. ^{a,b} Values within a row without a common superscript differ (P < 0.05)

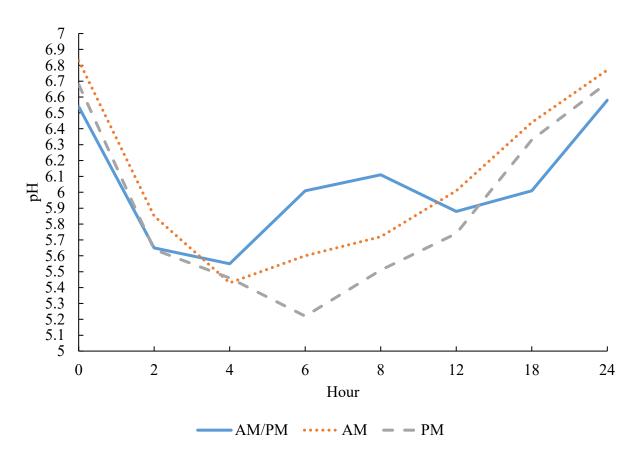


Figure 2-1 Ruminal pH over 24 hours

AM/PM: Half of diet delivered in morning and half of diet delivered in evening. AM: Full diet delivered in the morning. PM: Full diet delivered in the evening. Shuttle AM: Full diet delivered in morning; steers held in holding pens overnight. Shuttle PM: Full diet delivered in evening; calves held in holding pen throughout day.

Treatment effect (P < 0.35) hour effect (P < 0.0001) hour x treatment effect (P < 0.10).

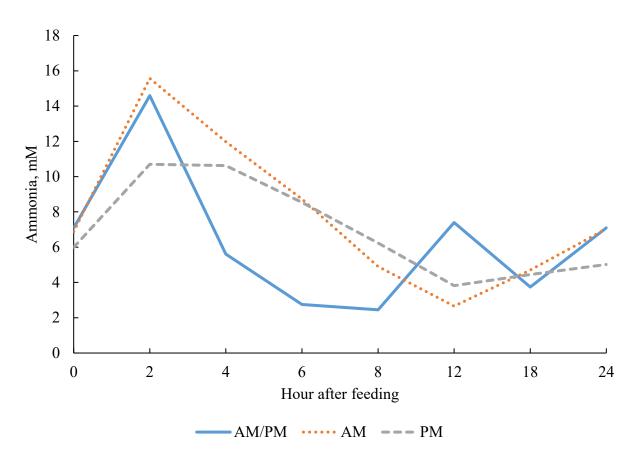
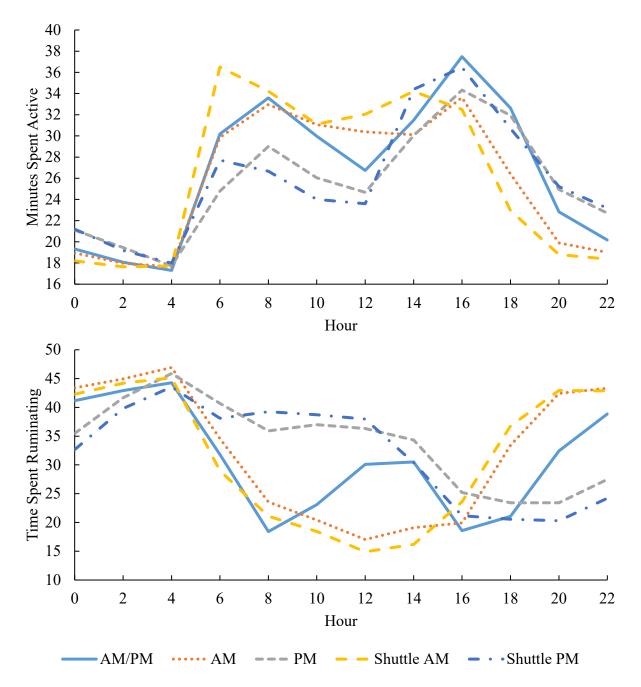


Figure 2-2 Ruminal ammonia production

AM/PM: Half of diet delivered in morning and half of diet delivered in evening. AM: Full diet delivered in the morning. PM: Full diet delivered in the evening. Shuttle AM: Full diet delivered in morning; steers held in holding pens overnight. Shuttle PM: Full diet delivered in evening; steers held in holding pen throughout day.

Treatment effect ($P \le 0.14$) period effect ($P \le 0.0006$) hour effect ($P \le 0.0001$) hour x treatment effect ($P \le 0.0001$).





AM/PM: Half of diet delivered in morning and half of diet delivered in evening. AM: Full diet delivered in the morning. PM: Full diet delivered in the evening. Shuttle AM: Full diet delivered in morning; steers held in holding pens overnight. Shuttle PM: Full diet delivered in evening; steers held in holding pen throughout day, n = 480. Activity: treatment effect (P < 0.0001) hour effect (P < 0.0001) treatment x hour effect (P < 0.0001). Rumination: treatment effect (P = 0.002) hour effect (P < 0.0001) treatment x hour effect (P < 0.0001).

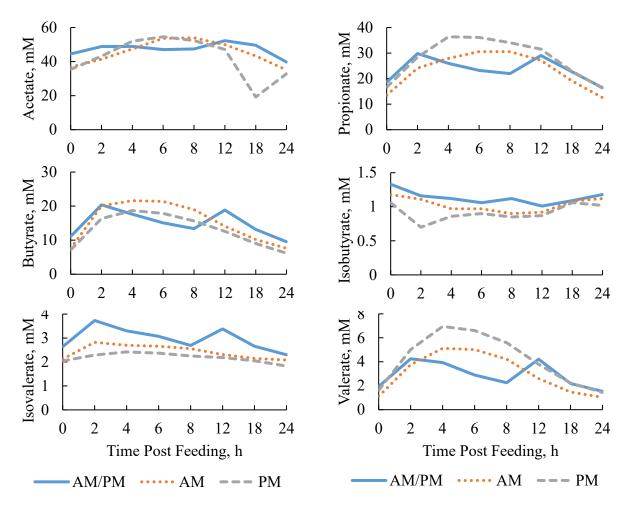


Figure 2-4 Ruminal VFA Concentrations over 24 h

AM/PM: Half of diet delivered in morning and half of diet delivered in evening. AM: Full diet delivered in the morning. PM: Full diet delivered in the evening. Shuttle AM: Full diet delivered in morning; steers held in holding pens overnight. Shuttle PM: Full diet delivered in evening; steers held in holding pen throughout day. For acetate, treatment effect (P < 0.13) period effect (P < 0.03) hour effect (P < 0.0001) treatment x hour effect (P = 0.0003). For propionate, treatment effect (P < 0.03) period effect (P < 0.001) hour effect (P < 0.0001) treatment x hour effect (P < 0.0001). For butyrate, treatment effect (P < 0.18) period effect (P < 0.02) period effect (P < 0.02) hour effect (P < 0.0001) treatment x hour effect (P < 0.0001) treatment x hour effect (P < 0.0001) treatment x hour effect (P < 0.0001). For isobutyrate, treatment effect (P < 0.02) period effect (P < 0.02) hour effect (P < 0.001) treatment x hour effect (P < 0.001). For valerate, treatment effect (P < 0.0001) treatment x hour effect (P < 0.001) treatment x hour effect (P < 0.0001) treatment x hour effect (P < 0.001) treatment x hour effect (P < 0.0001) treatment x hour effect (P < 0.0001) treatment x hour effect (P < 0.0001) treatment x hour effect (P < 0.0001).

Chapter 3 - Effects of feeding corn containing alpha-amylase gene and corn processing in limit-fed high-energy receiving diets on performance, health, and digestibility of newly received steers Introduction

Managing high risk calves such as newly weaned or received animals can prove difficult for producers involved in the cow-calf, stocker or feedlot segments of the beef industry. Calves are prone to low feed intake resulting from a multitude of stressors such as decreased immunity. Once adapted to their new environment and diet, maximizing feed efficiency becomes a key pillar in profitably growing and finishing cattle. Thus, diets should be formulated to satisfy the large nutritional demands of the animals to allow for sustained growth and proper immune function.

With the addition of grain to a ruminant diet, amylolytic activity of ruminal microbes has the capability to increase two-fold. Rojo et al., 2005 reported the efficiency of starch digestion can be increased when using external amylase enzymes. Limited work has been done on feeding corn hybrids containing an alpha amylase enzyme to cattle, and findings have been variable (Tricarico et al., 2005; DeFrain et al., 2005; Tricarico et al., 2007; Hristov et al., 2008). Yet work by Jolly-Breithaupt et al. (2018) demonstrated that feeding Enogen Feed Corn (EFC) to finishing cattle has the potential to improve feed efficiency by 5.5%. Johnson et al. (2019) also showed a 5.5% increase in feed efficiency when feeding EFC DRC containing and alpha-amylase enzyme.

Materials and Methods

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

Experiment 1. Performance Study

A total of 450 English crossbred bull and steer calves (BW= 288 kgs \pm 85 kgs) were purchased from Nazareth, Texas and assembled at a farm in Dimmit, Texas and then shipped 950 km to the Kansas State University Beef Stocker Unit on June 8, 2018. The steers were used in a completely randomized design with a 2 x 2 factorial arrangement of treatments to examine the effects of limit-feeding two corn types (Enogen Feed Corn (EFC) vs. yellow #2 corn (CON)) with two methods of corn processing (dry-rolled (DRC) vs. whole-shelled (WC)) on the performance of stocker cattle in a 90-day receiving and growing study. EFC DRC and CON DRC were analyzed for particle size (Kansas State University Swine lab, Manhattan KS), which was 2634 microns and 2196 microns, respectively. The four treatment diets, EFC/DRC, EFC/WC, CON/DRC, and CON/WC were formulated to provide 1.32 Mcal NEg/kg DM. Diets contained 35.57% corn, 6.43% supplement, 9% alfalfa hay, 9% prairie hay, and 40% wet distillers grains and solubles on a DM basis. All diets were limit fed at 2.2% of BW on a DM basis (Table 3.1). EFC containing the alpha-amylase enzyme was provided by Syngenta Crop Protection, LLC (Greensboro, NC). All diets had similar starch content. Upon arrival, calves were individually weighed using hydraulic squeeze chute on load cells (Silencer, Moly Manufacturing Inc., Lorraine, KS) and given an individual visual identification ear tag and a radio-frequency identification (RFID) button tag. Six bull calves, one morbid calf, as well as twenty-seven steers on the higher end of the weight spectrum were removed from the research population. The remaining 416 steers were stratified by individual arrival weight and randomly

assigned to 32 pens containing 13 steers each. Each of the 32 pens was provided ground prairie hay and ad libitum access to water via automatic waterers (Lil' Spring 3000; Miraco Waterers, Oswego, IL) for three days from arrival until treatment allocation. Pens were then randomly assigned to one of four treatments, which equaled 8 pens/treatment. Pens were soil surfaced and of equal size (9.1 x 15.2 m) with concrete bunks measuring 9.1-m in length attached to a 3.6-m apron.

The third morning after arrival (d 0), calves were weighed, ear tagged with a pen number, and vaccinated for viral diseases. Titanium 5 (Elanco Animal Health, Greenfield, IN), a modified-live vaccine protecting against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea types 1 and 2 (BVDI-II), parainfluenza 3 (PI3), and bovine respiratory syncytial virus (BRSV), was injected subcutaneously for protection against respiratory pathogens. The calves were also orally treated for internal parasites with Safe-Guard containing 10% Fenbendazole (Merck Animal Health, Madison, NJ) and topically treated for external parasite with Vetrimec containing 5mg Ivermectin/mL (VetOne, Boise, Idaho).

Animals were fed their respective diets once daily at approximately 0900 using a Roto-Mix feed wagon (model 414-14B), which was thoroughly cleaned between EFC and CON diets by auguring then scooping out excess feed into waste barrels. Feed delivery was increased weekly in accordance with pen weights to ensure restricted intake of 2.2% of calf BW on a DM basis. Individual animal weights were measured on d -3 (arrival), d 0 (initial processing), d 49 (fecal grab sampling) and d 91 (final weights). Fecal samples were obtained individually from steers in all pens on d 49 and analyzed for starch and organic matter the same week (Table 3.4). Pen weights were collected on day 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 91. Individual samples from each treatment were collected weekly and analyzed individually (dry matter [DM], crude protein [N × 6.25; AOAC International, 1997], acid detergent fiber (ADF; Van Soest et al., 1991), neutral detergent fiber (NDF; Van Soest et al., 1991), starch (Richards et al., 1995), calcium [Bowers and Rains, 1988], and phosphorus [AOAC International, 1997]; Table 3.1) by a commercial laboratory (SDK Laboratories, Hutchinson, KS). Animals were observed each day for signs of morbidity, such as depression, decreased appetite, and nasal or ocular discharge. Steers showing any of these signs were removed from the pen and quietly moved to the treatment area. Once restrained in the chute, rectal temperature was measured, and a clinical illness score (CIS) was assigned. CIS was assessed on a scale of 1 to 4: 1 = normal and healthy; 2 = slightlyill with mild depression/gauntness; 3= moderately ill with severe depression/ labored breathing/ ocular or nasal discharge; and 4 = severely ill to the point of death with little responses to human approach. Animals with a rectal temperature $> 39.9^{\circ}$ C and a CIS > 1 were treated. Treatment protocol was as follows; first treatment Resflor Gold (300 mg/mL florfenicol and 16.5 mg/mL flunixin meglumine; Merck Animal Health, Madison, NJ); second treatment, Baytril 100 (100 mg/mL enrofloxacin; Bayer Animal Health, Shawnee Mission, KS); third treatment, Biomycin (200 mg/mL oxytetracycline; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). Upon a third treatment, animals were considered chronic and removed from the research population.

Experiment 2. Intake and Digestibility Study

Eight ruminally cannulated beef steers (BW = $360 \text{ kg} \pm 58 \text{ kg}$) were used in a complete 4 x 4 Latin Square design to determine diet digestibility and digestion characteristics. Data from one steer in the second through fourth periods was removed due to issues with digestive health. Experimental diets were the same as in EXP. 1 (Table 3.1). The study consisted of four consecutive 15-d periods consisting of a 10-d diet adaptation, 4-d fecal collection, and 1 d for

ruminal fluid sampling. As the loads of feed were mixed daily for Exp. 1, the amount needed for Exp. 2 was removed from the beginning of each load and feed samples were analyzed independently from those in Exp. 1 (Table 3.1).

Animals were housed in individual outdoor pens (12.2 x 15.2 m). Each steer had ad libitum access to tank waterers, which were filled daily. Animals were fed once daily at approximately 1100 h. Diets were fed at 2.2% of BW on a DM basis. Total mixed ration (TMR) samples were collected on d 10 through 14 and composited for each period for analysis. TMR and weekly individual ingredient samples from Exp. 1 overlapping with the sampling week were sent to an independent laboratory for nutrient analysis following the same procedures as Exp. 2. On d 4 through 14, chromium oxide (Cr2O3) (10 g) was top-dressed and hand mixed into each animal's diet as a marker to calculate digestibility. Refusals were collected on d 11 through 15 and composited for each animal for each period. Fecal samples were also collected on d 11 through 14, taken from the rectum of the steers every 8 h with the sampling time increasing by 2 h each day so that every 2-h interval after feeding was represented. Fecal samples were stored frozen (-20°C) for later analysis. Refusal and fecal samples were composited for each steer in each period and sent to an independent commercial lab for analysis following the same procedures as Exp. 1. Refusal samples were dried at 55°C, air equilibrated, and ground through a 1-mm screen using a Wiley mill. Fecal samples were dried at 105°C and ground through a 1-mm screen using a Wiley mill. Fecal and refusal samples were weighed (0.5g) into 50-mL crucibles and ashed in a muffle oven at 600°C for 4 hours. Chromium concentrations were determined by atomic absorption spectrophotometry according to the procedures of Williams et al. (1962).

On d 15 of each period, ruminal fluid samples were collected form 4 different locations in the rumen at 0, 2, 4, 6, 8, 12, 18, and 24 h after feeding and pooled within sampling time.

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Following the 0 h sampling, 3 g of Co-EDTA (0.4 g Co) dissolved in 200 mL of water was dosed into the rumen. Rumen samples were analyzed for pH with a portable pH meter (Orion Model 230A (Beverly, MA)) and strained through 8 layers of cheesecloth. Strained rumen fluid was pipetted into four 2-mL micro-centrifuge tubes containing 0.25 mL of m-phosphoric acid and then frozen at -20°C for later analysis of VFA concentrations by gas liquid chromatography (GLC) and ammonia (Broderick and Kang, 1980). Additionally, 20 mL of strained rumen fluid was collected and frozen at -20°C for later analysis of Co concentration to determine liquid passage rate. Co concentrations were analyzed in the ruminal fluid and in the original dose by atomic absorption spectrophotometry.

Statistical Analysis

Performance data including ADG, DMI, G:F, F:G and total weight gain were analyzed using the MIXED procedure of SAS (v9.4, SAS Institute Inc., Cary, NC). Data were analyzed with corn variety, processing method, and variety x processing interaction included as fixed effects. Experimental unit was pen. Least Square Means were generated for all fixed effects comparisons. Ruminal pH was analyzed using the GLIMIX procedure of SAS. Fixed effects include corn, process, time, corn x process, corn x time, process x time and corn x process x time. Animal was treated as a random effect in the model and period was included as a repeated measure in the model. Kenward-Roger adjustment was made on denominator degrees of freedom. Least Square Means were generated for all fixed effects comparisons. Fecal analysis was analyzed using the GLIMIX procedure of SAS. Fixed effects included corn, process, and corn x process. Least Square Means were generated for all fixed effects included corn, process, and

VFA and Ammonia data were analyzed using the MIXED procedure of SAS. Fixed effects included period, corn type, processing method, corn x processing, hour, corn type x hour,

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processing method x hour, corn type x processing x hour. Hour within animal x period was included as a repeated measure. Animal was included as a random effect. Least Square Means were generated for treatment and hour parameters. Fixed effects included period, corn type, processing method, and corn x process. Animal was included as a random effect. Least Square Means were generated for corn type, processing method and corn x process.

Results and Discussion

Experiment 1. Performance Study

Low morbidity and mortality were observed in this experiment (Table 3.2). One steer was removed from the research population due to a digestive upset, one steer was removed for lameness concerns, and one steer was euthanized due anaplasmosis concerns.

Performance results from Exp. 1 are presented in Table 3.3. Over the entire 91-d trial, DMI of calves fed EFC was significantly lower (P<0.01) than calves fed CON. This difference was seen at all stages of the trial. Average daily gain (ADG) did not differ between EFC and CON treatments (P<0.56) nor did off-test weights (P<0.23), Gain: feed (G: F) or feed: gain (F: G) (P<0.95). There were no effects of corn processing observed for final DMI (P<0.86), body weight (P<0.53), ADG (P<0.30), F: G (P<0.24), or G: F (P<0.26).

Overall, ADG tended to be highest (P<0.08) for calves on the CON/DRC diets, and their off-test weights were significantly higher (P<0.03) than other treatments over the entire 91-d trial. G: F was greatest in steers fed CON/DRC diets (P<0.04). As early as d 56, F: G and G: F were improved for CON/DRC fed animals (P<0.04), but this trend was not consistent in all of the following weeks. When considering numerical differences, between DRC and WC, the DRC fed steers only exhibited an F:G 1.8% greater than WC fed calves.

Fecal starch analyses from d 49 (Table 3.4) show a source effect with CON leading to a greater fecal starch concentration than EFC (P<0.05), meaning less starch was digested and utilized by the animal when CON was fed. No effect was seen for processing, indicating the starch in WC was utilized as efficiently as DRC. Fecal starch is a good indicator of the site and extent of starch digestion in cattle (Fredin et al., 2014 and Zinn et al., 2007).

Wet distillers grains were included in the diet as a protein and fermentable fiber energy source to help limit the incidence of acidosis. Previous work has indicated that substitution of dietary corn with corn by-products that are high in fermentable fiber instead of starch can decrease the risk of acidosis in cattle while increasing animal performance (Krehbiel et al., 1995; Owens et al., 1997; Corrigan et al., 2009; Bremer et al., 2011).

Work by Johnson et al., 2019 saw a tendency for calves fed EFC to exhibit a lower DMI. Other researchers found no differences in DMI when feeding a ground corn hybrid (CA3272) containing an alpha-amylase enzyme when included at 0, 10, or 20% of the diet DM (Schoonmaker et al., 2014). Trial data showing no differences in ADG, d 91 BW, and feed efficiency between EFC and CON fed calves conflicts with results published by Jolly-Breithaupt et al. (2018) and Johnson et al. (2019), which showed 5.7 and 5.5% increases in feed efficiency, respectively, when feeding EFC DRC containing and alpha-amylase enzyme. However, work by Jolly-Breithaupt et al. (2018) revealed no differences in performance when cattle were fed EFC as DRC, which they concluded could be a result of specific growing conditions of the corn hybrid.

Similar performance between calves fed WC vs DRC is in agreement with research done by Siverson et al. (2014) who fed similar diets and found no significant differences in performance between DRC and WC when included at 29% of the diet DM in addition to 30%

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wet corn gluten feed (WCGF). These results suggest feeding WC results in nearly equal performance in 288 kgs growing calves as opposed to feeding DRC. These results also suggest that mastication by the animal is sufficient to break down WC kernels and that processing may not be necessary to optimize digestion and a lower cost of gain (COG). Work by Beauchemin et al. (1994) supports this inference after observing that the majority of corn kernels were broken during the consumption and mastication of WC when it was fed to calves. When considering cost, health and performance of the animal, the least amount of grain processing is best (Orskov, 1986).

In conclusion, the efficiency of feed conversion (F: G) of steers was not significantly altered by source of corn or processing method of the corn. There were no negative observations regarding the health or behavior of the steers when feeding any of the diets. This indicates producers can utilize either corn hybrid, and implement less processing to produce gain with greater economic efficiency.

Experiment 2. Intake and Digestibility Study

The results from this intake and digestibility trial supports the effects of treatments on growth efficiency in Experiment 1. Digestion and ruminal fermentation results are presented in Tables 3.5 and 3.6. Corn processing affected digestion and several ruminal parameters. pH was lower for DRC than for WC (P<0.01). Greater amounts of propionate were produced in DRC than WC fed steers (P<0.01). Greater amounts of butyrate were produced in WC than DRC fed steers (P<0.05). These results corresponded with the molar proportions of propionate being greater for DRC fed steers (P<0.04), while the proportion of butyrate was greater in WC fed steers (P<0.01), and the proportion of isobutyrate tended to be greater for WC fed steers (P<0.10).

Ruminal pH was not affected by corn hybrid (P<0.34), however, ruminal ammonia levels were greater for EFC fed steers (P<0.01). Ruminal ammonia and pH measurements over time after feeding can be seen in Figures 3.2 and 3.1, respectively. There was a treatment x time interaction for both ruminal pH and ruminal ammonia. NDF (P<0.03) and ADF (P<0.01) digestibility was greater for EFC than CON. Starch digestibility tended (P<0.09) to be greater for CON steers. The amount of butyrate produced tended (P<0.06) to be greater for EFC, as well as the amount of isovalerate produced (P<0.02). Similarly, the molar proportions of butyrate (P<0.05) and isovalerate (P<0.03) were greater for EFC than CON.

Treatment x time interactions for both ruminal pH and ruminal ammonia are to be expected based on eating behavior of the animals. As the steer consumes the specified treatment diet and fermentation begins, ammonia is produced and the pH declines in the rumen. This resulted in an increase in ammonia and a decrease in pH in the rumen after feeding. The tradeoff between starch and fiber digestibility between the CON vs EFC diets may explain the lack of results in the performance study, as more energy should be available to the animal when digestibility increases.

Implications

There were no negative observations regarding the health or behavior of the steers when feeding EFC. When compared with CON, there were no significant advantages in feed efficiency when feeding EFC. Under our circumstances, cattle fed WC had similar performance when compared to DRC. Processing costs of grain can be eliminated without sacrificing digestibility or performance, leading to potential benefits for the stocker/grower sector of the beef industry as a result of younger cattle's capability to masticate whole corn. Findings of this study suggest that digestibility of the corn grain was not increased with the addition of the alpha-amylase enzyme present in the EFC. Overall, the results of these studies indicate that using a corn containing an alpha-amylase enzyme does not improve feed efficiency in growing steers.

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| | | Corn grai | in source ² | | | |
|---------------------------------------|------------------------------|-----------|------------------------|-------|--|--|
| - | CO | DN | EI | FC | | |
| - | Corn processing ³ | | | | | |
| Ingredient, % of DM ¹ | DRC | WC | DRC | WC | | |
| Yellow #2 corn grain, dry rolled | 35.57 | - | - | - | | |
| Yellow #2 corn grain, whole shelled | - | 35.57 | - | - | | |
| Enogen Feed Corn grain, dry rolled | - | - | 35.57 | - | | |
| Enogen Feed Corn grain, whole shelled | - | - | | 35.57 | | |
| Wet distillers grains | 40.00 | 40.00 | 40.00 | 40.00 | | |
| Alfalfa hay | 9.00 | 9.00 | 9.00 | 9.00 | | |
| Prairie hay | 9.00 | 9.00 | 9.00 | 9.00 | | |
| Supplement ⁴ | 6.43 | 6.43 | 6.43 | 6.43 | | |
| Nutrient composition, % of DM | | | | | | |
| Exp. 1 | | | | | | |
| Dry matter, % | 58.4 | 58.9 | 58.5 | 58.2 | | |
| Crude protein | 16.1 | 16.0 | 16.6 | 16.8 | | |
| Neutral detergent fiber | 29.4 | 28.8 | 28.7 | 28.9 | | |
| Acid detergent fiber | 15.7 | 14.6 | 15.7 | 15.3 | | |
| Starch | 26.9 | 28.6 | 26.0 | 26.9 | | |
| Calcium | 0.87 | 0.88 | 0.90 | 0.90 | | |
| Phosphorus | 0.55 | 0.54 | 0.56 | 0.57 | | |
| Exp. 2 | | | | | | |
| Dry matter, % | 58.3 | 58.3 | 57.0 | 56.4 | | |
| Crude protein | 15.9 | 14.9 | 16.0 | 16.0 | | |
| Neutral detergent fiber | 27.1 | 26.0 | 31.3 | 29.5 | | |
| Acid detergent fiber | 11.3 | 10.7 | 14.3 | 12.4 | | |
| Starch | 28.3 | 31.0 | 27.5 | 25.1 | | |
| Calcium | 0.79 | 0.88 | 0.83 | 0.88 | | |
| Phosphorus | 0.44 | 0.53 | 0.50 | 0.51 | | |

Table 3.1 Ingredient and nutrient composition of diets (Exp. 1 and 2)

¹ Diets were formulated to contain 1.95 Mcal NEm/kg DM and 1.32 Mcal Neg/kg DM.

² CON, control; EFC, Enogen Feed Corn.

³ DRC, dry rolled corn; WC, whole shelled corn.

⁴ Supplement pellet was formulated to contain (DM basis) 11.09% crude protein, 8.50% calcium, 0.42% phosphorus, 5.50% salt, 0.80% potassium, 0.57% magnesium, 1.70% fat, 11.04% acid detergent fiber, and 331 mg/kg lasalocid (Bovatec; Zoetis, Parsippany, NJ).

| Diseases Diagnosis | Treatment Diet ¹ |
|--------------------|-----------------------------|
| Digestive upset | EFC/WC |
| Lameness | CON/DRC |
| Anaplasmosis | CON/DRC |

 Table 3.2 Effects of Enogen Feed Corn and corn processing on health (Exp. 1)

¹CON/WC: Yellow #2 corn/whole corn, EFC/WC: Enogen Feed Corn/whole corn, CON/DRC: Yellow #2 corn/dry-rolled corn, EFC/DRC: Enogen Feed Corn/dry-rolled corn

| | 2 | | | | | | | |
|-------------------|------------------|-------------------|------------------|----------------|------|---------|--------|------------------|
| | CC | \mathbf{DN}^{1} | EF | \mathbf{C}^2 | | | D | 1 |
| | | Corn P | rocessing | | | | P-va | llue |
| Item | DRC ³ | WC^4 | DRC ³ | WC^4 | SEM | Process | Source | Process x Source |
| No. of pens | 8 | 8 | 8 | 8 | | | | |
| No. of animals | 102 | 104 | 104 | 103 | | | | |
| Body weight, kg | | | | | | | | |
| d0 | 308 | 308 | 306 | 308 | 0.88 | 0.33 | 0.10 | 0.22 |
| d14 | 320 | 318 | 316 | 317 | 1.24 | 0.86 | 0.07 | 0.18 |
| d21 | 335 | 332 | 331 | 333 | 1.74 | 0.80 | 0.50 | 0.20 |
| d28 | 343 | 342 | 339 | 341 | 1.44 | 0.88 | 0.10 | 0.14 |
| d35 | 353 | 351 | 349 | 352 | 1.81 | 0.89 | 0.23 | 0.18 |
| d42 | 361 | 359 | 357 | 358 | 1.89 | 0.88 | 0.22 | 0.33 |
| d49 | 365 | 360 | 359 | 360 | 2.05 | 0.33 | 0.19 | 0.16 |
| d56 | 381 | 376 | 373 | 377 | 1.76 | 0.74 | 0.13 | 0.02 |
| d63 | 393 | 389 | 387 | 389 | 2.05 | 0.46 | 0.11 | 0.17 |
| d70 | 399 | 395 | 393 | 396 | 1.93 | 0.91 | 0.19 | 0.06 |
| d77 | 412 | 405 | 404 | 407 | 2.13 | 0.28 | 0.23 | 0.03 |
| d84 | 423 | 417 | 415 | 420 | 2.36 | 0.77 | 0.32 | 0.06 |
| d91 | 431 | 424 | 423 | 426 | 2.35 | 0.53 | 0.23 | 0.03 |
| ADG, kg/d | | | | | | | | |
| d 0-14 | 0.81 | 0.68 | 0.71 | 0.67 | 0.09 | 0.35 | 0.51 | 0.64 |
| d 0-21 | 1.25 | 1.13 | 1.21 | 1.20 | 0.07 | 0.37 | 0.82 | 0.46 |
| d 0-28 | 1.25 | 1.19 | 1.18 | 1.20 | 0.05 | 0.62 | 0.51 | 0.43 |
| d 0-35 | 1.29 | 1.23 | 1.23 | 1.25 | 0.05 | 0.70 | 0.68 | 0.44 |
| d 0-42 | 1.26 | 1.21 | 1.22 | 1.21 | 0.04 | 0.51 | 0.64 | 0.69 |
| d 0-49 | 1.16 | 1.06 | 1.09 | 1.07 | 0.04 | 0.17 | 0.57 | 0.39 |
| d 0-56 | 1.29 | 1.20 | 1.21 | 1.24 | 0.03 | 0.38 | 0.48 | 0.06 |
| d 0-63 | 1.35 | 1.28 | 1.29 | 1.28 | 0.03 | 0.24 | 0.38 | 0.40 |
| d 0-70 | 1.30 | 1.24 | 1.24 | 1.27 | 0.03 | 0.55 | 0.57 | 0.17 |
| d 0-77 | 1.35 | 1.25 | 1.28 | 1.29 | 0.03 | 0.13 | 0.60 | 0.08 |
| d 0-84 | 1.36 | 1.30 | 1.31 | 1.33 | 0.03 | 0.49 | 0.71 | 0.15 |
| d 0-91 | 1.35 | 1.27 | 1.29 | 1.31 | 0.02 | 0.30 | 0.56 | 0.08 |
| Average DMI, kg/d | | | | | | | | |
| d 0-14 | 7.17 | 7.39 | 7.07 | 7.10 | 0.02 | <.01 | <.01 | <.01 |
| d 0-21 | 7.31 | 7.45 | 7.14 | 7.17 | 0.02 | <.01 | <.01 | 0.02 |
| d 0-28 | 7.47 | 7.54 | 7.31 | 7.30 | 0.02 | 0.23 | <.01 | 0.08 |
| d 0-35 | 7.64 | 7.69 | 7.48 | 7.43 | 0.02 | 0.94 | <.01 | 0.04 |
| d 0-42 | 7.79 | 7.82 | 7.61 | 7.57 | 0.03 | 0.85 | <.01 | 0.13 |
| d 0-49 | 7.90 | 7.91 | 7.73 | 7.67 | 0.03 | 0.40 | <.01 | 0.22 |
| d 0-56 | 7.99 | 7.97 | 7.83 | 7.78 | 0.03 | 0.25 | <.01 | 0.61 |

Table 3.3 Effect of Enogen Feed Corn and corn processing on performance

| d 0-63 | 8.10 | 8.06 | 7.93 | 7.93 | 0.03 | 0.41 | <.01 | 0.52 |
|------------|-------|-------|-------|-------|-------|------|------|------|
| d 0-70 | 8.17 | 8.13 | 8.04 | 8.01 | 0.03 | 0.19 | <.01 | 0.89 |
| d 0-77 | 8.23 | 8.20 | 8.15 | 8.10 | 0.03 | 0.22 | <.01 | 0.76 |
| d 0-84 | 8.34 | 8.34 | 8.28 | 8.24 | 0.03 | 0.50 | 0.02 | 0.57 |
| d 0-91 | 8.47 | 8.49 | 8.41 | 8.38 | 0.03 | 0.88 | 0.01 | 0.62 |
| | | | | | | | | |
| F:G, kg/kg | | | | | | | | |
| d 0-14 | 8.93 | 10.87 | 10.00 | 10.64 | 0.27 | 0.21 | 0.80 | 0.51 |
| d 0-21 | 6.00 | 6.71 | 5.94 | 6.30 | 0.41 | 0.20 | 0.58 | 0.66 |
| d 0-28 | 5.95 | 6.33 | 6.17 | 6.10 | 0.13 | 0.46 | 0.83 | 0.36 |
| d 0-35 | 5.92 | 6.25 | 6.10 | 5.95 | 0.14 | 0.60 | 0.67 | 0.37 |
| d 0-42 | 6.21 | 6.45 | 6.25 | 6.25 | 0.14 | 0.42 | 0.55 | 0.57 |
| d 0-49 | 6.85 | 7.46 | 7.04 | 7.14 | 0.17 | 0.17 | 0.67 | 0.34 |
| d 0-56 | 6.21 | 6.62 | 6.49 | 6.29 | 0.11 | 0.48 | 0.68 | 0.04 |
| d 0-63 | 5.99 | 6.29 | 6.13 | 6.17 | 0.11 | 0.30 | 0.98 | 0.45 |
| d 0-70 | 6.29 | 6.54 | 6.45 | 6.33 | 0.10 | 0.67 | 0.78 | 0.14 |
| d 0-77 | 6.10 | 6.54 | 6.37 | 6.29 | 1.10 | 0.15 | 0.91 | 0.05 |
| d 0-84 | 6.13 | 6.41 | 6.33 | 6.21 | 0.10 | 0.52 | 0.85 | 0.08 |
| d 0-91 | 6.29 | 6.67 | 6.54 | 6.41 | 0.09 | 0.24 | 0.96 | 0.04 |
| | | | | | | | | |
| G:F, kg/kg | | | | | | | | |
| d 0-14 | 0.112 | 0.092 | 0.100 | 0.094 | 0.012 | 0.29 | 0.65 | 0.58 |
| d 0-21 | 0.170 | 0.153 | 0.166 | 0.169 | 0.010 | 0.32 | 0.53 | 0.45 |
| d 0-28 | 0.168 | 0.158 | 0.162 | 0.164 | 0.006 | 0.56 | 0.99 | 0.36 |
| d 0-35 | 0.169 | 0.160 | 0.164 | 0.168 | 0.006 | 0.70 | 0.77 | 0.34 |
| d 0-42 | 0.161 | 0.155 | 0.160 | 0.160 | 0.005 | 0.51 | 0.73 | 0.58 |
| d 0-49 | 0.146 | 0.134 | 0.142 | 0.140 | 0.005 | 0.18 | 0.92 | 0.33 |
| d 0-56 | 0.161 | 0.151 | 0.154 | 0.159 | 0.004 | 0.47 | 0.83 | 0.04 |
| d 0-63 | 0.167 | 0.159 | 0.163 | 0.162 | 0.004 | 0.26 | 0.87 | 0.43 |
| d 0-70 | 0.159 | 0.153 | 0.155 | 0.158 | 0.003 | 0.69 | 0.84 | 0.14 |
| d 0-77 | 0.164 | 0.153 | 0.157 | 0.159 | 0.003 | 0.15 | 0.99 | 0.04 |
| d 0-84 | 0.163 | 0.156 | 0.158 | 0.161 | 0.003 | 0.52 | 0.94 | 0.09 |
| d 0-91 | 0.159 | 0.150 | 0.153 | 0.156 | 0.003 | 0.26 | 0.95 | 0.04 |
| 1 | | | | | | | | |

¹Yellow #2 corn ²Enogen Feed Corn ³Dry-rolled corn ⁴Whole-shelled corn

| | | Corn S | Source | | | | | |
|-----------------|------------------|---------|------------------|--------|------|---------|---------|-----------|
| | CO | N^1 | EF | C^2 | | | | |
| | | Corn Pr | ocessing | | _ | | P-value | |
| Item | | | | | | | | Process x |
| | DRC ³ | WC^4 | DRC ³ | WC^4 | SEM | Process | Source | Source |
| DM, % | 21.2 | 20.7 | 20.3 | 19.4 | 0.49 | 0.17 | 0.03 | 0.70 |
| Starch, % of DM | 14.4 | 17.2 | 10.9 | 12.0 | 2.15 | 0.38 | 0.05 | 0.70 |

 Table 3.4 Fecal Analysis (Exp. 1)

¹Yellow #2 corn ²Enogen Feed Corn ³Dry-rolled corn ⁴Whole-shelled corn

| | | | Source | | | | | | |
|-----------------------------------|------------------|---------|------------------|--------|------|----------|----------|---------------------|--|
| | CO | | EF | C^2 | | | | | |
| - | | Corn Pr | ocessing | | | P-value | | | |
| Item | DRC ³ | WC^4 | DRC ³ | WC^4 | SEM | Process | Source | Process x Source | |
| Number of observations | 7 | 8 | 7 | 7 | | | | | |
| DMI, kg/d | 9.92 | 10.2 | 10.2 | 10.3 | 0.37 | 0.27 | 0.41 | 0.62 | |
| Ruminal | | | | | | | | | |
| pН | 6.24 | 6.35 | 6.15 | 6.35 | 0.14 | < 0.01 | 0.34 | 0.40 | |
| Ammonia, mM | 3.47 | 3.71 | 5.31 | 5.03 | 0.52 | 0.97 | < 0.0001 | 0.55 | |
| Total VFA, mM | 78.9 | 77.0 | 79.4 | 77.4 | 4.42 | 0.43 | 0.85 | 0.99 | |
| Acetate, mM | 43.7 | 44.4 | 44.3 | 43.3 | 2.69 | 0.94 | 0.87 | 0.61 | |
| Propionate, mM | 21.4 | 18.1 | 20.2 | 18.6 | 1.23 | < 0.0001 | 0.63 | 0.26 | |
| Butyrate, mM | 9.71 | 10.7 | 10.7 | 11.4 | 0.80 | 0.05 | 0.06 | 0.67 | |
| Isobutyrate, mM | 0.74 | 0.79 | 0.81 | 0.85 | 0.05 | 0.22 | 0.13 | 0.86 | |
| Valerate, mM | 1.88 | 1.42 | 1.60 | 1.61 | 0.23 | 0.24 | 0.82 | 0.21 | |
| Isovalerate, mM | 1.41 | 1.50 | 1.78 | 1.68 | 0.16 | 0.98 | 0.02 | 0.39 | |
| Digestibility, % (total tract) | | | | | | | | | |
| DM | 0.79 | 0.80 | 0.78 | 0.79 | 0.02 | 0.58 | 0.62 | 0.90 | |
| NDF | 0.70 | 0.68 | 0.74 | 0.72 | 0.02 | 0.34 | 0.03 | 0.90 | |
| ADF | 0.61 | 0.61 | 0.71 | 0.66 | 0.03 | 0.26 | 0.01 | 0.34 | |
| Starch | 0.90 | 0.93 | 0.84 | 0.88 | 0.03 | 0.29 | 0.09 | 0.88 | |

Table 3.5 Effects of Limit Feeding Enogen Feed Corn and processing on digestibility and ruminal characteristics

¹Yellow #2 corn ²Enogen Feed Corn ³Dry-rolled corn ⁴Whole-shelled corn

⁵Averages of values collected at 0, 2, 4, 6, 8, 12, 18, and 24 h after feeding.

| | | Corn | Source | | | | | |
|---|-----------------|------|--------|---------|------|----------|--------|------------------|
| | CON | | EFC | | - | | | |
| | Corn Processing | | | P-value | | | | |
| Item | DRC | WC | DRC | WC | SEM | Process | Source | Process x Source |
| Number of observations Ruminal VFA ¹ , % of total | 7 | 8 | 7 | 7 | | | | |
| Acetate | 55.7 | 57.9 | 56.3 | 56.3 | 1.25 | 0.32 | 0.62 | 0.30 |
| Propionate | 26.9 | 23.3 | 25.2 | 23.7 | 1.34 | 0.04 | 0.61 | 0.41 |
| Butryate | 12.1 | 13.9 | 13.2 | 14.6 | 0.57 | < 0.0001 | 0.05 | 0.63 |
| Isobutyrate | 1.02 | 1.08 | 1.05 | 1.16 | 0.09 | 0.10 | 0.29 | 0.62 |
| Valerate | 2.40 | 1.82 | 1.97 | 2.07 | 0.32 | 0.38 | 0.74 | 0.21 |
| Isovalerate | 1.90 | 2.00 | 2.24 | 2.26 | 0.17 | 0.65 | 0.03 | 0.77 |

 Table 3.6 Effects of Limit Feeding Enogen Feed Corn and processing on ruminal VFA profile

¹Average of values collected at 0, 2, 4, 6, 8, 12, 18, and 24 h after feeding, expressed as a percentage of total ruminal VFA concentration.

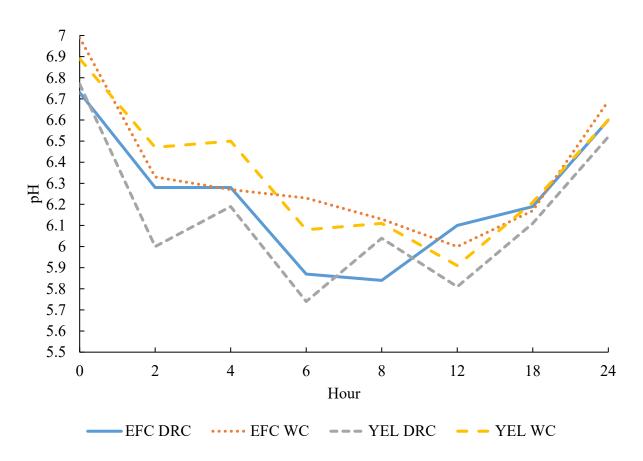


Figure 3-1 Ruminal pH over 24 hours

EFC DRC = Enogen Feed Corn/dry-rolled corn. EFC WC = Enogen Feed Corn/whole corn. YEL DRC = Yellow #2 corn/dry-rolled corn. YEL WC = Yellow #2 corn/whole corn. Corn effect (P < 0.34) processing effect (P < 0.001) corn x processing effect (P < 0.40) hour effect (P < 0.001) hour x corn effect (P < 0.36) hour x processing effect (P < 0.39) hour x corn x processing effect (P < 0.39).

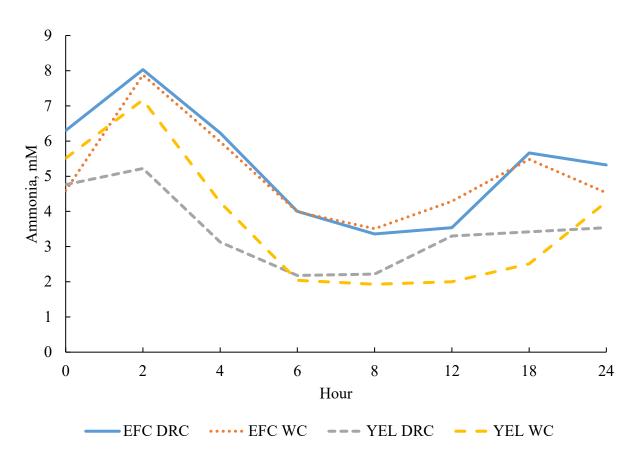


Figure 3-2 Ruminal ammonia over 24 hours

EFC DRC = Enogen Feed Corn/dry-rolled corn. EFC WC = Enogen Feed Corn/whole corn. YEL DRC = Yellow #2 corn/dry-rolled corn. YEL WC = Yellow #2 corn/whole corn. Corn effect (P < 0.0003) processing effect (P < 0.97) corn x processing effect (P < 0.55) hour effect (P < 0.0001) hour x corn effect (P < 0.42) hour x processing effect (P = 0.86) hour x corn x processing effect (P < 0.67) period (P < 0001).

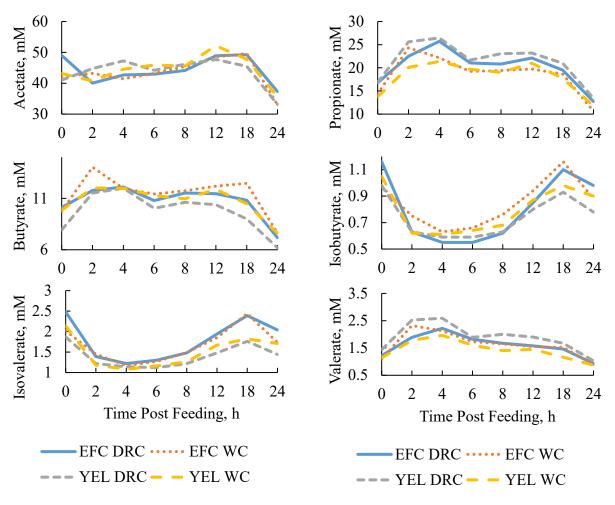


Figure 3-3 Ruminal VFA concentrations over 24 h

EFC DRC = Enogen Feed Corn/dry-rolled corn. EFC WC = Enogen Feed Corn/whole corn. YEL DRC = Yellow #2 corn/dry-rolled corn. YEL WC = Yellow #2 corn/whole corn. For acetate, corn effect (P < 0.87) processing effect (P < 0.94) corn x processing effect (P < 0.94) 0.61) hour x corn effect (P < 0.48) hour x processing effect (P < 0.86) hour x corn x processing effect (P < 0.09). For Propionate, corn effect (P < 0.63) processing effect (P < 0.002) corn x processing effect (P < 0.26) hour x corn effect (P < 0.99) hour x processing effect (P < 0.70) hour x corn x processing effect (P < 0.27). For butyrate, corn effect (P < 0.06) processing effect (P < 0.05) corn x processing effect (P < 0.67) hour x corn effect (P < 0.74) hour x processing effect (P < 0.52) hour x corn x processing effect (P < 0.39). For isobutyrate, corn effect (P < 0.52) 0.13) processing effect (P < 0.22) corn x processing effect (P < 0.86) hour x corn effect (P < 0.22) 0.36) hour x processing effect (P < 0.26) hour x corn x processing effect (P < 0.002). For isovalerate, corn effect (P < 0.02) processing effect (P < 0.98) corn x processing effect (P < 0.98) 0.39) hour x corn effect (P < 0.17) hour x processing effect (P < 0.94) hour x corn x processing effect (P = 0.004). For valerate, corn effect (P < 0.82) processing effect (P < 0.24) corn x processing effect (P < 0.21) hour x corn effect (P < 0.93) hour x processing effect (P < 0.86) hour x corn x processing effect (P < 0.06).