THESIS

DECREASING NITROGEN FOR VOLATILIZATION IN BEEF FEEDLOT CATTLE

Submitted by

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ABSTRACT

DECREASING NITROGEN FOR VOLATILIZATION IN BEEF FEEDLOT CATTLE

The effects of ractopamine hydrochloride (RAC) and a steroidal implant (IMP), on whole body N metabolism were evaluated in 24 Hereford x Angus steers (BW 554.4 ± 26.8 kg). The experimental design was a completely randomized block design with a 2 x 2 factorial arrangement of treatments. Factors included: 1) RAC (0.0 or 400 mg×steer^{-1}×d^{-1}) and 2) IMP (0.0 or 200 mg trenbolone acetate and 28 mg of estradiol benzoate). Steers were housed in individual pens and allowed ad libitum access to feed and water throughout the experiment. Steers were acclimated to the metabolism barn by bringing in, tying and currying for 12 d before the initiation of the experiment. Once cattle had been implanted for 48 d and had received RAC for 21 d, a nutrient balance study was conducted for 6 d. An IMP x RAC interaction tended ($P < 0.09$) to exist for DMI. Implanted steers receiving RAC tended to have lower DMI compared to non-IMP steers receiving RAC as well as IMP steers not receiving RAC. N intake ($P > 0.11$) and fecal N ($P > 0.18$) were not different due to treatment, yet numerically reflected the trend noted for DMI. Urinary N excretion was decreased by feeding RAC ($P < 0.01$). There tended ($P < 0.08$) to be an IMP x RAC interaction for urinary N excretion. Implanted steers receiving RAC tended to have less urinary N than steers receiving an implant only. Similarly, urine urea N excretion was decreased by RAC treatment ($P < 0.02$) and excretion tended to be decreased in steers that had also received IMP (IMP x RAC interaction; $P < 0.07$). Overall N retention was not affected by treatment ($P > 0.14$). These results indicate that urinary N excretion can be reduced by incorporating RAC according to labeled usage during the final phase of the finishing period. However, more studies will be required to elucidate the potential interactions of RAC with implant status and types of implants.

Key words: ractopamine hydrochloride, trenbolone acetate, estradiol benzoate, nitrogen balance, urinary nitrogen, urinary urea nitrogen

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I dedicate my thesis to my two beautiful daughters, Taya and Jessi. May you always believe in yourself and know the sky is the limit and education is the key to unlock your success.

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CHAPTER I

INTRODUCTION

Nitrogen is an essential component in the production of both plants and animals. Therefore, it is a common practice to provide nitrogen in surplus to ensure maximal yields. In beef production, the objective is to gain monetary profit by converting feed input dollars into muscle protein for human consumption. Feed costs can average from 60% to 70% of total production costs for beef cattle operations (Becker 2008). Excess nutrients are not utilized and are excreted by the animal, wasting feed dollars and diminishing profits. Digesting feedstuffs is an energy intensive process in that gut tissues are responsible for 17% to 25% of total whole body oxygen consumption (McBride and Kelly, 1990). Therefore, voiding the body of superfluous nitrogen is a metabolic energy drain in ATP. Cattle normally void 80% to 90% of nutrients they consume (McBride, 2003).

Costs associated with nutrient losses may go well beyond forfeitures in feed costs and meat sales. Ideally, N:P ratio of fertilizer is 5:1 however, when ammonia from manure is lost to volatilization, that ratio becomes commonly as low as 2:1 (Erickson et al., 1998). Then P from manure is either over-applied to fields when the proper amount of N is provided or N must be supplemented to correct the imbalance (Erickson et al., 1998). Estimates of 50% to 75% of excreted N are lost to volatilization (Bierman et al., 1999). From this volatilization additional expense is added to the cropping system in the purchase of fertilizer, labor hours and fuel required for application. Environmentally, nitrogen excesses can cause atmospheric, aquatic, and terrestrial pollution that is detrimental to the health and balance of ecosystems. Nitrogen monitoring in Rocky Mountain National Park over the past 20 years has determined that this high mountain ecosystem has suffered damages, defined as terrestrial eutrophication (Porter,

2007) due to excessive atmospheric nitrogen deposition. Governmental agencies, as explained in detail in Section I, are mandated to protect the national parks from such damage.

As the world population continues to grow from the current population of 6.9 billion to a projected 9.5 billion people by 2050 (United States Census Bureau, 2010), production agriculture has the onus to provide more food on less land, as urban areas expand, to maintain the food supply. Therefore, prudent usage of nitrogen inputs require novel ways to provide adequate nitrogen that is maximally retained by cattle to reduce environmental impacts. Numerous studies thus far have aimed to determine the most efficacious production practices, termed "Best Management Practices" (Colorado State University, 2011), to minimize nitrogenous losses that pollute the environment.

In 2010, a United States Department of Agriculture **(USDA)** National Institute of Food and Agriculture **(NIFA)** grant was awarded to Colorado State University to investigate possible solutions to maximize nitrogen retention in beef feedlot production with the goal of reducing NH_3 losses to the atmosphere. In typical beef cattle finishing rations, only 10% to 20% of the N consumed is retained in animal tissues, with 30% to 50% excreted in the feces and 40% to 70% excreted in the urine (Cole and Todd, 2009; Hristov et al., 2011). The current study utilizes two classes of growth promotants which are beta-adrenergic agonists **(β-AA)** and anabolic steroidal implants **(IMP)**. The β-AA utilized was ractopamine hydrochloride and the implant contained trenbolone acetate **(TBA)** combined with estradiol benzoate **(E2)**. These products were evaluated both singularly and in combination to examine how N retention is affected by and if there is a synergism between the repartitioning agents. If these growth modifiers are able to cause greater N retention in the carcass, then less will be excreted and consequently less available for volatilization into the environment. There has been no research to date comparing

the efficacy of the β-AA, ractopamine hydrochloride **(RAC)**, with and without effects of steroidal implants in feedlot steers.

The rationale for this study evolved from knowledge accumulated over the last 40+ years regarding the influence of adrenaline and adrenaline-like compounds on bodily functions. β-AA are involved in the fight-or-flight response in mammalian physiology. In human medicine, a broad spectrum of pharmacological uses of this class of drug has evolved. Examples include β-AAs being effective in the treatment of asthma as a bronchodilator, and prohibiting uterine contractions to prevent premature infant deliveries. Also, current interest is prevalent in investigating the use of β-AA in the treatment of diabetes and obesity due to its effect of increased cellular sensitivity to insulin and regulation of blood glucose levels.

Stemming originally from human pharmaceutical research involving obesity, a difference was noted in the research animal populations (Anderson, 2012) with treatment animals depositing less adipose tissue than controls. This effect was investigated and has transformed into an economic advantage in the animal feeding industry by capitalizing on the inclusion of β-AA drugs in the final phase of the feeding period to not only decrease fat, but also, increase muscle accretion. During the final phase of the feedlot period, cattle, by nature, have diminished growth due to less of the energy retained as muscle mass and more as adipose tissues, which have a greater caloric density than muscle tissue. When animals are given β-AA during this final phase of the feeding period, a greater percentage of gains are allocated to muscle mass, the cattle have greater ADG and feed efficiency which enhances cattle feeding profitability (Vestergaard et al., 1994; Schroeder et al., 2004; Dunshea et al., 2005; Avendaño-Reyes et al., 2006).

The rationale for hormonal inclusion in this study is hormones, by nature, induce the body into a metabolic mode of net gain. Anabolic implants enhance beef cattle performance

(Samber et al., 1996), and carcass muscle yield (Johnson et al., 1996). These findings make sense from a physiological perspective when considering female mammalian reproductive physiology requires a female meet not only an age, but also a body weight threshold that is congruent to a minimum body fat index before puberty can be reached (Winger, 2010). Furthermore, a net gaining status is required to maintain reproductive cyclicity (Beal n.d.; Beam and Butler, 1999; Winger, 2010,). Beef cattle are recommended to maintain a body condition score of 5 or greater to maintain consistency of the estrous cycle and decrease the amount of time required to breed the cow back (Rutter and Randel, 1984). This equates to approximately a 20% body fat index to keep the cow in a metabolic state capable of producing an estrous cycle (Rutter and Randel, 1984). Theoretically, hormonal impetus should drive the body to maximally retain nutrients which promotes the accretion of both muscle and fat. However, scientific findings are mixed whether hormonal implants affect marbling score and therefore quality grade (Smith et al., 2007). It is not definitive whether additional gains realized from implants promote an overall weight gain including muscle and adipose tissue verses muscle mass accretion only. Male hormones, such as testosterone and synthetic analogs, such as TBA, promote a metabolic gaining position. "Survival of the Fittest" dictates that hormonally active males, by nature, must achieve a greater muscle mass that promotes a competitive athletic advantage for breeding rights (Darwin and Beer, 2008). It is deductive that hormonal implants produce a net gaining response in both male and female derived hormonal implantation products.

The medicinal and economic benefits these drugs offer are well established. However, there is a paucity of data regarding the potential environmental benefits associated with the use of these growth promoting agents. The focus of the first experiment was to examine the effect of RAC (400 $mg \cdot steer^{-1} \cdot d^{-1}$ for the last 42 days on feed) and the steroidal implant Synovex Plus (SP)

(200 mg trenbolone acetate and 28 mg of estradiol benzoate) individually and in combination to examine the amounts of nutrients excreted during the entire finishing period in feedlot steers. Experiment 2 examined nutrient excretion and volatilization in a mass balance methodology utilizing two in pen mounding techniques.

This study's objectives were to:

- Determine if the growth promotant ractopamine hydrochloride will reduce urinary N content
- Determine if the growth promotant of steroidal implantation containing trenbolone acetate with estradiol benzoate will reduce urinary N content
- Determine if the growth promotants have a synergistic effect when used in combination in reducing urinary N content
- Determine if there is a difference in N volatilization levels utilizing two mounding techniques

A discussion follows of the environmental impetus to conduct this study, the metabolism of protein within the ruminant system, and how the growth promotants function metabolically.

CHAPTER II

REVIEW OF LITERATURE

SECTION I: THE ROMANS STUDY

Origins of the Rocky Mountain Atmospheric Nitrogen and Sulfur Study

In 1915, Congress passed legislation that established Rocky Mountain National Park **(RMNP)** (Memorandum of Understanding Agencies, 2007). In 1916, the Organic Act was passed which obligated the National Parks Service to protect this land "…for the benefit and enjoyment of the people of the United States…and for the preservation of the natural conditions and scenic beauties thereof" (Memorandum of Understanding Agencies, 2007). Furthering the plight to conserve this land in its pristine condition, the Wilderness Act of 1964 mandated the wilderness of the park be preserved so that it remains unimpaired for the future as a wilderness (Memorandum of Understanding Agencies, 2007). Lastly, the Prevention of Significant Deterioration Program (the Clean Air Act Amendment of 1977) aims to "preserve, protect, and enhance the air quality in national parks…and other areas of special national or regional natural, recreational, scenic or historic value" (Memorandum of Understanding Agencies, 2007). This plan declared as a national goal to prevent future, and correct any existing impairments of visibility in Class 1 federal areas that are resultant from anthropogenic air pollution (Memorandum of Understanding Agencies, 2007). As a designated National Park and Class 1 federally protected clean air area, the federal government is mandated by law to scrutinize the health of the ecosystem and protect the wilderness of RMNP from damages. Monitoring of RMNP's ecosystem began in 1980 (Memorandum of Understanding Agencies, 2010). Nitrogen

concentration in the park's precipitation has been increasing about 2.5% per year for the last two decades and is 15 to 20 times greater than natural levels (Baron et al, 2006). Documented changes include forest and soil biogeochemical changes, increased microbial activity in the soils, increased nitrogen in the lakes and streams, changes in surface water chemistry, altered tree chemistry and shifts in species of aquatic plants (Memorandum of Understanding Agencies, 2010).

Stemming from these documented changes, a petition was submitted to the Department of the Interior from Environmental Defense and Colorado Trout Unlimited on September 1, 2004 requiring "…the U.S. EPA and the state of Colorado to fulfill their legal responsibilities to lower NO_x and ammonia to protect human health, plants, and ecosystems, and scenic vistas at RMNP and to fully mitigate nitrogen deposition above the identified critical load" (Environmental Defense and Colorado Trout Unlimited, 2004) From this petition, the Rocky Mountain National Park Initiative **(RMNPI)** commenced through a collective effort by the three Memorandum of Understanding Agencies **(MOU)** which included the Colorado Department of Public Health and Environment, the National Park Service, and the U.S. Environmental Protection Agency (Memorandum of Understanding Agencies, 2010). The agencies' staff collaborated to create the policies and issuance of the Nitrogen Deposition Reduction Plan **(NDRP)** in 2007 which was endorsed by the Air Quality Control Commission (Memorandum of Understanding Agencies, 2010). This plan identifies the critical load limit as 1.5 kilograms of nitrogen per hectare per year wet deposition as the maximum value that can be absorbed without damaging the forest ecosystem (Memorandum of Understanding Agencies, 2010). As called for by the Colorado Air Quality Control Commission, a contingency plan was created in 2009 to take corrective actions should the goals set forth in the NDRP not be attained (Memorandum of Understanding

Agencies, 2010). The NRDP is a glide path approach that allows for the target reduction to be met over the next 25 years, culminating in 2032, with planned meetings of the associated agencies to evaluate the plan's progress every two years (Memorandum of Understanding Agencies, 2010). As explained in the Colorado Air Quality Control Commission Policy Resolution, the Plan is voluntary and imposes no enforceable requirement on any entity to make emission reductions currently, but does contemplate that the Commission may be presented with future proposals to adopt enforceable requirements to reduce nitrogen deposition in RMNP (Peterson, 2007).

The first challenge the RMNPI faced in diminishing nitrogenous depositions within RMNP was to determine which source regions and source types of nitrogen were contributing. During the spring and summer of 2006 the Rocky Mountain Atmospheric Nitrogen and Sulfur Study (**RoMANS)** undertook a field measurement campaign (Barna et al., 2009). A discussion of the findings from the RoMANS study follows an overview of nitrogenous reactions cycling through the atmosphere and ecosystem.

Origins of Total Reactive Atmospheric Nitrogen

Around 80% of the earth's atmosphere is comprised of nitrogen (Colorado State University IMPROVE model). Reactive nitrogen within the atmosphere occurs in two primary forms which are oxidized (NO_x) and reduced nitrogen (NH_3/NH_4^+) species (Memorandum of Understanding Agencies, 2010). Oxidized nitrogen results from any process that burns fuel (Memorandum of Understanding Agencies, 2010) and accounts for an approximate 63% of total reactive nitrogen within the atmosphere. Sources include motorized vehicles (33%), industry/power plants (18%), and oil/gas production (7%), lightning (2%) and volcanoes (3%) (Colorado State University IMPROVE model). Natural sources that contribute both NO_x and

NH_3 account for an estimated 14% to total atmospheric reactive nitrogen which include soils/natural vegetation (6%) and natural fires (8%). Reactive nitrogen in the reduced form accounts for approximately 23% of the total reactive nitrogen within the atmosphere. An estimated 15% comes from livestock production and 8% from the application of fertilizers in cropping systems (Colorado State University IMPROVE model). Other non-agriculturally derived ammonia sources exist such as urban use of synthetic fertilizers, oceans, biomass burning, flora decomposition in natural soil ecology and human waste treatment plants. Sources vary greatly in estimates of total ammonia emissions derived from agriculture. Colorado State University researchers estimate, in Colorado, 40% of reactive N is derived from animal manure and 20% from agricultural fertilizer application (Colorado State University, 2011). These estimates come with a great deal of uncertainty, however, and further research is aiming to elucidate more precisely the agriculture source contributions to understand environmental impacts and better manage production practices to reduce these contributions.

Atmospheric Reactivity of Nitrogen Molecules

Nitrogen gas and particulate matter from the previously mentioned sources are involved in a vast array of reactions within the atmospheric, terrestrial and aquatic environments due to the chemical properties of nitrogenous molecules. Ammonia's (NH_3) boiling point is -33.34˚C making it spontaneously volatilize into the gaseous state under natural conditions. Ammonia is lighter than air which dictates this gas is then readily taken up into the atmosphere (Fowler et al., 2009). Ammonia gas is highly polar making this molecule readily reactive with lower atmospheric H_2O. In the gaseous state, ammonia has a short atmospheric lifetime of only a couple of hours. If gaseous ammonia does not react with an acidic species, it will be deposited back to earth a short distance from its origination point (Colorado State University, 2011). If

ammonia does react with an acidic species, namely nitric acid or sulfuric acid (produced from combustible sources), $PM_{2.5}$ particles are formed as described below, that maintain a much longer atmospheric lifetime (around 15 days) (Colorado State University, 2011). Upon **UV** (ultra-violet) irradiation nitrogen dioxide **(NO_2)** dissociates, creating nitrogen oxide, and in the presence of hydrocarbons, reacts to form ozone **(O_3)**, nitric acid vapor **(HNO_3)**, organic species such as peroxyacetyl nitrate **(PAN)** and the greenhouse gas, nitrous oxide **(N_2O)** (Colorado State University IMPROVE model). NO, NO_2 and N_2O_5 react with OH^- to form nitric acid **(HNO_3)** which then can react with NH_3 to form ammonium nitrate **(NH_4NO_3)** (Colorado State University IMPROVE model). Ammonium nitrate molecules commonly bind together and also bind with water molecules creating particulate matter in the air, termed $PM_{2.5}$ which is particulate matter that is 2.5 microns or less (Colorado State University IMPROVE model). These particles form haze that obscures visibility and creates airborne respiratory irritants (Colorado State University IMPROVE model). Fine particles in the atmosphere create wet scavenging that is an efficient cleaning mechanism to rid the air of pollutants (Fowler et al., 2009). Depending on the particle's size, chemical make-up and ambient environment, the particle becomes the nucleus of a hydrometeor that attracts water. As water molecules surround the particle which collide and coalesce, the molecular mass eventually becomes greater than what can be supported by the updraft wind velocities (Fowler et al., 2009). At this point, the nucleated water droplet returns to the earth's terrestrial or aquatic environments, termed wet deposition, in either the form of rain or snow (Fowler et al., 2009). Alternatively, dry deposition, (via evaporation), is when the particulate matter is deposited settling out of the atmosphere devoid of incorporation with precipitation (Fowler et al., 2009). Most clouds are entrenched in a large scale system that covers areas of several thousand km^2 (Fowler et al., 2009). Therefore, air pollutants acquired in

one locale may then be transported and processed over large distances before the air is purged of the polluted precipitation (Fowler et al., 2009). Results from the RoMANS study speculate that this scenerio is what is occurring in RMNP via air masses originating in eastern Colorado as described in greater detail below.

The RoMANS Study Findings

Mountainous terrain is subject to high amounts of precipitation due to the updraft of air as it is pushed vertically over the elevated topography, causing cloud formation and resulting precipitation (Fowler et al., 2009). The RoMANS study determined nitrogenous depositions within RMNP occur primarily in the spring and summer months (Collett, 2010). Prevailing winds in Northern Colorado during this time of year originate from the west blowing easterly. This makes the probability of a significant amount of nitrogen deposition, which is speculated to originate from the eastern plains of Colorado, counterintuitive. Examining air mass movements along the front-range during the spring and summer months create scenarios that make eastern originating pollutants plausible, however. The RoMANS study determined two types of air trajectories that are thought to contribute to the eastern Colorado region's origination of nitrogen that is speculated to be deposited within RMNP. First, during the summer, there is a circadian air movement. Nocturnal air transcends down the mountain slopes onto the eastern plains as the air masses cool (Collett, 2010). Conversely, air masses vacate the eastern plains and ascend the mountainous elevations during the day as air masses warm and rise (Collett, 2010). Changes in elevation create pressure gradients which create clouds resulting in common daily precipitation events in the summer within RMNP. This regional air movement allows for small amounts of atmospherically suspended nitrogenous emissions to be carried by wind currents from the eastern

region and Front Range of Colorado up the mountainous slopes for precipitous deposition in high mountain terrain.

Although some nitrogenous deposition potentially occurs via summer air trajectories, it was assessed during the 2009 campaign that in RMNP over 90% of that year's nitrogen depositions transpired during a solitary upslope snow storm event occurring in the spring (Collett, 2010). During this time low atmospheric pressure cells developed on the eastern plains of Colorado. As high and low pressure cells collide, a counter current wind trajectory results that sweeps air masses and associated pollutants from the eastern plains southerly and ultimately upslope northerly on the Front Range. This is caused by the air mass encountering the junction of mountainous terrain. As the air mass lifts, a pressure gradient is created for a 'perfect storm' resulting in high precipitous fallout. More research is required and is occurring to determine pollutant origination sites to more accurately assess agricultural contributions to nitrogenous emissions.

Urease and Nitrogen Volatilization

During the spring, the soils are also equipped for nitrogenous emissions. Emissions of both NO and N_2O increase in a linear fashion as soil temperatures increase due to the positive effect of temperature on enzymatic processes (Fowler et al., 2009). Therefore, on soil surfaces which contain N, such as the case in animal feeding operations, bound N in the form of urine urea is released and volatilized to a much greater extent as temperatures and moisture increase in the spring. Steenhuis et. al. (1981) showed the greatest losses in manure N occurred during first melt because this water contained the greatest concentration of highly volatilizable N. Rhoades et al. (2008) reported at a feedlot in Texas that the greatest NH_3 fluxes occurred in April which is believed to be attributed to N accumulated in snow pack (Hristov et al., 2011).

N volatilization occurs because of how urea is processed within the environment. As described in more detail in the Ruminant Protein Metabolism section, urea is excreted from all mammals as a detoxification end product from the biodegradation of nitrogenous compounds within the body. Urease, which catalyzes the hydrolysis of urea, is ubiquitous within the environment because it is produced by numerous sources of ureolytic bacteria, yeasts, fungi, algae, and cell free enzyme from plants which are very stable within the soil (Mobley and Hausinger, 1989). Fecal material is abundant in ureolytic microbes. Therefore, when fecal matter is present and becomes mixed with urine on the feedlot surface, urine urea is broken down and converted to ammonia rapidly.

The complete hydrolysis of urea into NH_3 (or NH_4^+) begins immediately when bound N in urea is mixed with enzymatic urease. This occurs in a two-step process. Step one hydrolyzes urea into NH_3 and carbamic acid (Equation 1). Carbamic acid then spontaneously decomposes into one mole of CO_2 and one mole of NH_3 (Equation 2), thereby yielding 2 ammonia for every unit of urea excreted (Hristov et al., 2011).

$$NH_2(CO)NH_2 + H_2O \rightarrow NH_3 + NH_2(CO)OH \quad [1]$$

$$NH_2(CO)OH \rightarrow NH_3 + CO_2 \quad [2]$$

Fecal N contained in protein also goes through mineralization in a two-step process to convert protein into ammonium (NH_4^+). Step (1) involves proteases breaking the protein into component amino acids which then (2) are hydrolyzed converting the amino acid into organic acids and NH_4^+ (Hristov et al., 2011). Ammonium is not volatile. However, in an aqueous environment, NH_4^+ and NH_3 are in equilibrium (Equation 3) which is dependent on pH and environmental temperature (Hristov et al., 2011).

$$NH_4^+ \overset{\text{pH}}{\leftrightarrow} NH_3 + H^+ \quad [3]$$

The NH_4^+ surrogate is favored in environments of low pH and low temperatures. As temperature rises, NH_4^+ dissociates to NH_3 leading to increased volatilization (Hristov et al., 2011). At a pH below 4.5, there is essentially no free NH_3 and thus no volatilization (Hristov et al., 2011). Conversely, the greatest volatilization occurs as pH rises from 7 to 10, which is nearing the range of the pKa for ammonia. The pKa is defined as the pH in which a weak acid will ionize, releasing its proton. Therefore at a pH of 7 to 10, the NH_3 proxy is favored and readily volatilizes (Hristov et al., 2011). NH_3 only volatilizes on the surface of the manure. As urea is converted to NH_3/ NH_4^+, as illustrated in the equations above, CO_2 is liberated in the conversion of carbamic acid to NH_3. When CO_2 is exposed to ambient air, CO_2 is released more rapidly than NH_3. This leads to an increase in the surface pH of the manure thereby aggravating the conversion of NH_4^+ to NH_3 and expanding volatilization (Hristov et al., 2011). As cattle continually move throughout the pen, manure is mixed, maximizing the air/manure interface and consequently the opportunity for NH_3 to volatilize (Hristov et al., 2011). The primary area volatilization occurs within the feedlot pen is from urine spots, which provides the aqueous environment that allows NH_4^+ conversion to NH_3 that, when mixed with greater pH manure and warm temperatures, may complete emissions within 96 hours of urine deposition (Hristov et al., 2011).

The current scientific challenge is to get accurate measurements of ammonia emissions from feedlots because so many factors affect the rate and extent of volatilization. Because of the immense variation of environmental conditions where feedlots are located, the NRC suggests process based models are more appropriate. The USEPA currently proposes an emission factor of 13 kg•beef animal^{-1} annually for feedlot cattle or 23% of N entering the feedlot as cattle feed (Hristov et al., 2011). Due to the previously stated physical and chemical properties of the

reactants prove a universal across the board taxation without taking any environmental factors into account would not be a viable representation of accurate animal feeding operation emissions.

Terrestrial Reactivity of Nitrogen Molecules

Nitrogen is a crucial nutrient to sustain life for both plants and animals, but, excessive amounts are deleterious for all living organisms. In terrestrial ecology, nitrogen promotes plant growth. However, the balance is delicate, with subtle differences leading to vast ecosystem disturbances. Excess N leads to increased ecosystem productivity that can lead to increased production of non-native plant species which out-compete indigenous flora for nutrients and survivability. This over-fertilization is termed "terrestrial eutrophication," (Porter, 2007). Long term nitrogen deposition accumulates within the soil where NH_3 and H_2O are in equilibrium with $NH_4^{\pm}$ and OH^- (Colorado State University IMPROVE model). Bacteria convert $NH_4^{\pm}$ to NO_3, (nitrification) in a two part reaction that consumes O_2 and yields water and two hydrogen atoms for every NO_3 molecule produced (Fowler 2009, Colorado State University IMPROVE model). Nitrate (NO_3) acts as a plant fertilizer and the process of converting ammonium to nitrate acidifies the soil. The resultant excess H^+ then can react with the hydroxyl group attached to metals yielding water and an unbound metal. Chronic soil acidification may result if the alkaline earth metal, calcium (a natural buffering agent) is leached. Liberated metals such as aluminum, which is toxic to plants, may then leach into ground waters causing shortening and swelling of plant roots.

Once the soil O_2 has been depleted via the nitrification process, NO_3 is then anaerobically reduced to N_2O and N_2, the process termed denitrification (Fowler et al., 2009). Elevated nitrogen levels can cause an imbalance of essential nutrients by creating a decreased root to

shoot ratio in plants (Tarnay et al., 2001). Dry nitrogen deposition on canopy leaves decreases "stromatal control over water loss, forcing early leaf senescence in drought stressed conifers" (Tarnay et al., 2001). These foliage stressors lead to trees becoming more susceptible to insect infestations, diseases, drought and cold temperature damage leading to forest die back (Colorado State University IMPROVE model). Long term nitrogen deposition leads to nitrogen saturation in which there is more available nitrogen than the plants can utilize for growth thereby resulting in the leaching of nitrogen into neighboring aquatic ecosystems and ground water creating further ecological complications (Tarnay et al., 2001).

Aquatic Reactivity of Nitrogen Molecules

If the exorbitant ammonium nitrate molecules are deposited from the atmosphere back to a water source, H_2O causes the molecule to dissociate into the component ammonium (NH_4^+) and nitrate (NO_3^-) ions resulting in a cascade of events. Most ammonium is bacterially converted to nitrate ions. During this conversion, dissolved oxygen is consumed and hydrogen ions are released, thereby acidifying the water source. The resultant nitrate is utilized as a food source by the algal community which then grows exponentially in accordance with the abundance of nitrate provisions. This further diminishes the levels of dissolved oxygen which is consumed during diatomite's growth periods and also during the population's decomposition after the completion of its short lifespan. As the water source acidifies and O_2 levels diminish, aquatic species of plants and animals perish.

SECTION II: RUMINANT PROTEIN METABOLISM

Protein is a critical nutrient serving vast functions throughout the mammalian body. It is a dynamic compound ever changing in form and function to maintain a balance between accretion and degradation as metabolic demands push and pull the nitrogen flux attempting to fulfill the ever changing physiological requirements. The energy costs of these processes are high with estimations of no less than 20% of total energy expenditure to maintain the perpetual flux (Reeds, 1982). Lobley (1992) discovered a 500 kg steer actually degrades and resynthesizes at least 2550 g of protein to accrete a net daily gain of 150 g of protein. Daily net protein accretion only accounts for approximately 5.5% of total daily protein synthesis (Grubb, 2009).

Proteins found in tissues include collagen and elastin (which both increase as the animal ages), myofibrilar proteins of the sarcoplasm, contractile proteins that assist in muscle contraction, and keratins involved in production of hair, wool, feathers, hooves, horns, claws, and beaks (Pond et al., 2005). Protein components of the blood are expansive. They consist of serum proteins including albumin, which serves to maintain osmotic pressure and acts in a carrying capacity, globulins which serve in immunological response as well as numerous other actions, thromboplastin and fibrinogen for blood coagulation, hemoglobin for oxygen transport, and apoproteins that assist in the transfer of constituents in metabolic reactions (Pond et al., 2005). Enzymes are also composed of proteins which function to catalyze hundreds of specific metabolic reactions (Pond et al., 2005). The endocrine system is regulated by hormones, many of which are proteins that are key regulators in critical functions throughout the body (Pond et al., 2005).

Sources of N that are consumed include nucleic acids, amino acids, proteins, peptides, amines, amides, nitrates, nitrites, urea, and ammonia and sources recycled within the body derived from sloughed cells and urea that re-enters the rumen across the ruminal epithelium or in

saliva (Huntington and Archibeque, 1999). After mastication and deglutition, feedstuffs are delivered into the rumen where degradation of food particles via microbial digestion begins. Protein is consumed in two forms. The first is termed **RUP** (rumen undegradable protein) which is protein that bypasses microbial breakdown, arriving in the abomasum and later the small intestine, intact for gastric digestion and absorption by the animal. The second form is **RDP** (rumen degradable protein) which is protein that the microbial population is able to break down and utilize for maintenance, growth and reproduction. Unlike other mammals, ruminants do not depend solely on the provision of a balance of specific amino acids. Microbial crude protein **(MCP)** provides the host animal with 40% to 80% of its daily amino acid requirement (Owens and Bergren, 1983; Sniffen and Robinson,1987). Therefore, there are two animal nitrogen requirements that must be satisfied by the diet; first is the microbial population and second the host animal's requirements.

The microbial population is highly diverse. It consists of bacteria (the most abundant microorganism in the rumen), protozoa, fungi, and yeast species (Sniffen and Robinson, 1987). The makeup of the microbial population depends on the type of diet which the host animal is consuming (Russell et al., 1992). The microbial population varies within regions of the rumen along with diet preference. The amylolytic species prefer sugars and thrive on high concentrate diets, whereas the cellulolytic strains prefer fibrous compounds and predominate on a high roughage diet, while still other species are cross feeders that have an equal affinity for both types of carbohydrates (Sniffen and Robinson, 1987). Fungi specialize in the degradation of lignified cell walls. Protozoa consume sugars and prey upon bacteria i.e. predation within the rumen ecosystem (Sniffen and Robinson, 1987), are usually absent in high concentrate diets due to the lack of the floating fibrous mat which keeps the protozoa from "washing out" (Cheeke, 1991).

The microbial population requires provision of a nitrogen, carbon, and sulfur source to flourish; however, the structure of these essential nutrients is inconsequential. The micro flora meets their own nutritional requirements through degradation of food particles and endogenous substances. The microorganism community possesses enzymatic capabilities that their hosts do not (Fuller and Reeds, 1998). This allows the microorganisms to utilize substrates, such as highly fibrous complex carbohydrates, that are resistant to mammalian digestive enzymes (Fuller and Reeds, 1998). Therefore, regarding carbon source, either type of carbohydrate (complex or simple) is a viable ration option. So long as diet composition changes are gradual, the flux of the microbial population is able to shift to either to a cellulolytic or amylolytic based population (Pond et al., 2005). Similarly, the microbial population (and therefore the host animal) does not depend on the provision of a particular balance of amino acids for a nitrogen source. The microbial population solely depends on the provision of RDP in either the form of protein or non-protein nitrogen **(NPN)** (Owens and Bergren, 1983). NPN, such as in synthetic feed urea or endogenously recycled urea, is broken down by bacterial urease which makes additional nitrogen available for microbial growth (Fuller and Reeds, 1998). Although provision of NPN as the sole source of nitrogen allows the ruminant animal to survive and produce on a minimal level (Owens and Bergren, 1983), peak production, such as lactation and animals capable of outstanding growth performance, may be hindered because microbial synthesis of limiting amino acids may be insufficient. The demands of genetically superior animals may benefit from the addition of RUP to meet specific amino acid requirements (Pond et al., 2005).

A shortage of RDP has been shown to reduce microbial carbohydrate digestion, feed intake, synthesis of microbial protein (Griswold et al., 2003), and decrease cattle weight gains (Zinn et al., 2003). This is due to the cellulolytic bacteria's inability to degrade the fibrous

compounds of a high roughage diet without the addition of a nitrogen source. Cellulolytic bacteria preferentially favor the N source of free ammonia over pre-formed amino acids (Russell et al., 1992). If given an adequate N source, such as NPN, the bacteria are able to efficiently break down and utilize high roughage diets. However, if a N source is not present, the large food particles will remain intact and in the rumen for extended periods, leaving the animal without a source of nutrition because digesta will not pass out of the rumen and into the abomasum until it reaches a 2 mm particle size (Sniffen and Robinson, 1987). High concentrate rations are not limited by particle size. However, with high concentrate diets that are deficient in RDP, the animal will still become protein deficient because the microbial population will fail to flourish without an adequate source of nitrogen. Microbial bodies are thought to have a better amino acid profile which more closely approximates, albeit not precisely meets, the animal's requirements than nearly any other feedstuff (NRC, 2000).

Excessive levels of RDP can be as equally injurious as inadequate consumption. Animal performance is hindered by decreased fertility rates (McCormick et al., 1999) and potentially decreased weight gains and milk production, possibly due to energy expended in voiding the body of surplus N (NRC, 2000). High protein diets have been shown to create excess ammonia in the rumen, which is transferred to the liver for processing to be recycled or, in high protein diets, is primarily converted to urea, fated for excretion (Van Soest, 1999). When elevated ammonia levels exceed the liver's capacity to process and excrete the surplus, ammonia toxicity results (Owens and Bergren, 1983). When this happens, blood pH becomes elevated resulting in alkalosis which may produce death within hours of the onset of symptoms (Pond et al., 2005). Further details of hepatic processing of surplus ammonia, along with the associated metabolic costs, will be covered in a subsequent section. The aforementioned details explain the

importance of meeting, without exceeding, the metabolic requirements for nitrogen and why there is such a delicate balance.

When adequate RDP is provided, the microbes produce an array of fermentation products for the host animal to utilize in a symbiotic relationship. With RDP, the first task the microbes employ is to cleave the amine group from the nitrogen source, creating a source of ammonia. Ammonia is also derived from the breakdown of amino acids and other body proteins (Reynolds, 1992). Due to the pH within the gastrointestinal tract of a ruminant, essentially all ammonia is in the protonated NH_4^+, allowing ammonia to serve as a buffering intermediate in metabolic acid/base balance (Huntington and Archibeque, 1999). Ammonia absorption is maximized at a greater pH as a result of NH_3 passing freely across membranes whereas NH_4^+ does not (Pond et al., 2005). Most ruminal bacteria utilize NH_3 and many, especially cellulolytic bacteria require it (Russell et al., 1992; Pond et al., 2005). The bacterium are able to then incorporate NH_3 into MCP. Ammonia may also be absorbed across the ruminal epithelium or lower part of the GI tract wall and into the host animal's portal vein for hepatic processing. Enzymatic microbial urease converts urea to ammonia, which microbes utilize to synthesize amino acids (Reynolds, 1992). With microbial utilization, as long as the rumen environment contains a carbon structure and sulfur, the microbial population is able to utilize the amine group to reincorporate the carbon structure, allowing for microbial reproduction and therefore a supply of MCP (Pond et al., 2005).

Short chain organic fatty acids (**VFA**'s or volatile fatty acids) are a co-product of fermentation produced by rumen microbes which are absorbed through the rumen wall to provide the host animal with a form of metabolizable energy. Since ruminants do not produce enzymes to break down sugars to the extent that non-ruminant animals are capable of, they rely heavily on the provision of VFA'a as their primary energy source to enter the citric acid cycle for

energy production (Owens and Bergren, 1983). Additionally, ruminants derive energy from the liver via gluconeogenesis to meet the animal's glucose requirements. Gluconeogenesis creates glucose by the process of deaminating amino acids to provide carbon skeletons to deliver into the citric acid cycle for ATP production (Reynolds, 1992).

As the chyme passes out of the rumen, it is transferred into the abomasum to begin gastric digestion of the remnant food particles (Russell et al., 1992). Ruminants function from the abomasum through the hindgut much like monogastric animals, such as pigs and humans (Huntington and Archibeque, 1999). Gastric juices, including hydrochloric acid and proteolytic enzymes secreted by the gastric chief cells, produce an acidic environment which kills the microbes and serves in the acid hydrolysis of the proteins including RUP and MCP (Sniffen and Robinson, 1987, Pond et al., 2005). As the pH drops to around 2.5, the hydrogen from the acid becomes associated with the protein, yielding the protein with a positive charge. This allows association with polar H_2O and begins the unfolding of the quaternary structure of the protein into a linear form, exposing the peptide bonds that join the amino acids together. This prepares the protein sources for further digestion and absorption within the small intestine.

As the chyme passes through the pyloric sphincter and into the duodenum, the pancreas empties its proteolytic enzymes and the gall bladder adds bile salts. The proteolytic enzymes further detach protein bonds creating peptides, dipeptides, and nucleic acids. Then at the luminal wall of the ileum, and to a lesser extent, the jejunum, brush border aminopeptidases, dipeptidases and nucleotidases complete degradation by splitting them into individual amino acids, purine and pyrimidine components (Pond et al., 2005). The addition of bile salts is essential in the digestive process because most intestinal proteases function optimally at a pH of around 7 (Webb and Mathews, 1994). Because the chyme emptying into the duodenum has a pH of approximately 3,

bile salts from the gall bladder are released, and act as a base, gradually raising the pH of the environment in the small intestine. Therefore, it is not until the gradual pH increase culminates somewhere around the ileum that protease activity is maximized and the absorption of the majority of amino acids occurs via active transport (Williams, 1969).

Active transport sights on the intestinal brush border membrane are highly specific and do not have equal affinities for amino acids. There are reportedly 12 transport systems that are selectively based on type of amino acid and charge. Neutral and negatively charged amino acids have separate transporters (Christensen, 1984). There is competition between amino acids for transport sights where transport of one amino acid may prevent another variety of amino acid from being absorbed (Pond 2005). Essential amino acids are selectively absorbed with methionine having the most preferential status (Webb and Mathews, 1994).

After active transport to the basolateral side of the intestinal membrane, amino acids are unable to be stored and therefore must be metabolically used immediately for protein synthesis or broken down for recycling or excretion (Boron and Boulpaep, 2009). Whole body protein turnover is an ever changing rate of the difference between protein synthesis and degradation. This is influenced by countless biological factors such as stage of growth, production, tissue injury, and illness. It is also in a state of constant maintenance of tissues throughout life such as skin and intestinal mucosa which both have a high turnover rate due to the wear and tear of continual use.

In protein synthesis, specific sequences of nitrogenous bases of purines and pyrimidines make nucleotide sequences that encode instructions for amino acid to synthesize proteins (Boron Boulpaep, 2009). Peptide bonds are employed to join amino acids together forming chains that

eventually lead to complete protein molecules that are sequenced to perform a specific function within the body (Boron and a Boulpaep, 2009).

Alternatively, transamination is the process by which an existing amino group from one amino acid is passed to a second amino acid. This is a process that allows for the synthesis of nonessential amino acids through metabolic intermediates (Murray et al., 2009). Transamination allows for dietary amino acids to be converted to non-protein nitrogen derivatives, tissue proteins, glucose via gluconeogenesis, acetyl CoA, to enter the citric acid cycle becoming oxidized to produce CO_2, and ketone bodies that may be oxidized for the synthesis of fatty acids or to the common amino nitrogen acceptor, glutamate, which proceeds to deamination.

Muscle mass accounts for over 50% of body mass, which allows a large reservoir of attainable energy for the production of glucose, and therefore ATP, allowing for maintenance of critical bodily functions during a fasted state (Murray et al., 2009). Muscle generates over half of the free amino acid pools from endogenous proteins (Murray et al., 2009). Muscle catabolism occurs when pyruvate from glycolysis of muscle glycogen becomes transaminated, forming alanine. Alanine is then transported to the liver where it becomes transaminated back to pyruvate where it can enter the citric acid cycle for gluconeogenesis (Murray et al., 2009).

The terminal sector of nitrogen flux in mammals is handled by the urea (ornithine) cycle primarily in the liver and, to a lesser extent, the kidney (Reynolds, 1992). Many ruminants absorb more nitrogen via ammonia than from α-amino acids (Reynolds, 1992). Ammonia, which is produced and used to a great extent by the rumen microbes, is fairly toxic to the host animal. Therefore, the liver removes and detoxifies this ammonia, primarily by converting it into urea which is released into the vena cava (Reynolds, 1992).

Nitrogen flux is a result of intake protein, degradation of tissue proteins, sloughed intestinal lining cells, residues of digestive enzymes and other body proteins (Owens and Bergren, 1983). Excess amino acids are not able to be stored and are not excreted in the amino acid structure. Therefore, amino acids must be used immediately for protein synthesis or broken down for recycling or excretion (Boron and Boulpaep, 2009). Transamination allows nitrogen from one amino acid to be passed to alpha-ketoglutarate, becoming the non-essential amino acid glutamate (Murray et al., 2009). This is followed by oxidative deamination when the irreversible action of glutamate dehydrogenase oxidatively captures the N from glutamate as ammonia. Ammonia is then converted to urea in the urea cycle in the liver and to a lesser degree in intestinal cells (Pond, 2005) for excretion via the kidney (Cammarata and Cohen, 1950).

The urea cycle begins within the mitochondria where specific carriers bring in the required CO_2 to condense with ammonia and ATP to form Carbamoyl phosphate (Murray et al., 2009). Ornithine then reacts with carbamoyl phosphate which is transformed to L-Citrulline and is transported out of the mitochondria and back into the cytosol (Murray et al., 2009). L-Aspartate (from glutamate derived from the citric acid cycle (Pond et al., 2005) along with ATP convert L-Citrulline to Argininosuccinate (Murray et al., 2009). As Argininosuccinate is converted back to L-Arginine, a Fumarate is released that is available to return back to the Citric Acid Cycle (Murray et al., 2009). Urea is released in the conversion of L-Arginine back into Ornithine (Murray et al., 2009). Ornithine is then transported back into the mitochondria ready to complete another circuit of the urea cycle (Murray et al., 2009). Urea is then ready to be transported to the kidney for excretion in the urine which is the chief route for excretion in mammals (Murray et al., 2009) or in the case of ruminants, can be either excreted or used for recycling into the digestive tract (Reynolds, 1992).

Ruminant urea recycling augments low nitrogen diets (Owens and Bergren, 1983). Rather than being excreted from the body as a waste product, 40% to 60% of urea is instead recycled and reintroduced into the lumen of the digestive tract (Reynolds, 1992). Urea can re-enter various portions of the digestive tract, by diffusion into saliva or directly from the blood across the luminal wall of the rumen or gut (Huntington and Archibeque, 1999). Of the plasma urea, 23% to 92% is recycled to the digestive tract (Owens and Bergren, 1983) with ruminal ammonia concentrations being negatively associated with urea recycling and positively related to plasma urea concentrations and organic matter digestion in the rumen (Owens and Bergren, 1983). Excreting urea is not without a metabolic cost. The synthesis of one mole of urea requires four moles of ATP (McBride, 1990) and, therefore, it is energetically pragmatic for the animal to recycle this resource. Recycled urea is an important source of N entering the digestive system that provides a constant source of ammonia to support microbial fermentation (Huntington and Archibeque, 1999). Feeding regimens that capitalize on this recycling proficiency are another area receiving research to lessen nitrogenous excretions. As research continues to illuminate the inner workings of the ruminant metabolic system, further efficiencies will be promoted, hopefully resulting in sustainable production practices that will keep pace with world demands.

SECTION III: GROWTH MODIFIERS

Strategies to encourage frugal metabolic usage of N requires an understanding of how various physiological systems operate in building muscle mass. Numerous growth modifiers promote muscle accretion targeting various metabolic pathways. Beta-adrenergic agonists and steroidal implants are two such repartitioning agents that affect muscle accretion in two separate systems which will be discussed next.

Beta-Adrenergic Agonists

Beta-androgenic receptors encourage the accretion of muscle mass during the final stages of the finishing period which, by nature, is predominated by fat deposition. Manipulating this metabolic pathway is a possible means to promote N retention and thereby lessen N excretion from the ruminant system.

The term "adrenergic" encompasses compounds associated with adrenaline or by definition, is activated by epinephrine (adrenaline) or any substances having epinephrine like activity (i.e. epinephrine, norepinephrine and a myriad of synthetic adrenergic agonists) (Dictionary.com, 2011). Epinephrine and nor-epinephrine (along with Dopamine which has different receptors and will not be covered) are classified as catecholamines that are produced from the amino acid tyrosine (Boron and Boulpaep, 2009). Catecholamines are released in response to the sympathetic nervous system stimulation involved in the flight-or-fight response originating from the adrenal medulla, exclusively in the case of epinephrine. Norepinephrine is produced in other organs and tissues throughout the body as well (Murray et al., 2009).

Adrenergic receptors **(AR)** are a class of G protein-coupled receptors that are present on most mammalian cells. Adrenergic receptors are the targets of catecholamines (Boron and Boulpaep, 2009) and β-AA. βeta-adrenergic agonists are organic molecules that bind to the AR

located on the cell surface of tissues including skeletal muscle and adipose tissue (Mersmann, 1998). After binding, a series of reactions cause the phosphorylation of several intra cellular proteins (Mersmann, 1998). In adipocytes, this promotes hydrolysis of triglycerides and a decrease in fatty acid and triglyceride synthesis (Mersmann, 1998). In muscle fibers, binding affects the activity of calpains and calpastatins which causes decreased proteolytic capacity and myofibrillar breakdown resulting in more muscle accretion (Bardsley et al., 1992). βeta-adrenergic agonists also cause increased blood flow which increases nutrients delivered to muscle mass as well as increased levels of insulin which drives nutrients into the cells for increased protein synthesis (Mersmann, 1998).

Adrenergic receptors are sub-classed into alpha (2 sub-classes) **(α-AR)** and beta (3 sub-classes (β-1, β-2, β-3)) **(β-AR)** (Marieb, 1995). Epinephrine and norepinephrine can each bind to either sub-class of receptor but each type of receptor has a greater affinity for one or the other type of catecholamine. Generally, binding to α-AR tends to create stimulatory responses whereas β-AR tends to result in inhibitory outcomes (Marieb, 1995). There are exceptions to this general rule because both hormones work in conjunction to get the body equipped for exertion, which means certain bodily functions have to engage while others must decline working simultaneously to promote survival activities. To further the complexity, opposite outcomes may result at varying concentration levels of the same hormone (i.e. epinephrine can have both an inhibitory and stimulatory responses on the same receptor which is dose dependent) (Boron and Boulpaep, 2009).

During flight-or-fight response, 80% of catecholamine released is epinephrine which produces a more powerful stimulation of the heart and metabolic activities while norepinephrine has greater influence on peripheral vasoconstriction (Marieb, 1995). Organs and tissues have

differing concentrations of adrenergic receptor varieties dependent on the organ's function within the body. Alpha-adrenergic receptors are responsible for vasoconstriction and decreased motility of smooth muscle in the gastro-intestinal tract, which are the primary targets of nor-epinephrine (Boron and Boulpaep, 2009). βeta-adrenergic receptors have a greater affinity for epinephrine. The β-ARs are present in all organs and tissues associated with growth, such as skeletal muscle, adipose tissue and some neuro-endocrine organs (Yang and McElligott, 1989). β_1-adrenergic receptors are primarily associated with cardiac contraction. β_2-adrenergic receptors are the counter-balance of the α_1 subclass, as these receptors cause relaxation of smooth muscle (including bronchial muscles), dilate arteries to skeletal muscle, increase lipolysis in adipose tissue, increase glycogenolysis/gluconeogenesis and create anabolism in skeletal muscle (Yang and McElligott, 1989). β_2-adrenergic receptor is the most abundant subtype in bovine skeletal muscle and adipose tissues (Sillence 1994, Baxa 2010). β_3-ARs enhance lipolysis in adipose tissue (Marieb, 1995; Boron and Boulpaep, 2009).

Phenethylamines are a class of compounds referred to as beta-adrenergic agonists (β-AA) that are similar in structure and action to naturally occurring catecholamines. Phenethylamines are utilized to modify the rate and composition of growth in livestock by increasing muscle synthesis, carcass weight, carcass leanness, improve efficiency of gain, and increase rate of gain by repartitioning dietary energy toward muscle rather than adipose tissues (Avendaño-Reyes et al., 2006; Abney et al., 2007; Allen et al., 2009). β-AA have varying affinities for the three subclasses of β-ARs dependent on composition of the drug and the function of the receptor it binds with (Avendaño-Reyes et al., 2006). Pharmacological classifications are based on their potencies when compared to epinephrine/norepinephrine (Yang and McElligott, 1989). Some pharmacological agents block or stimulate a specific sub-class of receptor whereas others are

non-specific and bind to numerous receptor types simultaneously. β-AA are potent growth promoters (Abney et al.,2007). Numerous studies on several animal species have demonstrated that feeding β-AA during the final phase of the finishing period causes animals to allocate a greater percentage of weight gains to muscle protein rather than fat accretion (Ricks 1984; Yang and McElligott, 1989; Abney et al, 2007). Skeletal muscle hypertrophy is a result of changes in protein synthesis and degradation rates (Beermann, 2002) In fact, not only is there greater muscle accretion, but there is also an accompanying decreased lipogenesis and increased lipolysis (Mersmann, 1998; Avendaño-Reyes et al., 2006; Baxa 2010).

The metabolic/physiological reasoning for these drugs to produce such an effect is apparent when considering the natural metabolic happenings of an animal experiencing stress which releases catecholamines in response. When the **CNS** (central nervous system) is triggered, nerve impulses stimulate the hypothalamus to release **CRH** (corticotropin releasing hormone) which in turn stimulates the anterior pituitary to release **ACTH** (adrenocorticotropic hormone). ACTH then stimulates the adrenal gland to release a number of hormones that up-regulate the body for a fight-or-flight response. This includes epinephrine and nor-epinephrine which trigger adrenergic receptors throughout the body. β_2 –adrenergic receptors activate vasodilation in the circulatory system to increase blood flow in tissues that lead to skeletal muscle. This allows oxygen to be delivered to the muscles that are readying for intense exertion. β_2-adrenergic receptors are also responsible for relaxing tissues around the bronchioles to prepare for increased ventilation to provide the needed oxygen to skeletal muscles. The α_1-ARs constrict the veins and arteries going to the heart to increase blood pressure making blood plentifully available to pump the required oxygen to the large muscle groups in the extremities. Similarly, β_1-ARs increase the cardiac rate and output as well as down-regulates the muscles going to the

gastrointestinal tract. This diverts blood away from the digestive system making more blood available for use in skeletal muscles. Insulin levels become down-regulated in a two- step process of α and β-ARs to maintain blood glucose levels for the brain to function while simultaneously up-regulating glycogenolysis in liver and muscle tissues. Lipolysis in adipose tissue is also up-regulated to provide glucose and free fatty acids as exertion of the muscle tissue demands.

Therefore, the logic surrounding the use of synthetic β-AA in food animal production is to capitalize on selectively stimulating the β_2 -AR provisions without (or minimally) stimulating the remainder of the adrenergic receptors. This allows the animal to dilate arteries going to skeletal muscle, thereby increasing blood flow which provides more nutrients and oxygen to devote to muscle growth. Additionally, β_2-ARs activate lipolysis and glycogenolysis which provides additional energy to the animal through the metabolism of fat and glycogen stores, allowing more energy to be diverted to the muscle mass.

One such phenethanolamine is manufactured under the trade name of Optaflexx™ by Elanco Animal Health (Indianapolis, IN), which contains ractopamine hydrochloride (RAC). Ractopamine HCl is an orally active β-AA that was approved for use in finishing beef cattle in the United States in 2003 (Food and Drug Administration, 2003). Inclusion levels up to 430 $mg \cdot steer^{-1} \cdot d^{-1}$ top dressed during the last 28 to 42 days of the feeding period increases protein accretion, improves growth performance and decreases adipose tissue deposition in livestock (Smith, 1987; Abney et al., 2007). Average daily gain **(ADG)**, feed efficiency and hot carcass weight **(HCW)** have been reported to improve in numerous studies when beef cattle are fed RAC (Anderson et al., 1989; Abney et al., 2007; Schroeder et al., 2004). Steers improved by 24% greater ADG, consumed less dry matter, improved gain to feed **(G:F)**, carcasses were 5%

heavier and yielded greater than did control steers (Avendaño-Reyes et al., 2006). Utilizing ten experiments conducted in varying regions of the United States, Schroder et al. (2004) determined overall that animals receiving RAC in the final phase of the finishing period improved ADG by 26%, increased total body weight **(BW)** gain by 20%, improved efficiency of gain by 20.5% and improved HCW by 8.3 kg compared with non-supplemented controls. Longissimus muscle area increased with increasing RAC treatment levels (Schroeder et al., 2004; Abney et al., 2007) and decreased yield grades (Anderson et al., 1989, Abney et al., 2007).

RAC reaches an efficacy plateau in beef cattle around day 35 that renders no further increase in ADG from day 35 to 42 of the Food and Drug Administration **(FDA)** label approved feeding period (Moody, 2000; Johnson, 2004; Avendaño-Reyes et al., 2006; Abney et al., 2007). In the Abney et al. (2007) study, ADG was 14.8% greater for 35 vs. 28 d but no further increase was observed as the feeding duration increased beyond that time to the 42 d maximum labeled feeding duration. Numerous studies have indicated that RAC increases growth rapidly at the onset of treatment. However, as a plateau is reached in the growth curve, it is speculated that the β-AA receptors become desensitized due to chronic exposure resulting in a down-regulation of the receptors thereby diminishing the performance improvements achieved early on in the drug's administration (Moody, 2000; Johnson, 2004; Abney, 2007). The diminishing returns realized in RAC treatment are lessened as drug levels are decreased with optimal results occurring at day 28 with a 200 $mg \cdot steer^{-1} \cdot d^{-1}$ inclusion rate with continued improvements to d 42 with animals administered the 100 $mg \cdot steer^{-1} \cdot d^{-1}$ feeding level (Abney et al., 2007).

Abney et al. (2007) determined that final BW, ADG, G:F ratio and HCW increased linearly as dose of RAC increased. For treatment groups of steers fed varying RAC dosage levels Schroeder et. al. (2004) reported increased ADG by 17.1% for 100 $mg \cdot steer^{-1} \cdot d^{-1}$, 19.6%

for 200 mg·steer^{-1}·d^{-1} and 25.7% for 300 mg·steer^{-1}·d^{-1}. Total weight gain in this study was increased by 7.1 kg for the 100 mg·steer^{-1}·d^{-1}, 7.8 kg for the 200 mg·steer^{-1}·d^{-1} and 10.9 kg for the 300 mg·steer^{-1}·d^{-1} above controls. G:F ratios for these three treatment groups also increased by 13.6% for the 100 mg·steer^{-1}·d^{-1}, 15.9% for the 200 mg·steer^{-1}·d^{-1} and 20.5% for the 300 mg·steer^{-1}·d^{-1}. HCW increased by 2.9 for the 100 mg·steer^{-1}·d^{-1}, 6.4 for the 200 mg·steer^{-1}·d^{-1} and 8.3 kg for the 300 mg·steer^{-1}·d^{-1} compared to controls (Schroeder et al., 2004).

Abney et al. (2007) also reported an increased time to consume 50% to 75% of daily intake relative to control steers, however no other positive or negative metabolic or performance effects were correlated with this deviation in consumption. Johnson (2004) reported unpredictable results for ADG ranging from a 9% decrease to a 30% increase.

Steroidal Implants

Anabolic steroidal implants (implants) shift the composition of gain in cattle by hormonally stimulating an increase in protein deposition, thereby decreasing the percentage of gain to body fat (Guiroy, 2002). Growth hormones containing estradiol 17-β have been reported to decrease urinary N by 28% (Cecava and Hancock, 1994). Manipulation of the hormonal metabolic pathway is therefore another possible means to promote N retention and thereby lessen N excretion from the ruminant system.

Since the 1950's, steroidal implants have been approved by the United States Food and Drug Administration for use in steers and heifers destined for slaughter to enhance growth rate, feed efficiency and lean tissue accretion (United States Food and Drug Administration, nda). Implants have been shown to increase carcass weight (Roeber, 2000) and carcass muscle yield (Johnson, 1996). Implants are termed a repartitioning agent because, as defined by the NRC, net energy for gain is the energy content of the tissue accreted, which is derived from the

relationship of the proportion of fat and protein in the empty body tissue gain (NRC, 2000). The energy retained from feed consumed is directed to become either protein or fat (NRC, 2000) because when progenitor cells are directed toward the myogenic pathway, their entry to the adipogenic pathway is blocked (Johnson, 2007). Therefore, if metabolic signals can direct more of that energy to be retained as protein accretion, then less energy will be allotted for fat deposition, so energy is repartitioned to build muscle mass.

Implants are classified as estrogenic, such as estradiol **(E_2)**, gestagenic, such as progesterone, androgenic, such as testosterone, or xenobiotic such as trenbolone acetate **(TBA)** or a myriad of combinations of the aforementioned substances. Mammalian bodies by nature produce and metabolize anabolic compounds. Estrogenic compounds are derived primarily from the granulose cells of the ovary and in the testes of the male. Androgens are primarily derived from the Leydig cells of the male testes or in females, the ovary and the adrenal cortex (Boron and Boulpaep, 2009). Synthetic forms (xenobiotics) are also employable metabolites which stimulate body systems similarly as do their anabolic counterparts.

Reviews of androgenic versus estrogenic derived compounds suggest their modes of action within the body differ yet, when utilized in combination, provide synergistic gains in muscle accretion (Unruh, 1986). Assessments of how each class of implant functions point to estrogenic compounds acting indirectly through their action on the pituitary gland, adrenal cortex, thyroid gland and pancreas involving growth hormone, insulin and thyroid hormone production. Androgenic compound's action seem to be more directly on the muscle cell proper, although both classes have receptors on muscle tissue (Hutcheson, 1994).

Trenbolone acetate, a xenobiotic, is the most commonly used androgenic implant that is 10 and 50 times more active than testosterone propionate and testosterone respectively

(Hutcheson, 1994). Industry concerns have been noted due to decreased marbling with the percentage of carcasses grading USDA Choice decreased by 25% when cattle were implanted with TBA (Morgan, 1997). Herschler et. al. (1995) and Scheffler et al. (2003) found no difference in **KPH** (kidney, pelvic, heart) percentage when comparing nonimplanted and implanted steers although other studies have shown a decrease (Johnson, 1996).

Androgenic implants affect corticosteroid, thyroid hormone, insulin, growth hormone, **(IGF-I)** and estrogen levels by making cells more responsive to these growth factors (Hutcheson, 1994). One possible mode of action is through inhibition of the glucocorticoids from binding to their receptors which thereby prohibits cortisol, whose action is to promote muscle degradation (Hutcheson, 1994). Similarly, reduction in thyroid hormones may contribute to the decreased rate of muscle breakdown and decrease the amount of energy required for maintenance thereby increasing the amount of energy available for body mass growth (Hutcheson, 1994). Androgen's direct effect on muscle accretion is derived from the receptor-hormone complex attaching to an acceptor site on the DNA causing mRNA synthesis which ultimately culminates in an increase in skeletal muscle protein synthesis (Hutcheson, 1994).

In 1992 the FDA approved the use of the combination of implants that contain an androgen (trenbolone acetate; TBA) with an estrogen (estradiol; E_2) (Johnson, 1996), in which previous research indicated improved feed efficiency by 20% and growth rate by 15% (Johnson, 1996). The combination of estradiol and TBA resulted in synergistic improvement by doubling the protein content of gain than did estradiol implantation alone (Bartle et al., 1992). A review by Dolezal (1997) reported the greatest increase in LM and HCW resulted from the use of combination implants in yearling steers. Early implantation of trenbolone acetate estradiol benzoate has been shown to decrease intra muscular fat deposition within the **LM** (longissimus

muscle) (Bruns, 2005). Feedlot steers implanted with anabolic steroids containing TBA in combination with estradiol-17β (E_2) are reported to have improved feedlot performance and increased muscle accretion (Johnson, 1996; Pampusch et al., 2003). Johnson (1996) observed that steers implanted with TBA/E_2, from the day of implantation to d 40, had a 10% to 12% increase in muscle accretion compared to that of non-implanted steers fed the same number of days. Following d 40 post implantation Johnson (1996) noted that muscle accretion gains leveled with that of non-implanted steers. Muscle accretion is inversely associated with N excretion. As noted above, Cecava and Hancock (1994) realized a 28% decrease in urinary N in steers implanted with estradiol 17-β while Rumsey and Hammond (1990) discovered implants containing TBA with E_2 (Synovex-S) decreased urinary N excretion by 8%. Overall, literature depicts that with steroidal implant usage, digestibility of feed N remains constant resulting in unaltered fecal N content, yet, urinary N is decreased (Archibeque, 2008). Decreased urinary N reveals post absorptive changes of metabolism in how energy is being allocated towards muscle accretion, thereby using more N for building muscle protein, rather than fat deposition which does not require N (Archibeque, 2008). Therefore, adipose accretion leaves more N available for excretion.

When considering another β-AA; Zilpaterol Hydrochloride, Baxa (2010) determined the combination of Zilpaterol Hydrochloride with steroidal implant Revelor S (RS; 120 mg of TBA and 24 mg of E_2 17-β) additively contributed to BW and carcass gain in finishing feedlot steers and decreased marbling scores, USDA quality grades and fat thickness. There is no data currently on the comparative efficacy of β-agonist RAC in combination with steroidal implants. Therefore, this study aimed to determine if there is a synergistic effect when utilizing the RAC in combination with Revelor S steroidal implant.

SECTION IV: BEST MANAGEMENT PRACTICES FOR FEEDING CATTLE

Colorado State University Cooperative Extension in conjunction with the Colorado Department of Agriculture, Colorado Department of Public Health and Environment, USDA Soil Conservation Service and numerous other governmental agencies are charged with addressing the Agricultural Chemicals and Groundwater Protection Act (SB 90-126). This act mandates the protection of groundwater and the environment from damages or degradation due to the improper use of agricultural chemicals, yet promotes their proper use and approved applications (Waskom, 1994). Rather than leveraging overly restrictive measures on producers and industry professionals, Colorado has elected to train and educate individuals associated with agricultural production on a voluntary basis on Best Management Practices to hopefully prevent pollution and contamination so that further regulation will be unnecessary (Waskom, 1994). To document compliance with Colorado Department of Public Health and Environment Regulation 61 and 81 (ground and surface water requirements), are the Best Management Practices informational bulletins compiled by Colorado State Cooperative Extension on topics ranging from fertilizer application rates, manure handling and numerous other topics to prevent ground water contamination and environmental pollutants. Currently factsheets are available from the Colorado State University Cooperative Extension website that discusses Best Management Practices for Reducing Ammonia Emissions (Lupis et al., 2010). The intent of this study is to further research that allows for the determination of the best approaches to offer producers alternatives to voluntarily reduce emissions which negate the necessity for formal governmental regulation.

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CHAPTER III

MATERIALS AND METHODS

This study was collaboration between the United States Department of Agriculture, National Institute of Food and Agriculture and the Department of Animal Sciences at Colorado State University in 2010 to investigate possible solutions to maximize nitrogen retention in beef feedlot production. All procedures involving live animals were conducted within the guidelines of and approved by the Colorado State University Animal Care and Use Committee.

EXPERIMENT 1

Experimental Design and Experimental Treatments.

Twenty-four yearling steers were used in a balance trial to measure nutrient intake, excretion, balance, digestibility, retention, carcass merit, and if there are synergistic effects among growth promotants when utilizing hormonal implants with and without beta agonists in beef feedlot cattle. This study used 24 steers in a completely randomized block design with a 2 x 2 factorial arrangement of treatments. The factors included the addition or lack of a ractopamine hydrochloride topdress **(RAC)** (Optaflexx, Elanco Animal Health, Indianapolis, IN) during the final 42 days of the feeding period (400 $mg \cdot steer^{-1} \cdot d^{-1}$) or the use of Synovex-Plus Implants **(IMP)** (200 mg trenbolone acetate and 28 mg of estradiol benzoate, Fort Dodge Animal Health, Fort Dodge, IA). These factors led to the following 4 treatment groups and 6 replications; Treatment 1- Control (no-RAC/no-IMP), Treatment 2- (RAC/no-IMP), Treatment 3- (no RAC/IMP), Treatment 4- (IMP/RAC).

Cattle, Pen Parameters and Sample Collections

Thirty-six yearling Hereford x Angus steers were selected from the Colorado State University (CSU) Beef Improvement Center (Rouse Ranch) cow herd for Experiment 1. Steers arrived at the CSU Agriculture Research, Development and Education Center **(ARDEC)** March 11, 2010 with an average arrival BW=235 $\pm$ 16 kg. Steers were vaccinated prior to their arrival. Day 49 steers were given injectable 1% ivermectin for parasite control (Noromectin, Norbrook Laboratories Limited, Newry, Northern Ireland).

Steers were initially housed as a group in a large soil surfaced, dry lot pen and offered a starter ration ad libitum for 42 days to allow for slow growth while the animals were adapted to close human contact and balance trial procedures. Prior to the initiation of the trial, steers were gradually adapted to a high concentrate diet utilizing a five step-up ration method (Table 1). Nutrient composition data for the final finisher ration is provided in Table 2. Ration samples were gathered weekly and ration dry matter **(DM)** was determined via 2 d in a 60˚ C forced air drying oven.

On d 22 the steers were moved and randomly assigned to five 40 m x 6.1 m soil surfaced pens to facilitate desensitization to close human contact and training for the application of the fecal collection apparatuses (Hastings Canvas & MFG. CO., Hastings, NE). Steers were trained on a random schedule from d 2 through the start of the trial on d 185 to maintain desensitized behavior. Steers were culled due to excessively excitable temperaments or chronic health issues such as bloat and respiratory disease. The final twenty-four steers were selected for the trial based on consistency of feed intake and temperament.

Steers were weighed on days 0, 49, 83, 97, 111, 125, 132, 137, 144, 157, 164, 171 to monitor gains for scheduling the coordination of implants, initiation of RAC treatments and subsequent start of the trial when animals entered the metabolism barn. The hormonal

implantation strategy involved projecting weights 49 d prior to the start of the balance trial. This strategy was employed to maximize efficacy of the hormonal treatment levels during the balance trial due to estimated performance half-life of the hormonal implant. On d 137 the steers were randomly selected from the trained group of cattle and were split into heavy (Group 1) and light (Group 2) classifications that entered the metabolism barn on d 185 and 192 respectively. Within each group, steers were stratified by weight to achieve equal weights within treatment groups of implanted verses non-implanted animals. The implanted treatments in groups 1 and 2 were given Synovex-Plus on d 137 and d 144 respectively. The implantation dates were staggered by one week so that each group of steers would be at the same respective level of hormone therapy at the time each group of steers entered the metabolism barn. In addition, on d 144 ultra-sound was performed on all steers, between the 12^{th} and 13^{th} ribs, to measure intramuscular fat and the rib eye surface area of the longissimus dorsi. A 10.0% permethrin was poured on for fly control (Brute, Y-Tex Corporation, Cody, WY). After weighing and when applicable, implantation on day 137 (group 1) and on day 144 (group 2), steers were moved to an alleyway and randomly sorted into individual pens, also measuring 40 m x 6.1 m, to facilitate individual feeding and administration of RAC treatments 42 d prior to finish. Animals were offered 18.14 kg of finisher ration upon entry into the individual pens. Refusals were pulled, weighed and recorded daily between 0430 and 0630 hours with subsequent deliveries based on the previous days consumption and delivery increases amounting to no more than approximately .75 kg DM to minimize acidosis and bloat issues.

Premix bags of 449.6 g of finely ground corn (no RAC treatments) and 449.6 g finely ground corn plus 4.36 g Optaflexx (RAC treatments) were prepared. RAC treatment premixes were administered as a ration top dress of 400 mg of ractopamine·steer^{-1}·d^{-1}. To acclimate steers

to the RAC or no RAC treatments, on d 160 all steers began receiving 449.6 g of finely ground corn top dressed on approximately 3.63 kg of finisher ration. Steers were not fed the remainder of their ration until the partial ration with top dress was consumed. Group 1 steers on d 164 and group 2 steers on d 171 were weighed and randomly assigned to RAC or no RAC treatment groups. Treatment groups were assigned by randomly stratifying treatments by weight in accordance with previous implantation category and by considering previous average DM intakes. RAC and no RAC ground corn treatments continued to be top dressed in the same fashion as was delivered during the acclimation period.

Steers began acclimation period to entering the metabolism barn on d 173. All steers were sequentially allowed access to the barn for approximately an hour for 12 d prior to the start of the trial. Steers were returned to their respective individual pens after their desensitization training each day.

For the balance trial, steers were weighed upon their entry and exit date from the metabolism barn on days 185/191 (Group 1) and 192/198 (Group 2). Steers were taken into the metabolism barn and were led into randomly pre-assigned rubber matted stalls numbered 1 through 12 which measured 1 m x 3 m. Stalls were assigned based on alternating treatments of RAC or no RAC to avoid confounding location with treatment. The time the steer entered the stall was recorded. The steer was fitted with a fecal collection harness, a urine collection apparatus and a halter or collar which was attached to the front of the stall by a .71 m chain that was adjusted for the steers to lie down and stand up comfortably, yet did not allow them to turn around in the stall. The urine apparatus was attached via flexible tubing to a vacuum system which deposited each steer's urine into individual 50 liter Nalgene carboys which were attached to an additional 20 liter Nalgene carboy in case of overflow. Daily, 100 ml of 6 N HCl was

added to each urine carboy to prevent ammonia volatilization. Collections were performed at the same time each day. Total masses of urine, feces and feed refusals were measured. For each animal's fecal, urine and orts specimens, a 10% subsample was retained daily and accumulated in a composite bag for the week. Feed samples were collected daily and composited for each feeding period. Fecal bags were washed and allowed to dry 48 hours between collection intervals. Steers were fed 3.625 kg of finisher ration with the RAC or no RAC ground corn top dress as soon as refusals were gathered out of the bunk. The remainder of the ration was not fed until the steer had consumed the initial 3.625 kg of finisher ration with top dress. Feed consumption decreased markedly on some animals and, consequently, feed offerings were gradually and conservatively decreased (no more than 1.47 kg/d DM) to ensure steers had adequate feed available at all times. As steers would come back on feed, increases of no more than .73 kg/d DM were also adhered to prevent acidosis or bloat issues.

Steers remained in the barn for 6 consecutive collection days, except for 1 steer in Group 1 which had to be removed from the trial on the first day, and 1 steer which was removed from the trial 1 d early (5 d on trial) due to the animal repeatedly becoming entangled in his stall. One animal's samples also had to be eliminated from the trial due to a sample collection error. In all, 22 specimen samples were viable (Control-6, RAC-5, IMP-6, RAC/IMP-5). Steers were taken off trial on d 6, removing fecal and urine apparatuses at precisely the same time that they were put on 6 days previous. Steers were a total of 204 dof at the ARDEC facility.

Carcass Evaluation

A final weight was obtained on all steers on the final shipping date, d 204. At the end of the feeding period, each treatment was shipped to a commercial abattoir USDA-inspected facility (Cargill Meat Solutions, in Fort Morgan, Colorado). At harvest, HCW's were recorded. Carcass

data was recorded by Diamond T Livestock Services, Inc. including marbling score, percent yield grade **(PYG)**, fat thickness, % kidney/pelvic/heart **(KPH)**, rib eye area, quality grade, and calculated yield grade as determined by a USDA grader.

Sample Evaluation

Subsamples of feed, feces and orts were dried in forced air drying ovens at 60˚ C until 2 identical consecutive weights were obtained. Samples were finely ground in a Thomas-Wiley laboratory mill with a 1 mm screen. Urine subsamples were placed into 50 ml conicals.

Composite samples of ground, dried feed, fecal and orts and urine samples (LECO Corporation, St. Joseph, MI) were analyzed with the LECO TruSpec CN (St. Joseph, MI) for N and C content. Urine urea N was analyzed utilizing the Stanbio Laboratory (Boerne, TX 78006) Enzymatic Urea Nitrogen (blood/urea/nitrogen **(BUN)**) Assay Kits with the Gen 5 plate reader by Biotek (Winooski, VT 05404) at 20% and 50% dilution rates dependent on the concentration of the urine. The standard curve for the urine urea N was based on 30, 15 and 7.5 mg/dl standards. Ground, dried feed, fecal and orts samples and urine samples dried with cellulose were analyzed for energy content utilizing the Model 1261 Parr Instrument Company (Moline, IL.) bomb calorimeter. Urine subsamples and approximately 100 g of dried, ground composite feed, fecal and ort were sent to Michigan State University Diagnostic Center for Population and Animal Health Laboratory for a Ruminant Proximate Analysis panel and wet chemistry Panel C analysis for mineral concentrations.

Calculations

The following equations were used in calculations:

$$Nutrient\ Intake = As\ fed\ consumed * Nutrient\ \%\ DM - Orts\ remaining * Orts\ \%\ DM$$

$$Fecal\ and\ Feed\ nutrients = Fecal\ (or\ Feed)\ DM * Fecal\ (or\ Feed)\ Nutrient\ g/g$$

$$Digestibility = \frac{Nutrient\ Intake\ DM - Fecal\ Nutrient\ DM}{Nutrient\ Intake\ DM}$$

$$Balance = Nutrient\ Intake - Fecal\ Nutrient - Urine\ Nutrient$$

$$Metabolic\ Body\ Weight\ (BW) = \left(\frac{BW\ Beginning + BW\ Ending}{2}\right)^{.75}$$

$$Balance\ Retention = \frac{Nutrient\ Balance}{Metabolic\ BW}$$

Statistical Analysis

Data were analyzed using the MIXED procedures of SAS (Version 9.3, SAS Inst. Inc., Cary, NC). Steer was the experimental unit. Significance was determined utilizing least square means with an F-test ($P \leq 0.05$), and tendencies were declared when ($P \leq 0.10$). The model included the fixed effects of implantation status, ractopamine status and the combination of implant with ractopamine. Block was considered a random effect in the model.

EXPERIMENT 2

This study was a collaboration between the United States Department of Agriculture, National Institute of Food and Agriculture, the Department of Animal Sciences and the Department of Soil and Crop Sciences at Colorado State University (Fort Collins, Colorado) in 2010 to investigate differences in fecal mounding technique on calculated nitrogen volatilization. All procedures involving live animals were conducted within the guidelines of and approved by the Colorado State University Animal Care and Use Committee.

Experimental Design

To model feedlot pen precipitation runoff, 187 purebred Red Angus, Black Angus and crossbred bulls on a feeding test were used to measure DM, N and P intake, fecal excretions and digestibility. Also, DM, N and P Fecal excretions and precipitation runoff content per unit of

intake were determined. The study design was a balanced randomized arrangement grouped into 2 treatments with 3 replications.

Experimental Treatments

The treatments consisted of fecal mounding technique variations. Treatment 1- Control (CON) was a standard round based conical manure mounding technique located in the center of the pen, Treatment 2- (LONG) was a long, narrow mound that ran the length of the middle of the pen. Pens were randomly assigned to treatment classification with numerical ordering of pens assigned alternating treatment categories (even numbered pens, replicates 2, 4 and 6, were CON, odd numbered pens, replicates 1, 3 and 5, were assigned to LONG. There were 6 pens total, 3 pens per treatment and 30 to 32 bulls per pen.

Cattle

Privately owned purebred Red Angus, Black Angus and crossbred bulls (187 total) were delivered to the Agriculture Research Development and Education Center, Fort Collins, Colorado, for a breeding bull feed test April 12, 2011. Upon arrival, bulls were weighed with an average arrival BW = 388 $\pm$ 44 kg. Bulls were **EID** (Electronic Identification) tagged and sorted into 6 outdoor pens according to pure verses crossbred classification and then stratified by weight designations decided by the cattle owner. Pens started the trial with 32 bulls per pen. During the trial, due to animals being euthanized for health issues or gates opened by cattle, bull counts per pen per day were used for total bull counts and then divided by total days to get average bull count per pen. Average bull counts used in calculations were: Pen 53: 30.10, Pen 54: 32.20, Pen 55 32.40, Pen 56: 30.63, Pen 57: 32.40, Pen 58, 31.63.

Pens

Pens were located in the Feed Intake Unit. Each of the 6 soil surfaced pens measured approximately 156' by 53' 4". Each pen had an individual water trough. Cement dividers ran the length of each pen keeping excrement and precipitation separated between individual feedlot pens. Photos were taken of cleaned pens before the start of the trial for comparative reference for pen cleaning at the end of the trial.

For the initial soil sample, due to the hardness of the pen soil, a corer could not be used. Therefore, random samples of the top 2 inches of soil were taken with a shovel from each pen, composited and a 45 g subsample of the composite was taken for laboratory analysis. During the trial, pens were scraped and mounded with 4 areas core sampled (5 inches deep by 1.5 inches in diameter) in pens 54, 56 and 58 and taken pre and post scraping on 23 May, 2011 and 30 June, 2011.

Feed, Deliveries and Ration Sample Collections

Each pen contained 4 feed bays measuring approximately 3' 10" by 2' 9" which utilized the Grow Safe™ electronic feed weighing system. Fresh feed was milled for each feeding and bulls were fed 2 to 3 times per day dependent on feed level within the grow safe bunks. Bunk calls were designed to keep feed available in the bunks at all times for proper operation of the Grow Safe™ feeding system. Feed deliveries were performed with the use of a single axle truck with Mohrlang™ Mixer Feeder Model 4525. Refusals were cleaned out of bunks on 21 June, 2011 which were weighed and recorded. Bulls were fed a grower ration ad libitum for a total of 81 d. Ration composition is detailed in Table 3 and feed analyses are presented in Table 4. Diets were formulated to meet or exceed the NRC (2000) requirements for growing bulls for all vitamin and minerals. The grower ration was designed to contain 80.65% DM, 12.98% Protein,

1.11% NPN, 63.10 Mcal/cwt, 80.86% TDN, .4% Ca, .33% P, .88% K, .17% S, .16% Mg, 1519.25 IU/lb. Vit A, 1.21 IU/lb. Vit E, and 25.80 g/ton Monensin on a DM basis.

Ration samples were gathered daily and frozen at 20˚ C. The total feeding period ration sample was composited and ration DM was determined via 2 d in a 60˚ C forced air drying oven. Feed intake data were collected through bunks with suspended load cells and extrapolated to correlate with recorded feed deliveries on days when the load cells were inoperable. Dry Matter Intake **(DMI)** was determined utilizing total as fed feed deliveries multiplied by the total feeding period average percent DM of the diet as determined by weekly samples.

Cattle Weighing

Bulls were weighed and recorded on d 0, 27, 42, 55, 62, 70 and 77 and a final projected weight based on weight regression on d 81.

Runoff Collections

Precipitation was measured and recorded in a standard rain gauge for each runoff event. Precipitation runoff samples were collected in cement collection pits at the base of each pen with an average collection runoff volume capacity of 480283.2 cm^3. Runoff events occurred on d 2, 9, 13, 29, 30, 31, 36, 37, 38, 47, 58, 59 and 66. For each pit collection, total depth of precipitation was measured in each pit corner. Total precipitation collection volumes were calculated utilizing individual pit dimensions along with the depth of each of the four pit corners which were then averaged for the total pit volume. Pits were stirred to homogenize runoff collections and then samples were collected in 50 ml. conicals.

Manure Collections

After bull were shipped on d 81, pens were scraped and mounded into one central pile where random manure samples were taken and stored in gallon zip lock bags. Samples were frozen at 20˚C until analysis. Total fecal collections were loaded on dump trucks and weighed at Horton Feedlot Scales (Wellington, CO) on an as is basis. Actual total manure collections were compared to calculated total fecal output (FO) obtained from the following equation:

Dry Matter Excretion Equation

$DME_T = \sum n_{x=1} DMI_x * DOF_x * (1\text{-}DMD_x/100)$

Long Mound:

$$= 11738\ g/d * 81d * (1 - \frac{60}{100})$$

DM excreted per bull : Theoretical = 380311.2 g Actual = 382966.5 g

Normal Mound:

$$= 11778\ g/d * 81d * (1 - \frac{31}{100})$$

DM excreted per bull: Theoretical = 658272.4 g Actual = 661494.0 g

Nitrogen Excretion Equation

$N_{E\text{-}T} = \sum n_{x=1} (DMI_x * C_{cp\text{-}x} * DOF_x * / 6.25) - [0.0412 * (LW_f - LW_s)] + [0.000243 * DOF_t * [LW_f + LW_s/2)]^{0.75} * (SRW / (LW_f * 0.96))^{0.75} * [LW_f\text{-}LW_s/DOF_t]^{1.097}]$

Theoretical N Excretion for Long Mound:

$$= \frac{11738\ g * .1222 * 81d}{6.25} - [0.0412 * (514.8 - 370.9)] + [0.000243 * 81d * [\frac{514.8 + 370.9}{2}]^{0.75} * \left(\frac{478}{514.8 * 0.96}\right)^{0.75} * \left[\frac{514.8 - 370.9}{81}\right]^{1.097}]$$

=18587.1638 g N excreted per bull

Theoretical N Excretion for Normal Mound:

$$= \frac{11778\ g * .1222 * 81d}{6.25} - [0.0412 * (541.4 - 386.6)] + [0.000243 * 81d * \left[\frac{541.4 + 386.6}{2}\right]^{0.75} * \left(\frac{478}{541.4 * 0.96}\right)^{0.75} * \left[\frac{541.4 - 386.6}{81}\right]^{1.097}]$$

=18650.34297 g N excreted per bull

Sample Evaluation

Subsamples of feed and feces were dried in forced air drying ovens at 60˚ C until 2 identical consecutive weights were obtained. Samples were finely ground in a Thomas-Wiley laboratory mill with a 1 mm screen.

All composite samples of ground, dried feed, fecal and soil were analyzed for total N by combustion method using a N analyzer (LECO TruSpec CN, LECO Corp., St. Joseph, MI). Runoff samples were freeze dried before analysis to concentrate sample adequately for N and C analysis by the LECO TruSpec.

Approximately 100 g of dried, ground composite feed and fecal samples as well as 20 mls. of wet runoff samples were sent to Michigan State University Diagnostic Center for

Population and Animal Health Laboratory (MSU) for a wet chemistry Panel C analysis for minerals.

Nutrient Balance

A nutrient balance was conducted for the 6 open feedlot pens. Nutrient digestibility (DM, N and P) was arrived at using the total nutrient intake minus total fecal nutrient content divided by total nutrient intake. N intake was calculated using analyzed N content of composited feed sample multiplied by DMI and corrected for N content of feed refusals. N and P retention were estimated from individual animal performance using the following equations (NRC, 2000; Cole et al., 2006):

$$Shrunk\ Body\ Weight\ (SBW) = Weight * 0.96$$

$$Empty\ Body\ Weight\ (EBW) = SBW * 0.891$$

$$Shrunk\ Weight\ Gain\ (SWG) = Average\ Daily\ Gain * 0.96$$

$$Empty\ Body\ Gain\ (EBG) = SWG * 0.956$$

$$Retained\ Energy\ (RE) = 0.0635 * (EBW^{0.75} * EBG^{1.097})$$

$$Protein\ Retained\ (ProtRE) = (268 * SWG) - (29.4 * RE)$$

$$Phosphorus\ Retained = ProtRE \pm 0.039$$

and

$$Nitrogen\ Retained\ (NRE) = \frac{ProtRE}{6.25}$$

Where **SWG** = shrunk weight gain (kg/d); **EBG** = empty body gain (kg/d); **RE** = retained energy (Mcal/d); **EQEBW** = equivalent empty BW (kg); ProtRe = protein retention (g/d); PhosRe = P retention (g/d); and NRe = N retention (g/d). Urinary N and P excretion were estimated as the difference between nutrient intake and fecal + retained nutrients (NRC, 2000, Cole et al., 2006).

To estimate the volume of N lost to the atmosphere, diet and fecal N percentages were determined utilizing the LECO combustion method detailed above which were then multiplied by the respective DM content each. N lost in runoff was calculated as the quantity of runoff multiplied by the N concentration of the runoff. N content of the soil was determined by the difference of N content before and after the cattle were in the pens. The amount of N volatilized was calculated as the difference between the amount of N excreted, and the amount removed in the manure, runoff and N incorporated into the pen soil.

Statistical Analysis

Data were analyzed using the MIXED procedures of SAS (Version 9.3, SAS Inst. Inc., Cary, NC.). Pen was the experimental unit. The model included the fixed effect of mound type. Significance was determined utilizing least square means with an F-test ($P \leq 0.05$).

CHAPTER IV

RESULTS

EXPERIMENT 1

Results for intake, excretion and metabolic retention are presented in Table 5 for nutrients and Table 6 for selected minerals.

For total period intakes, there was a synergistic (RAC x IMP) effect on less total DMI (P=0.05) for steers that received both IMP and RAC treatment. Consequently, along with decreased DMI, the synergism of growth promotants (RAC x IMP) also lessened total C intake (P=0.05), total energy (E) intake (P=0.05), total ADF intake (P=0.01), total NDF intake (P=0.05), total crude fat intake (P=0.03), total Ca intake (P=0.05) and total P intake (P=0.03). The combination of RAC with IMP tended to decrease total N intake (P=0.06) and total K intake (P=0.07). Growth promotants singularly had no effect ($P \geq 0.10$) on total intakes except for RAC only treatment which decreased intakes of ADF (P=0.05) and Lignin (P=0.04).

On an intake per d basis, there was a synergistic effect (RAC x IMP) on less daily ADF intake (P=0.03) and tended to decrease daily intakes of DM (P=0.09), C (P=0.09), NDF (P=0.09), E (P=0.10), crude fat (P=0.07), Ca (P=0.08) and P (P=0.06). RAC only treatment tended to decrease daily lignin intake (P=0.06). No other treatments were observed to have an effect on daily intakes of nutrients ($P \geq 0.10$).

On a fecal excretion per d basis, synergistically (RAC x IMP) there tended to be less Ca (P=0.07) eliminated. Less P tended to be excreted when both IMP and RAC were used (P=0.06) as well as when RAC treatment was administered without implant (P=0.08). No other nutrients were affected by growth promotant status ($P \geq 0.10$) for daily fecal excretion levels.

Total urinary excretions were affected by RAC only treatment with decreases in total urinary DM (P =0.02), N (P =0.01), and E (P =0.01). IMP only treatment tended to decrease

total N (P =0.09) and E (P =0.09) levels in urine while there was an interaction by which the RAC and IMP, when used together, tended to also decrease total N (P =0.07) and E (P =0.07) in urinary excretions. No other nutrients were affected by growth promotant status ($P \geq 0.10$) on total urinary excretions.

On a urinary excretion per d basis, RAC treatment lessened daily urinary DM (P =0.03), N (P =0.01) and E (P =0.03). Synergistically (RAC x IMP), daily urinary excretions tended to be lessened also for N (P =0.08) and E (P =0.09). No other nutrients were affected by growth promotant status ($P \geq 0.10$) for daily urinary excretions.

Digestibility of nutrients as well as digestibility per unit of intake were not affected by treatment ($P \geq 0.10$) except for RAC only treatment which lessened ash (P =0.01), and P (P =0.02) digestibilities for both total and per unit of intake parameters.

Synergistically (RAC x IMP), daily nutrient balance tended to be decreased for C (P =0.07) and E (P =0.09). RAC only treatment decreased the balance of ash (P =0.01) and P (P =0.05).

Synergistically (RAC x IMP), balance per kg of metabolic body weight tended to decrease C (P =0.08) and E (P =0.10) retention. RAC treatment decreased P balance per kg of metabolic body weight (P =0.05).

There were no carcass differences found by the interaction of RAC x IMP treatments. There were, however, noted carcass differences for IMP only treatment group. IMP only treatment produced carcasses with greater ending body weight (P = 0.01) and greater metabolic weight (P = 0.01). Steers receiving IMP only treatment also tended to receive greater yield grade scores (P = 0.07) along with greater body fat thickness (P = 0.07).

EXPERIMENT 2

Table 8 provides mean data and statistical results for comparison of LONG versus CON style mounding techniques. Statistical analysis concluded intakes of DM (P = 0.93), N (P = 0.93) and P (P = 0.93) g·steer^{-1}·d^{-1} were similar among treatments. Metabolic body weights were not different among treatment groups (P = 0.57). Total runoff values that were extrapolated back to total g·steer^{-1}·d^{-1} for DM (P =0.16), N (P =0.19) and P (P =0.80) were not different among treatments. Similarly, runoff per unit of intake was not different among treatments for DM (P = 0.19), N (P = 0.22) or P (P = 0.79).

Fecal output, or actually manure collected from pens (g·steer^{-1}·d^{-1}) was different between treatments groups for DM (P = 0.01), N (P = 0.01) and P (P = 0.03) with CON pens retaining more (DM, N and P) in the pen mound than did the LONG treatment pens. There were differences among treatment groups for less fecal output per unit of intake for DM (P = <0.01), N (P = <0.01) and P (P = 0.02) in LONG verses CON mounded pens. Less apparent digestibility was observed for LONG verses CON mounded pens for DM (P < 0.01), N (P < 0.01), and P (P = 0.02). These differences were also reflected in digestibility per unit of intake being greater for LONG verses CON mounded pens in DM (P < 0.01), N (P < 0.01) and P (P = 0.02).

Volatilization is considered on a per pen basis. Per pen, intakes of DM (P = 0.97), N (P = 0.97) and P (P = 0.97) were similar among treatments. Fecal DM (P = 0.01), N (P = 0.01) and P (P = 0.03) were different between treatments with LONG mounds having less of those nutrients than CON mounds. There were no statistical differences in runoff DM (P = 0.13), N (P = 0.15) and P (P = 0.72) or soil N retention (P = 0.86) per pen. There were also no statistical differences in calculated E (P = 0.53), P (P = 0.39) or N (P = 0.39) g·steer^{-1}·d^{-1} retention. No statistical difference occurred between calculated N excreted (P = 0.86) in either total or in

g·steer^{-1}·d^{-1}. There was however a statistically significant difference in total g·steer^{-1}of N (P = 0.01) that was volatilized.

CHAPTER V

DISCUSSION

EXPERIMENT 1

The combination of growth promotants decreased the intake of DM, C, E, ADF, NDF, crude fat, Ca and P and tended to decrease N consumption. The Avendaño-Reyes et al. (2006) study also reported decreased DMI. The combination of growth promotants, however, did not create a decrease in final BW or metabolic BW. Improvement in feed efficiency with the use of either RAC or IMP is well documented in numerous studies (Johnson et al., 1996; Pampusch et al., 2003; Schroeder et al., 2004; Bruns, 2005; Avendaño-Reyes, et al., 2006; Abney et al. 2007). In this study all treatments realized better gains than did the CON group. This indicates that the cattle were able to gain more on less feed thereby achieving improved feed efficiency similar to the findings of Baxa (2010) when he examined the synergism among Zilpaterol Hydrochloride **(ZH)** with Revelor S implants (RS; 120 mg of TBA and 24 mg of E_2 17-β). Although actual F:G calculations are not realistic in a metabolism barn study due to the environmental stress the cattle experience, control cattle were not as efficient in their feed conversions when compared to treatment groups.

Similar to Johnson's findings (2006) the IMP only treatment was the only group that realized a statistically significant improvement in final and greater metabolic BW, however, this group also had the greatest mean averages of nutrient consumptions. Parr et al. (2010) reported that implanting increased BW ADG and G:F especially when using a longer duration release implant. Herschler et. al. (1995), Johnson et. al. (1996) and Bruns et. al. (2005) reported TBA + E2 often have no effect on 12^{th} rib fat thickness and can result in no change or decreased KPH. Similarly, this study found no differences in KPH however, the IMP only carcasses tended to have a greater fat thickness regardless of RAC status indicating the hormonal receptors drove the

entire body to retain overall nutrients, not only muscle mass accretion. Because mammalian reproductive physiology requires a status of net metabolic gain to maintain reproductive functionality, hormonal impetus to retain nutrients dictates the retention of both muscle as well as fat. This study indicated this net gaining tendency as reflected in greater subcutaneous fat thickness in IMP only treatments. Marbling scores, however, did not simultaneously improve for this group. In fact, yield grades were the poorest for the IMP only group indicating the additional gains in fat did not equate to additional muscle or intramuscular fat deposition. Duckett and Andrae (2001) found implanting once with TBA + E2 decreased marbling score by 4% and re-implanting decreased the marbling score by 6 to 11%. Morgan (1997) reported decreased marbling with the percentage of carcasses grading USDA Choice decreased by 25% when cattle were implanted with TBA. Nitrogen balance, digestibility and retention parameters did not reflect a greater N retention in the IMP only group which also indicates the additional body mass gained in this group was not due to muscle mass accretion. Fecal N was not different among any treatment groups. The IMP only group did have the greatest mean of N in their urine. This indicates that the gains of additional nutrients consumed were metabolically driven into lipogenic pathways rather than muscle accretion. Therefore, N was not utilized in the creation of muscle proteins and consequently became surplus N which was then excreted in the urine. This is contrary to the findings reported by Cecava and Hancock (1994) who realized a 28% decrease in urinary N in steers implanted with estradiol 17-β while Rumsey and Hammond (1990) discovered implants containing TBA with E_2 (Synovex-S) decreased urinary N excretion by 8%. Therefore, from this study, it appears that the implant only treatment will promote better gains yet does not achieve our goal to reduce urinary N.

Alternatively, RAC only treatment group, although gains were slightly better (1.72%) than controls, did not attain a statistically significant improvement in ending or metabolic BW. This is contrary to Schroder et al's. (2004) findings who reported increased total BW gain by 20% when compared to controls. The RAC only group had average nutrient intakes on all measured nutrients except ADF and lignin. Dry matter intake was not different for this group indicating these steers may have been somehow selectively sorting the TMR (total mixed ration) because this group achieved statistical significance in the lesser amount of ADF and lignin consumed. Avendaño-Reyes (2006) reported decreased DMI and Abney (2007) reported an increased time to consume 50% to 75% of daily intake relative to control steers. Duration of consumption periods were not measured for this study however, when considering Abney's results, our findings may indicate that treatment with RAC may somehow increase selectivity in eating behaviors which would simultaneously lengthen feeding duration periods in cattle.

None of the carcass traits achieved statistical significance for the RAC only treatment groups however, numerically, the means of the data are indicative of the differences in the way nutrients were metabolically driven within the cattle. The RAC only treatments had the lowest mean quality numerical and also had the lowest mean marbling numerical, the second lowest fat thickness (CON was the lowest) yet had the best calculated YG. This indicates metabolically, that any gains steers treated with RAC receive are being allocated to muscle mass accretion. Similarly, Avendaño-Reyes (2006) also reported greater yield grades than did control steers. Dry matter digestibility, did tend to be somewhat lower for RAC treated steers although digestibility per unit of intake was not affected. This indicates that the decreased intake of the RAC only treated steers might have somewhat impacted the apparent digestibility. The most significant differences realized with RAC only treatment is in urine parameters. Statistical significantly less

urine DM, both per day as well as total produced for the feeding period, was excreted. Of this lessened DM content, a large percentage of the decrease is attributable to less N being present in the urine. Urine N is decreased in per day and total feeding period production as well as the urine contained less total urine urea N. There is also less E excreted in the urine for RAC only treated animals. Therefore, the differences in N and E elimination from this treatment group indicate that although the differences are not large enough to reveal statistical difference in the balance and retention parameters, we do see statistically different changes in the urine that is being produced. These nutrients are not metabolically being driven to additional fat production as reflected by the poor marbling and fat scores. These steers are accreting more muscle mass as indicated by the improved calculated yield grade which is the measurement of total saleable meat product. Energy is retained for either fat or muscle accretion. Nitrogen is retained to build proteins and if not utilized, is excreted. Because muscle is made of protein, additional muscle utilizes additional N. There is no evidence of accreted fat on these carcasses combined with the lack of N and E being excreted in the urine indicating that these animals are utilizing surplus energy to build additional muscle mass. Other studies have also reported greater muscle gains through reporting greater LM area with increasing RAC treatment levels (Schroeder, 2004; Abney et al., 2007)

The greatest differences are observed in the combination of IMP x RAC. Steers receiving this combination had statistically significant less DMI, N, C, E, ADF, NDF and crude fat, ash, Ca, P and K intake yet, achieved the second greatest mean body weight (although not statistically significant). Previous research has also indicated improved feed efficiency by 20% and growth rate by 15% when combining growth promotant treatments of implant with beta-agonists (Johnson, 1996). Parr et al. (2010) reported that steer performance and carcass traits

suggest different modes of action for steroidal implants when combined with another beta agonist, Zilpaterol Hydrochloride **(ZH)**.

Although carcass merit did not achieve statistical significance, the synergism of the growth promotants caused this treatment group to achieve the greatest mean marbling numerical, the second greatest fat thickness, the largest mean rib eye area, and the greatest quality numerical. When Baxa et al. (2010) was investigating the interactions of ZH with Revelor S, the researchers determined the combination additively contributed to BW and carcass gain in finishing feedlot steers however, contrary to this study, he reported decreased marbling scores, USDA quality grades and fat thickness.

The treatment combination also realized the second greatest mean ending body weight and tied with the IMP only group for the greatest metabolic BW. There were not differences found in fecal eliminations with this group except there tended to be less NDF, ash, Ca and P in the feces. The most significant statistical differences were discovered in urine parameters with the combination of growth promotants. The mean total urine N production for the feeding period was 36% less than the greatest group (IMP only cattle) and 35% less than CON with daily urine N production following similar trends. Urine urea N was 43% less than the greatest group (IMP only cattle) and 36% less than CON. This combination group of cattle also tended to show statistical significance in 16% less urine urea N per urine N content than did the greatest (IMP only group) and 10% less than controls. Nitrogen digestibility was not different among treatment groups. Nitrogen balance and retention did not show statistical significance however, the numerical means showed there was 64% greater balance and a 60% improvement in retention of N per kg of metabolic BW, when comparing the combined treatment with the CON groups. There was statistical significance in less C tending to be retained by the combined treatment

steers. There was also a statistically significant difference in less E tended to be in urine of steers treated with the combination of growth modifiers. The trends observed with this data set implies hormonal impact was driving these steers to retain more BW while the β-AA was driving them to metabolically put the additional weight to both muscle and fat all with less consumed feed when compared to the CON. The synergism of growth modifiers allows for better gains which improve the bottom line of the cattle feeder yet, because the β-AA drives muscle accretion rather than only providing additional fat, the carcass traits are actually improved which benefits the meat packer as well as the consumer. These benefits to the meat industry also realize better environmental stewardship by retaining more nitrogen within the carcass resulting in less N excreted and volatilized into the environment.

EXPERIMENT 2

The LONG fecal mounding technique resulted in a mean decrease of 42% less fecal DM collected from the pens, 38% less N and 42% less P contained in the feces when compared to the CON mounded pens. Rations were identical. Intakes, ADG, starting and finishing weights were not statistically different. Therefore, this indicates the animals were not producing less feces or less nutrients within the feces in the LONG mounded pens. Rather, the LONG mounds were allowing more mass of nutrients to leave the pens thereby diminishing the quantity of apparent feces available for collection from the pens. When comparing apparent fecal output per unit of intake, only 40% of DM intake resulted in feces in LONG mounded pens versus 69% of intake resulted in feces in normally mounded pens (P <0.01). The decreased quantity of fecal nutrients in the LONG mounded pens artificially inflates the apparent digestibility by 48% for DM, 54% improved for N and an 182% improvement in P compared to CON mounded pens. Likewise, 38% less N in the feces in LONG compared to CON mounded pens indicates the LONG mounding technique allows for increased volatilization or possibly leaching. Calculated N volatilization was greater in LONG verses CON mounding with LONG mounded pens reflecting a more expected value of 48% (P = 0.01) whereas CON mounded pens showed 16% volatilization. Erickson et al. (1998) reported percent volatilization ranges of 65 to 74% in a study looking at varying dietary treatments. Therefore, this study's findings of 16% volatilization for the CON mounded pens are surprisingly low. In the LONG mounded pens, there was more total surface area for the manure to air interface and therefore, more conversion of urea to ammonia which then volatilized. This study, however, cannot rule out increased leaching in the LONG mounded pens because soil sampling was not performed on a per pen basis so differences in soil accumulations cannot be determined. This is especially true when considering the diminished P content of the manure. Nitrogen differences can be explained

through increases in volatilization however, P does not volatilize. Therefore, P must either be leached or in runoff. There was no statistical significance in differences between runoff values among treatments, although numerical means reflected the LONG treatment groups showed greater amounts of DM and N in the runoff than did the CON groups. However this trend is not reflected for P and therefore does not account for the differences seen in decreased fecal P values. Further research in examining soil samples from pens individually is needed to determine if LONG mounding increases P and N leaching in the soil base. Furthermore, we could not rule out differences in total mound DM mass were not attributable to differences in DM content of each mound. Therefore, an alternative sampling technique utilizing a core sampler may prove advantageous to rule DM content differences out as a possible source of error.

Table 1. Experiment 1. Step-up Ration Composition on As Fed basis

	Ration Composition, %					
		Step			Finisher	
	Developer	2	3	4	AF	DM
Ground Alfalfa Hay	25.07	23.67	18.00	18.00	14.13	16.15
Corn Silage	56.69	45.00	35.00	20.00	15.00	5.74
Whole Corn	15.49	0.00	0.00	0.00	0.00	0.00
Cracked Corn	0.00	28.34	43.66	58.05	66.97	73.80
Supplement	2.75	2.99	3.34	3.95	3.89	4.31

Table 2. Experiment 1. Finisher Ration Nutrient Composition on Dry Matter Basis

Nutrient	Finisher Ration Period 1	Finisher Ration Period 2
Dry Matter, %	81.58	81.47
Crude Protein, %	11.6	11.6
E, KJ/g	18.12	17.84
ADICP, %	1.0	0.6
Soluble Protein (% of CP)	35.0	34
ADF, %	13.3	10.6
NDF, %	18.4	22.0
Lignin, %	1.9	2.8
NFC, %	62.6	59.2
Crude Fat, %	3.6	3.3
Ash, %	3.68	3.95
Calcium, ppm	3878	4092
Phosphorus, ppm	2476	2550
Magnesium, ppm	1574	1603
Potassium, ppm	7990	9912
Sodium, ppm	1203	1276
Sulfur, ppm	1148	1284
Copper, ppm	21	17
Iron, ppm	88	120
Zinc, ppm	79	73
Manganese, ppm	36	41
Molybdenum, ppm	.657	.766
Cobalt, ppm	<.500	<.500

Table 3. Experiment 2. Grower ration composition

	Ration Composition, %	
Commodity	AF	DM
Ground Alfalfa Hay	15.00	5.74
Corn Silage	14.13	16.15
Cracked Corn	66.97	73.80
Supplement	3.89	4.31

Table 4. Experiment 2. Grower Ration Nutrient Composition on Dry Matter Basis

Nutrient	Grower Ration
Dry Matter, %	59.2
Crude Protein, %	12.22
Nitrogen, %	1.96
Phosphorus, ppm	2154
Calcium, ppm	7150
Magnesium, ppm	1892
Potassium, ppm	15905
Sodium, ppm	581
Sulfur, ppm	1741
Copper, ppm	12.8
Iron, ppm	383
Zinc, ppm	43.6
Manganese, ppm	44.4
Molybdenum, ppm	<1.00
Cobalt, ppm	<.500

Table 5. Experiment 1. Results for selected nutrient intakes and excretion of finishing cattle with or without ractopamine hydrochloride and with or without hormonal implants

Item	Non-Implanted		Implanted		SE	P-values		
	No Rac	Rac	No Rac	Rac		IMP	RAC	IXR
DM								
Intake, g	44164	46139	52325	40863	3275	.65	.15	.05
Intake, g·d^{-1}	7361	7675	8721	7090	566	.48	.24	.09
Feces, g	12726	13833	14967	12930	1307	.60	.71	.23
Feces, g·d^{-1}	2121	2301	2494	2231	216	.47	.84	.30
Urine, g	3089	2840	3174	2158	261	.25	.02	.14
Urine, g·d^{-1}	515	473	529	373	43	.31	.03	.18
Digestibility, %	72	70	71	69	1	.62	.10	.67
Digestibility per Intake	.99	.93	.83	1.06	.11	.87	.44	.18
N								
Intake, g	765	804	909	717	61	.63	.20	.06
Intake, g·d^{-1}	128	134	152	124	10	.48	.31	.11
Feces, g	330	352	371	314	26	.96	.48	.12
Feces, g·d^{-1}	55	59	62	54	4	.77	.63	.18
Urine, g	372	343	378	242	28	.09	.01	.07
Urine, g·d^{-1}	62	57	63	42	5	.12	.01	.08
Urine urea N g·d^{-1}	25	23	28	16	3	.34	.02	.07
Urine urea N per Urine N	40	41	43	36	2	.69	.21	.08
Urine N, g·d^{-1} per N Intake, g·d^{-1}	50	44	43	36	5	.16	.19	.93
Digestibility, %	57	55	59	56	2	.48	.24	.75
Digestibility per Intake, g·d^{-1}	46	42	39	48	4	.98	.54	.13
Balance, g·d^{-1}	10	18	27	28	9	.14	.60	.73
Balance per Intake	7	11	15	20	6	.16	.43	.99
Retention per kg Metabolic BW	.10	.16	.24	.25	.08	.15	.63	.74
Balance per Digested, %	11	18	24	35	11	.17	.42	.85
C								
Intake, g	19243	20115	22824	17836	1431	.64	.15	.05
Intake, g·d^{-1}	3207	3346	3804	3094	247	.48	.24	.09
Feces, g	5605	5967	6568	5620	578	.58	.60	.52
Feces, g·d^{-1}	934	993	1095	969	95	.46	.72	.33
Urine, g	919	968	898	745	78	.12	.50	.19
Urine, g·d^{-1}	153	161	150	129	13	.17	.61	.26
Digestibility, %	71	70	71	69	1	.64	.20	.61

Continued Table 5. Experiment 1. Results for selected nutrient intakes and excretion of finishing cattle with or without ractopamine hydrochloride and with or without hormonal implants

Item	Non-Implanted		Implanted		SE	P-values		
	No Rac	Rac	No Rac	Rac		IMP	RAC	IXR
C								
Digestibility per Intake, g·d^{-1}	2.27	2.14	1.90	2.42	.25	.86	.42	.19
Balance, g·d^{-1}	2120	2192	2560	1996	169	.46	.15	.07
Balance per Intake	66	65	67	64	1	.99	.18	.54
Balance per kg Metabolic BW	19	20	22	17	1	.74	.11	.08
Balance per Digested, %	93	93	94	94	.73	.25	.51	.67
Energy								
Intake, KJ	800024	838093	949519	756863	56594	.54	.17	.05
Intake, KJ·d^{-1}	133337	139411	158253	131295	9776	.38	.28	.10
Feces, KJ	233995	252454	275449	235991	23925	.59	.65	.22
Feces, KJ·d^{-1}	38999	42004	45908	40700	3950	.47	.77	.29
Urine, KJ	5959	5489	6041	3870	451	.09	.01	.07
Urine, KJ·d^{-1}	5793	5505	6681	4850	454	.79	.03	.09
Digestibility, %	71	70	71	69	1	.86	.26	.88
Digestibility per Intake, KJ·d^{-1}	.054	.051	.045	.057	.006	.79	.45	.18
Balance, KJ·d^{-1}	88545	91903	105664	85745	6670	.40	.21	.09
Balance per Intake	67	66	67	65	1	.93	.39	.91
Balance per kg Metabolic BW	802	818	921	746	57	.67	.16	.10
Balance per Digested, %	94	94	94	94	.63	.77	.50	.92
ADF								
Intake, g	5311	5585	6465	4807	291	.76	.05	.01
Intake, g·d^{-1}	885	929	1044	840	54	.50	.13	.03
Feces, g	2644	2907	3051	3680	582	.30	.43	.75
Feces, g·d^{-1}	441	484	508	628	95	.26	.38	.68
Digestibility, %	50	47	51	24	10	.29	.14	.24
Digestibility per Intake	6	5	5	3	1	.24	.29	.58

Table 5 Continued

Item	Non-Implanted		Implanted		SE	P-values		
	No Rac	Rac	No Rac	Rac		IMP	RAC	IXR
NDF								
Intake, g	8968	9258	10396	7918	679	.95	.11	.05
Intake, $g \cdot d^{-1}$	1495	1540	1733	1370	119	.77	.18	.09
Feces, g	4386	4806	5129	4294	344	.73	.53	.07
Feces, $g \cdot d^{-1}$	731	800	855	742	57	.55	.69	.12
Digestibility, %	50	45	49	44	4	.82	.23	.99
NFC								
Digestibility per Intake	3	3	3	3	.33	.86	.92	.14
Lignin								
Intake, g	1072	1025	1276	963	85	.40	.04	.12
Intake, $g \cdot d^{-1}$	179	171	213	165	15	.31	.06	.17
Feces, g	797	824	768	699	93	.40	.82	.60
Feces, $g \cdot d^{-1}$	133	137	128	121	15	.48	.92	.70
Digestibility, %	22	4	31	25	9	.11	.18	.52
Digestibility per Intake, $g \cdot d^{-1}$	12	-11	11	14	9	.16	.25	.13
Crude Fat								
Intake, g	1560	1636	1833	1456	102	.64	.14	.03
Intake, $g \cdot d^{-1}$	260	272	305	253	18	.44	.24	.07
Feces, g	348	353	373	350	30	.71	.77	.63
Feces, $g \cdot d^{-1}$	58	59	62	60	5	.55	.91	.78
Digestibility, %	78	78	80	75	2	.65	.20	.17
Digestibility per Intake, $g \cdot d^{-1}$	30	29	26	31	2	.65	.37	.13

Table 6. Experiment 1. Results for selected mineral intakes and excretion of finishing cattle with or without ractopamine hydrochloride and with or without hormonal implants.

Item	Non-Implanted		Implanted		SE	P-values		
	No Rac	Rac	No Rac	Rac		IMP	RAC	IXR
Ash								
Intake, g	1612	1717	1942	1522	140	.62	.25	.07
Intake, g·d^{-1}	269	286	324	264	24	.48	.36	.11
Feces, g	1066	1449	1289	1242	121	.94	.16	.08
Feces, g·d^{-1}	178	241	215	215	20	.78	.11	.11
Digestibility, %	32	14	32	16	6	.87	.01	.80
Digestibility per Intake, g·d^{-1}	12	4	9	5	2	.72	.01	.39
Ca								
Intake, g	149	166	193	146	15	.43	.32	.05
Intake, g·d^{-1}	25	28	32	25	3	.33	.43	.08
Feces, g	165	203	183	160	15	.39	.61	.05
Feces, g·d^{-1}	27	34	31	28	3	.50	.48	.07
Urine, g	2.34	2.17	2.97	3.12	1.04	.44	.99	.88
Urine, g·d^{-1}	.39	.36	.50	.53	.17	.43	1	.86
Digestibility, %	-15	-28	2	-16	11	.18	.15	.81
Digestibility per Intake, g·d^{-1}	-82	-132	-2	-117	69	.49	.23	.63
P								
Intake, g	109	116	130	100	8	.75	.15	.03
Intake, g·d^{-1}	18	19	22	17	1	.58	.24	.06
Feces, g	70	108	87	82	10	.66	.11	.05
Feces, g·d^{-1}	12	18	15	14	2	.78	.08	.06
Urine, g	35	24	19	20	9	.27	.53	.50
Urine, g·d^{-1}	5.76	3.93	3.21	3.37	1.44	.28	.55	.48
Digestibility, %	35	5	31	16	9	.69	.02	.37
Digestibility per Intake, g·d^{-1}	198	11	133	83	49	.94	.02	.17
Balance, g·d^{-1}	.72	-2.65	3.97	-0.31	1.87	.14	.05	.80
Balance per	5	-15	16	-6		.33	.05	.92
Intake								
Balance per kg Metabolic BW	.006	-.023	.035	-.003	.017	.15	.05	.80
Balance per Digested, %	10	84	-9	143	150	.89	.44	.79
K								
Intake, g	395	410	468	365	32	.65	.17	.07
Intake, g·d^{-1}	66	68	78	63	5	.52	.25	.12
Feces, g	59	64	77	77	11	.16	.83	.78
Feces, g·d^{-1}	10	11	13	13	2	.13	.77	.84

Table 6 Continued. Experiment 1. Results for selected mineral intakes and excretion of finishing cattle with or without ractopamine hydrochloride and with or without hormonal implants.

Item	Non-Implanted		Implanted		SE	P-values		
	No Rac	Rac	No Rac	Rac		IMP	RAC	IXR
K								
Urine, g	295	345	279	220	43	.10	.91	.20
Urine, $g \cdot d^{-1}$	49	57	47	38	7	.12	.98	.23
Digestibility, %	85	84	83	80	2	.13	.29	.62
Digestibility per Intake, $g \cdot d^{-1}$	137	129	112	138	15	.56	.53	.25
Balance, $g \cdot d^{-1}$	6.82	.15	18.57	12.03	8.31	.16	.42	.99
Balance per Intake	9	-3	19	19	11	.16	.57	.58
Balance per kg Metabolic BW	.06	-.001	.16	.10	.07	.17	.39	.97
Balance per Digested, %	10	-4	22	22	14	.16	.60	.59

Table 7. Experiment 1. Results for carcass performance parameters of finishing cattle with or without ractopamine hydrochloride and with or without hormonal implants

	No Implant		Implant			P-values		
Item	No Rac	Rac	No Rac	Rac	SE	IMP	RAC	IXR
Marbling numerical	465	434	460	476	28	.49	.78	.38
Yield grade	3.47	3.51	3.65	3.61	.08	.07	.98	.64
Fat Inches	.59	.60	.66	.65	.03	.07	.98	.64
KPH	2	2	2	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
Rib eye area	11	11	11	12	.37	.36	.12	.51
Quality Numerical	421	405	414	426	12	.53	.87	.26
Calculated Yield Grade	4	3	4	4	.14	.15	.17	.42
Ending BW, kg	524	533	553	549	8	.01	.76	.38
Metabolic BW, kg	111	112	115	115	1	.01	.65	.47

[1] No differences detected

Table 8. Experiment 2. Nutrient intake, excretion and precipitation runoff of growing cross and pure bred beef bulls with fecal mounding lengthwise or traditionally rounded in feedlots pens

Item	Long Mound	Normal Mound	SE	P-value
DM				
Intake, g·pen^{-1} Total	30093705	30023449	1070284	.97
Intake, g·bull^{-1}·d^{-1}	11738	11778	323	.93
Feces, g·pen^{-1} Total	12113090	20827533	1378998	.01
Feces, g· bull $^{-1}$·d^{-1}	4703	8175	512	.01
Runoff, g·pen^{-1} Total	57251	38218	7116	.13
Runoff, g· bull $^{-1}$·d^{-1}	23	15	3	.16
Runoff /Intake, g	.19	.13	.03	.19
Feces /Intake, g	40	69	3	<0.01
Digestibility	60	31	3	.003
Digestibility/Intake	.51	.26	.04	0.01
N				
Intake, g·pen^{-1} Total	588392	587018	20926	.97
Intake, g· bull $^{-1}$ Total	18590	18653	512	.93
Intake, g· bull $^{-1}$·d^{-1}	230	230	6	.93
Feces, g·pen^{-1} Total	271557	441044	28359	.01
Feces, g·bull^{-1} Total	8548	14022	852	.01
Feces, g·bull^{-1}·d^{-1}	106	173	11	.01
Runoff, g·pen^{-1} Total	1704	1249	181	.15
Runoff, g·bull^{-1} Total	54	39	6	.19
Runoff, g·bull^{-1}·d^{-1}	.67	.49	.08	.19
Runoff/Intake, g	.29	.21	.04	.22
Feces /Intake, g	46	75	3	<0.01
Digestibility	54	25	3	.004
Digestibility/Intake	24	11	1.8	0.01
Soil Retention, g Total	4.98	5.02	.17	.86
P				
Intake, g·pen^{-1} Total	64836	64684	2306	.97
Intake, g·bull^{-1}·d^{-1}	25	25	.70	.93
Feces, g·pen^{-1} Total	46569	79658	7437	.03
Feces, g·bull^{-1}·d^{-1}	18	31	3	.03
Runoff, g·pen^{-1} Total	227	222	9	.72
Runoff, g·bull^{-1}·d^{-1}	.09	.09	.004	.80
Runoff /Intake, g	.35	.34	.02	.79
Feces/Intake, g	72	123	10	.02
Digestibility	28	-23	10	.02
Digestibility/Intake	112	-88	38	.02

Table 9. Experiment 2. Physical parameters and calculated retentions of nutrients in growing cross and pure bred beef bulls with fecal mounding lengthwise or traditionally rounded in feedlots pens

	Long Mound	Normal Mound	SE	P-value
Pen Bull Count	31.6	31.5	.63	.88
Start Weight, kg	11732	12172	501.4	.57
Final Weight, kg	16572	17168	714	.59
Metabolic BW, kg	1297	1333	41	.57
ADG, kg	1.77	1.66	.04	.10
Calculated Nutrient Retention				
Retained Energy, $g \cdot bull^{-1} \cdot d^{-1}$	11	12	1	.53
Phosphorus Retained, $g \cdot bull^{-1} \cdot d^{-1}$	155	147	5	.39
Average Total Protein Retained, kg	154.5	147.3	5.35	.39
N Retained, $g \cdot bull^{-1} \cdot d^{-1}$	25	24	.86	.39

Table 10. Experiment 2. Calculated excretion and feeding period N volatilization

Daily N Excretion Per Bull				
	Control Mounding	Long Mounding	SEM	P-Value
Number of Pens	3	3		
N Intake, g·bull^{-1}·d^{-1}	230	230	6	.93
N Retention, g·bull^{-1}·d^{-1}	24	25	.86	.39
N Excreted, g·bull^{-1}·d^{-1}	207	205	7	.86
Total Calculated Feeding Period Emissions Per Bull (g)				
	Control Mounding	Long Mounding	SEM	P-Value
N Excreted, g·bull^{-1}	16744	16587	576	.86
Manure N, g·bull^{-1}	14022	8548	852	.01
Soil Average .03% N Retained, g·bull^{-1}	4.98	5.02	.17	.86
N Runoff, g·bull^{-1}	40	54	6.48	.19
Total N Volatilized, g·bull^{-1}	2677	7981	543	<0.01
Total N Excretion Volatilized, %	16	48	3.52	<0.01
N Volatilized per Intake, %	14	43	3	<0.01