EFFECTS OF RACTOPAMINE HYDROCHLORIDE ARE NOT CONFINED TO MAMMALIAN TISSUE: EVIDENCE FOR DIRECT EFFECTS OF RACTOPAMINE HYDROCHLORIDE SUPPLEMENTATION ON FERMENTATION BY RUMINAL MICROORGANISMS

by

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B.S., California State University Chico, 2002
M. S., Kansas State University, 2008

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Department of Animal Sciences and Industry
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Abstract

Beta-adrenergic agonists, which are synthetic catecholamines, increase rate of gain, improve feed efficiency, and decrease carcass fat when fed to cattle before slaughter. However, little attention has been given to the potential effects of beta-adrenergic agonists on the rumen ecosystem. Natural catecholamines, such as norepinephrine, epinephrine, and dopamine, have been observed to stimulate bacterial growth. The objectives of this research were to determine if ractopamine hydrochloride (RAC) a synthetic catecholamine has direct effects on growth and fermentation products of ruminal bacteria, and to determine the effects of protein source on ruminal fermentation and proteolysis when cattle are fed RAC. The effects of varying concentrations of RAC on ruminal fermentation were evaluated *in vitro*. Ractopamine hydrochloride had a quadratic effect on in vitro gas production ($P < 0.05$). Total VFA production was not changed with RAC ($P > 0.50$). Different concentrations of RAC were evaluated *in vitro* with different nitrogen sources to determine effects of nitrogen degradability on response to RAC. There was an interaction between RAC and nitrogen substrate ($P < 0.01$), with more degradable forms of nitrogen eliciting greater changes in *in vitro* dry matter disappearance (IVDMD) with RAC supplementation. Significant main effects also were detected for RAC, substrate, and hour ($P < 0.001$). *In vitro* analysis of proteolysis revealed that RAC lowered ammonia and amino acid concentrations ($P < 0.001$). *In vivo* ruminal ammonia concentrations also were lower when RAC was fed in combination with dry-rolled corn, but not when fed in conjunction with steam-flaked corn (grain processing × RAC, $P < 0.01$). Addition of RAC, steam-flaked corn, and distiller's grains (DG) all resulted in lower ruminal ammonia concentrations ($P < 0.01$). Amino acid concentrations were decreased when RAC was added to diets with DG but were unchanged in diets without added DG (DG × RAC, $P < 0.05$). Results from these studies suggest that RAC affects fermentation by ruminal microflora. However, there were no differences in growth or fermentative end products of pure bacterial cultures with the addition of RAC ($P > 0.10$). Overall beta-adrenergic agonists alter ruminal fermentation, which could have important implications for diet formulation.

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Dedication

I would like to dedicate this dissertation to my grandfather, James O'Leary. He encouraged me to attend Kansas State University and I am forever grateful for his love.

CHAPTER 1 - Literature Review: Effect of Catecholamines on Gut Microflora and Potential for Beta-adrenergic Agonists to Impact Ruminal Fermentation

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Abstract

Catecholamines are produced by chromaffin cells of the adrenal medulla and adrenergic and dopaminergic neurons from tyrosine. Catecholamines regulate many vital physiological and metabolic responses because of the location of receptors. The impact of catecholamines is not limited to mammals; direct effects of natural catecholamines on bacteria have been researched extensively to understand the potential impact of these compounds on bacterial infections in humans. Catecholamines have increased the growth of bacteria, virulence-associated factors, and adhesins and increased biofilm formation. Beta-adrenergic agonists are similar in structure and pharmacological properties to natural catecholamines. Beta-adrenergic agonists enhance performance of finishing cattle during the final days before harvest. Responses to β-adrenergic agonists include increased average daily gain, improved feed efficiency, and increased carcass lean. These responses have been observed as a direct effect to the animal; however, a review of the literature suggests that the response to β-adrenergic agonists also could be mediated by a direct or indirect effect on ruminal microorganisms. Ractopamine hydrochloride increased fermentation *in vitro*, particularly with increased amounts of degradable intake protein. Inclusion of ractopamine hydrochloride *in vivo* decreased ruminal concentrations of ammonia and amino acid. The rumen is host to a large population of diverse microorganisms, and a direct impact of a synthetic catecholamine on the microbial population could potentially alter fermentation and the ruminant performance. Reviewing literature on catecholamines and their direct impact on microorganisms could lead to improved decisions regarding dietary supplementation of β-adrenergic agonists, thereby increasing the growth performance response in ruminants.

Introduction

Catecholamines, which are naturally present in ruminants and other mammals, have a direct effect on the animal's major organs, gut, and other tissues. However, the impact of catecholamines is not limited to animals; bacteria have been observed to be directly influenced by the presence of catecholamines. Beta-adrenergic agonists, which are synthetic catecholamines, currently are used to enhance cattle performance before harvest. Beta-adrenergic agonists are orally active and have been noted for their ability to repartition energy from adipose tissue to lean tissue. Orally administered products enter the rumen and can potentially interact with ruminal microorganisms, thereby influencing fermentation. However, there is limited research regarding the effects of β-adrenergic agonists on the rumen and its microorganisms. By better understanding how this compound affects ruminal fermentation, nutritionists can potentially enhance its use in livestock diets.

Catecholamines

Natural catecholamines, which include epinephrine, norepinephrine, and dopamine, contain a catechol nucleus formed by a benzene ring with adjacent hydroxyl groups and an amine group [1]. Epinephrine, norepinephrine, and dopamine are synthesized by chromaffin cells of the adrenal medulla. Norepinephrine and dopamine are synthesized by adrenergic and dopaminergic neurons. Catecholamines are synthesized from tyrosine, which is obtained from dietary sources or synthesized from phenylalanine in the liver and other tissues. Catecholamines are stored in secretory granules in the adrenomedullay cells. Norepinephrine and epinephrine secreted by the adrenomedullay cells bind to receptors on adipose, cardiovascular, hepatic, muscular, and pancreatic tissues to regulate metabolic processes and also to nerve cell receptors to influence neurogenic responses [1]. The primary source of epinephrine is the adrenal medulla, whereas norepinephrine is synthesized in the adrenal medulla and by adrenergic neurons distributed throughout the body [1]. Catecholamine receptors are classified as α- and β-adrenergic receptors. These receptors have been further classified as α_1, α_2, β_1, and β_2 on the basis of the physiological response they mediate in animals and the identification of chemical

antagonists [1]. These receptors are found throughout the body, but the presence of β-adrenergic receptors in the gut is of particular interest for this review paper.

Beta-adrenergic Agonists

Beta-adrenergic agonists are phenethanolamine compounds that physically and pharmacologically resemble natural catecholamines, such as norepinephrine and epinephrine [2]. Critical activities affected by natural catecholamines and possibly by synthetic catecholamines, such as ractopamine hydrochloride, include inotropic and chronotropic effects on heart contractions, vasoconstriction and dilation of blood vessels, contractions of and secretions by the gastrointestinal tract, secretion of insulin from the pancreas, and stimulation of lipolysis, glycogenolysis, and glycolysis [3]. Because of their involvement in heart contractions and vasoconstriction and dilation of blood vessels, β-adrenergic agonists have been a research focus in human health to relieve asthma and alter cardiovascular function, leading to the development of synthetic compounds that bind to β-adrenergic receptors. Beta-adrenergic receptors are located in the plasma membrane of almost all types of mammalian cells and are stimulated physiologically by catecholamines [4]. Beta-adrenergic receptors consist of seven membrane-spanning regions with three internal and three external loops. The β-adrenergic agonist binds to the receptor located in the center of the seven transmembrane domains, forming an agonist-receptor complex that activates the Gs protein. The α-subunit of the Gs protein then activates adenylate cyclase, and this enzyme, along with adenosine triphosphate (ATP), creates cyclic adenosine monophosphate (cAMP). Cyclic adenosine monophosphate binds to the regulatory subunit of protein kinase A, causing its activation and leading to phosphorylation of intracellular proteins. The phosphorylation activates some intracellular proteins and inactivates others, leading to increased muscle accretion and decreased adipose deposition [4]. Effects of the binding of β-adrenergic agonist receptors include stimulation of glycogen phosphorylase and inhibition of glycogen synthesis, which result in production of glucose from glycogen stores and stimulation of lipolysis, causing the release of free fatty acids from adipose tissue [5]. Beta-adrenergic receptors are categorized into three subtypes (β1, β2, and β3), but few compounds bind exclusively to one type of receptor [5]. Responses to β-adrenergic agonists seem to be greater in ruminants than in single-stomached animals [5]. Mersmann [4] suggested that species that had been intensively selected for growth may have less response to β-adrenergic agonists

because they are closer to their maximal growth potential. Also, β-adrenergic agonists may not be as effective at targeting specific tissues in some species compared with others. Bell *et al.* [2] found that maximum response to β-adrenergic agonists is not achieved when they are used in conjunction with diets that are inadequate in total protein or amino acids. There is less response to β-adrenergic agonists in young, rapidly growing animals, in which muscle growth is rapid and lipid accretion is low. Response to β-adrenergic agonists in adipose tissue appears to be driven by the tendency of finishing animals to deposit carcass fat at a higher rate than lean tissue [5].

Beta-adrenergic agonists are fed during the last 20 to 42 days before harvest to increase muscle accretion and reduce fat deposition [4]. Researchers have observed that β-adrenergic agonists improve average daily gain, efficiency, and carcass weight in cattle [6-8]. The two β-adrenergic agonist compounds approved by the U.S. Food and Drug Administration for use in cattle are ractopamine hydrochloride (Optaflexx®, Elanco Animal Health, Greenfield, IN) and zilpaterol-HCl (Zilmax®, Intervet Inc., Millsboro, DE). Ractopamine-HCl and zilpaterol-HCl have been noted to increase rate of gain, improve feed efficiency, and decrease carcass fat when fed during the final 28 to 42 days [6, 9] and final 20 to 40 days [8, 10], respectively, before slaughter.

Catecholamines and Bacteria

In the 1920s, the first purified catecholamine, adrenaline, was used to treat a variety of illnesses. However, not long after it first use, patients with no prior bacterial infections began to develop bacterial sepsis [11]. The development of bacterial infection in these patients was linked to contaminated glass syringes, but it was noted that the dose of *Clostridium perfringens* needed to cause infection was reduced more than four logs in the presence of therapeutic levels of adrenaline [11]. Reports dating as far back as the 1930s noted increased bacterial proliferation following adrenaline administration. The change in bacterial growth centered solely on the impact of adrenaline on the host, described as changes in host immunity or vasoconstriction that could facilitate the proliferation of bacteria. Reports of the influence of stress on bacterial infections also have pointed to the ability of catecholamines to suppress the immune system as the mode of action for increased bacterial growth. However, over the past two decades, endocrinologists have researched the direct effects of catecholamines on bacterial growth [12]. This novel research has revealed that catecholamines including norepinephrine, epinephrine,

dopamine, and dopa directly increase growth of Gram-negative bacteria, including *Escherichia coli*, *Yersinia enterocolitica*, and *Pseudomonas aeruginosa*. Freestone *et al*. [13] evaluated a greater range of bacterial species and observed that the growth response to catecholamines was widespread among Gram-negative and Gram-positive bacteria. However, the increase in growth depended on the type and concentration of catecholamine to which the bacteria were exposed. O'Donnell *et al*. [14] observed that *in vitro* bacterial growth response to norepinephrine was dependent on the inoculum concentration of the bacteria [14]. Freestone *et al*. [15] observed that norepinephrine and dopamine were more potent at inducing growth of *E. coli* O157:H7 and *Salmonella enterica*, whereas epinephrine was an antagonist of norepinephrine and dopamine growth responsiveness in *Yersinia enterocolitica*. Freestone *et al* [15] speculated the norepinephrine and dopamine were more stimulatory as a result of being released from norepinephrine- and dopamine-containing neurons in the enteric nervous system. De Champlain [16] administered 6-hydroxydopamine (6-OHDA) to rats, resulting in an increased concentration of noradrenaline, and found that the neurophysiologic conditions of the host led to major shifts within microflora in the gastrointestinal tract. Twenty-four hours after administering 6-OHDA to rats, De Champlain [16] noted a three to five log increase of Gram-negative bacteria in the gut. Other researchers noted similar stimulatory effects of naturally occurring catecholamines on growth of Gram-negative bacteria [17, 18]. Belay and Sonnenfeld [18] evaluated the effects of catecholamines on *in vitro* growth of pathogenic bacteria and noted that norepinephrine and dopamine increased growth to the greatest extent in *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Epinephrine and isoproterenol also increased growth of these bacteria, but to a lesser degree. Growth of *Staphylococcus aureus* increased in the presence of norepinephrine, but to a lesser extent than Gram-negative bacteria. Belay and Sonnenfeld [18] concluded that growth was enhanced by the addition of catecholamines but was dependent on the catecholamine and the bacterial species. In contrast, Belay *et al*. [19] tested other pathogenic bacterial species, including *Porphyromonas gingivalis*, *Bacteriodes fragilis*, *Shigella boydii*, *Shigella sonnie*, *Enterobacter sp.*, and *Salmonella cholerasuis*, and found no enhanced growth with addition of catecholamines. These results further support the conclusion that catecholamines' influence on bacterial growth is dependent on bacterial species. Catecholamines also are known for their ability to influence populations of oral bacteria [20]. Roberts *et al*. [20] studied bacteria common to the oral cavity of humans and found that supplementing bacteria with natural catecholamines produced by the

human body resulted in increased growth in more than half the bacteria tested. They concluded that natural catecholamines have a direct effect on oral bacteria, again suggesting that response varies among bacterial species. Besides inducing bacterial growth, catecholamines have been observed to increase production of virulence-associated factors such as Shiga-like toxins [21], increase expression of K99 pilus adhesions and virulence-related factors [22], and increase biofilm formation [23].

Researchers also have observed the presence of mammalian hormones in microbes and speculated the role of catecholamines in microbial cells involves intercellular communication [24, 25]. Lyte and Ernest [12] stated the mechanism for Gram-negative bacterial growth induced by catecholamines was non-nutritional and possibly receptor mediated. Kinney *et al*. [17] observed that catecholamines function as siderophores by chelating iron, which gives an advantage to bacteria that are able to recognize and use siderophores. This is in agreement with observations of Freestone *et al*. [26], who used *E. coli* strains and observed that a functional siderophore system is a key mechanism by which bacteria assimilate iron made available by the interaction of catecholamines with the host iron-binding protein. O'Donnell *et al* [14] suggested that norepinephrine could act as an exogenous siderophore to liberate iron from iron-binding proteins. Freestone *et al*. [27] observed that norepinephrine stimulated bacterial growth in a nutrient-poor medium when transferrin or lactoferrin were present. Norepinephrine was able to break the bonds between iron and transferrin, or between iron and lactoferrin, providing the bacteria an available source of iron.

Catecholamines also might serve as a type of environmental cue that microorganisms use to sense their surroundings and initiate cellular processes, including growth [11]. Lyte *et al*. [28] observed that catecholamine-induced bacterial growth is the result of noradrenaline-induced production of an autoinducer of growth, which eliminates the need for any additional catecholamine exposure to further increase proliferation. Freestone *et al*. [26] evaluated specific catecholamine receptor agonists to determine if the increase in bacterial growth was a result of catecholamine binding a bacterial receptor. Only α-adrenergic antagonists were capable of blocking norepinephrine- and epinephrine-induced growth, and dopamine-induced growth was blocked by dopaminergic antagonists. Freestone *et al*. [26] hypothesized that the adrenergic antagonist could be inhibiting catecholamine uptake by the bacteria.

Beta-adrenergic Agonists and Bacteria

Beta-adrenergic agonist compounds share similar pharmacological and structural properties with the endogenous catecholamines norepinephrine and epinephrine [3]. Because many important physiological and metabolic responses are regulated by catecholamines, most mammalian tissues and organs contain receptors for these compounds. The binding of natural or synthetic catecholamines to β-adrenergic receptors promotes similar effects in the animal, including increased lipolysis in adipose tissue and increased glycogenolysis and gluconeogenesis in the liver [1].

Naturally occurring catecholamines, such as epinephrine and norepinephrine, affect gut motility and secretory responses in mammals [29-31]. This can directly affect the amount of time feed remains in the rumen, which influences feed digestion by ruminal microorganisms. Change in the passage rate of the digesta from the rumen can alter the population of microorganisms in the rumen. As passage rate increases, microorganisms that grow at slower rates will be subject to washout from the rumen. Researchers have observed that β-adrenergic agonists reduce the frequency and intensity of ruminal contractions [29,30,32]. Ruminal contractions are a vital part of digestion in the rumen; they mix ruminal digesta and aid in digestion of the diet by ruminal microorganisms. Ruminal contractions also are the mechanism for eructation of ruminal gases; inhibition of eructation leads to digestive bloat, resulting in mortality. Montgomery *et al*. [33] observed increased mortality in steers fed zilpaterol-HCl compared with steers fed no zilpaterol ($P < 0.01$); six mortalities among 1,878 steers fed zilpaterol were due to digestive bloat compared with one among steers not fed zilpaterol. Research also suggests that β-adrenergic agonists increase absorption in the digestive tract [31, 34, 35]. Aschenbach *et al*. [35] found in sheep that β_2 adrenergic agonists increased ruminal glucose uptake via sodium-glucose-linked transporter. Glucose typically is found at low concentrations in the rumen; however, concentrations increase after cattle consume large amounts of rapidly fermented carbohydrates, predisposing cattle to acidosis. Increasing the removal of glucose from the rumen can reduce acidosis. Aschenbach *et al*. [35] did not observe the same increase with dobutamine, a β_1 adrenergic agonist.

To this author's knowledge, the only research that has examined the impact of synthetic catecholamines on gut microflora of livestock was conducted by Edrington *et al*. [36,37], Poletto *et al*. [38], and Walker and Drouillard [39]. Edrington *et al*. [36,37] examined the effects of

ractopamine hydrochloride on *E. coli* O157:H7 and *Salmonella* in experimentally inoculated sheep and swine [36] and feedlot cattle [37]. Edrington *et al*. [36] observed that sheep administered ractopamine hydrochloride before and after oral inoculation of *E. coli* O157:H7 increased shedding of the pathogen ($P < 0.01$) and tended to have increased cecal populations ($P = 0.08$) of the pathogen. Edrington *et al*. [36] found a different result when examining the effect of ractopamine-HCl in pigs experimentally inoculated with *Salmonella*. Pigs fed ractopamine-HCl had decreased fecal shedding ($P < 0.05$) and fewer liver samples that tested positive for the challenge strain of *Salmonella* ($P = 0.05$) than pigs not fed ractopamine hydrochloride. Edrington *et al*. [37] found that cattle administered ractopamine shed fewer *E. coli* O157:H7 ($P = 0.05$) but tended to shed more *Salmonella* ($P = 0.08$) than cattle not administered ractopamine hydrochloride. Poletto *et al*. [38] found that pigs fed ractopamine hydrochloride for 4 weeks shed less *Enterobacteriaceae* at slaughter than control pigs ($P < 0.05$). Although researchers have demonstrated that natural catecholamines increase growth of *E. coli* O157 [28, 40, 15], more research needs to be conducted to determine the potential impact of β-adrenergic agonists on pathogenic bacteria.

Walker and Drouillard [39] observed a quadratic effect on *in vitro* gas production with the addition of ractopamine hydrochloride to buffered ruminal fluid ($P < 0.05$; 177, 181, 185, 190, and 170 mL water displaced by gas for 0, 0.226, 2.26, 22.6, and 226.0 mg ractopamine hydrochloride /L, respectively). However total volatile fatty acids (VFA) production was not changed ($P > 0.50$). Walker and Drouillard [39] also evaluated the impact of ractopamine hydrochloride on *in vitro* dry matter disappearance with isonitrogenous combinations of corn and soybean meal; corn and urea; or corn, soybean meal, and urea as substrates. There was an increase in *in vitro* dry matter digestibility with the addition of ractopamine hydrochloride ($P < 0.001$), and changes in dry matter disappearance ($P < 0.01$) were more pronounced when ractopamine was used in conjunction with more degradable forms of nitrogen (i.e., urea). These results suggest ractopamine hydrochloride affects ruminal microorganisms, potentially altering nitrogen requirements or proteolytic activity and degradation or dietary nitrogen sources. Walker and Drouillard [39] evaluated the direct impact of ractopamine hydrochloride on proteolysis *in vitro* and observed lower concentrations of ammonia and amino acids when ractopamine hydrochloride was added to fermentation tubes ($P < 0.001$). Walker and Drouillard (unpublished data) found a similar decrease in concentrations of ammonia and amino acids when salbutamol

was added to fermentation tubes ($P < 0.01$). Ractopamine hydrochloride lowered ruminal ammonia and amino acid concentrations *in vivo*, but the response was dependent on the diet [39]. This could explain results of Walker [41] and Beermann [42], in which β-adrenergic agonists elicited a greater response in ruminants fed protein sources that were more readily degraded by ruminal microbes.

Ruminal Bacteria

The ecosystem of the rumen is diverse, and bacteria play the dominant role in ruminal fermentation. Ruminal bacterial numbers have been reported to be 10^{10} cells per gram of contents [43]. Ruminal bacteria can be divided into categories based on the digestive function performed in the rumen: amylolytic, proteolytic, fibrolytic, lipolytic, etc. Bacterial species in the rumen that are responsible for normal fermentation of starch, lactate, and protein as well as biohydrogenation of fatty acids are mostly Gram negative.

Ruminal bacterial species are interdependent. Microorganisms of one species rely on other species to produce substrates essential for their survival. This is known as cross-feeding and is an important feature of the ruminal ecosystem. Several end products produced by ruminal microorganisms are not measurable in the rumen because they are rapidly assimilated and used as substrates by other species of ruminal microbes. These products are referred to as intermediates. For example, most of the propionate produced in the rumen is produced from succinate by organism such as *Ruminobacter amylophilus*, which is decarboxylated to propionate by organisms such as *Selenomonas ruminantium* [44,45]. Methanogens use hydrogen and carbon dioxide produced by other microorganisms to generate methane as an end product. This benefits the methanogens and removes hydrogen from the rumen, thus ensuring survival of ruminal microorganisms. The ability of microorganisms to interact in the rumen leads to improved digestion of complex feeds [46]. An example of interdependence is digestion of plant cell wall material containing pectin, hemicellulose, cellulose, protein, and lignin in which the physical arrangement can hinder microbial access to the cellular components. The ability of one microbial species to degrade a physical barrier that otherwise impeded another microbe enables more complete digestion [47]. Another example of interdependence occurs between saccharolytic microbes and cellulolytic and amylolytic species; enzymes secreted by the cellulolytic and

amylolytic species are nutrients for the saccharolytic species, which, in turn, form essential nutrients for the former species [46].

Ruminal bacteria are the main starch-fermenting microorganisms in the rumen [47]. Amylolytic and dextrinolytic microbial species vary the greatest in number because of the variation in starch content and solubility of diets [46], and breakdown of starch begins with bacterial attachment to the feed particle. The major starch-fermenting bacteria in the rumen are Gram negative and include *Ruminobacter amylophilus* and *Selenomonas ruminantium*. Kotarski *et al*. [48] identified 15 strains of amylolytic bacteria and characterized eight amylolytic enzymes. Not all bacteria were equipped with the complete range of enzymes; thus, maximal breakdown of starch to glucose requires coordination among bacteria species. Cotta [49] found the coculture of *Streptoccocus bovis*, *Butyrivibrio fibrisolvens*, *Bacteriodes ruminicola*, and *Selenomonas ruminatium* resulted in the greatest bacterial growth rates and complete breakdown of starch compared to pure cultures of these organisms.

Protein degradation in the rumen is initiated by attachment of microorganisms to feed particles, after which, cell-bound microbial proteases are activated [50]. An estimated 70 to 80% of ruminal microorganisms are attached to feed particles [51], and 30 to 50% of the attached microorganisms have proteolytic activity [52]. In the ruminal system, there is no specific microorganism that occupies the protein fermentation niche as many ruminal microorganisms possess proteolytic activity and ferment amino acids or peptides [46]. Russell *et al*. [53] found *Streptococcus bovis* to be very proteolytic. Fulghum and Moore [54] identified *Butyrivibrio sp*., *Succinivibrio sp*., *Selenomonas ruminantium*, *Borrelia sp*., and *Bacteroides sp*. as major proteolytic bacteria. Atwood *et al*. [55] tested pasture-grazed dairy cows, deer, and sheep for hyper-ammonia producing bacteria and identified *Clostridium aminophilum*, *Clostridium sticklandii*, *Peptostreptococcus anaerobius*, and *Fusobacterium necrophorum* as major hyper-ammonia producing bacteria present in the rumen. Scheifinger *et al*. [56] found that ruminal degradation of dietary amino acids is a result of extensive bacterial interaction. They evaluated amino acid degradation activity in *Megasphaera*, *Streptococcus*, *Selenomonas*, *Butyrivibrio*, and *Eubacterium* and found that each of the bacterial species was capable of degrading amino acids. However, total degradation of amino acids is a result of the combined deaminating activity of bacteria. Wallace [57] found that growth of *Butyrivibrio alactacidigens*, *Butyrivibrio fibrisolvens*, *Selenomonas ruminantium*, and *Streptococcus bovis* in medium containing casein as

the sole nitrogen source was greater when the organisms were cultured together than when each was inoculated singly, in which case growth was poor or nonexistent. In addition to ruminal bacterial species, protozoa are proteolytic and contribute to the breakdown of protein in the rumen. Veira [58] stated there was an increase in protein degradation in faunated ruminants compared with defaunated ruminants. A higher concentration of ruminal ammonia has been observed in faunated animals compared with ciliate-free animals [59-61]. Hino and Russell [62] evaluated the relative contributions of ruminal bacteria and protozoa in degradation of protein in an *in vitro* experiment. They observed that soluble proteins were primarily degraded by bacteria, whereas protozoa contributed to the degradation of insoluble particulate proteins. In the *in vitro* experiment, protozoa were limited in their ability to assimilate peptides or amino acids. Bacteria also were better able to degrade low-molecular-weight particles compared with protozoa. The researchers observed that the combination of bacteria and protozoa had a synergistic effect on increasing ammonia and decreased ($P < 0.05$) non-ammonia, non-protein nitrogen. Forsberg *et al*. [63] observed that protozoal proteolytic activity was primarily due to cysteine proteinases and aspartic proteinases and that aminopeptidase activity was higher than deaminase activity. Protozoa predate ruminal bacteria, engulfing them and releasing free amino acids and ammonia into the rumen [64].

Factors that affect proteolysis in the rumen include solubility of dietary protein, structure of the protein, level of intake by the animal, and particle size of the feedstuff. Sniffen *et al*. [65] fractioned protein contained in ruminant feedstuffs into three categories according to solubility in the rumen: Category A consisted of non-protein nitrogen and was rapidly converted to ammonia. Category B was true protein and was broken into B_1, B_2, and B_3; B_1 was rapidly degraded in the rumen, B_2 was intermediately degraded in the rumen, and B_3 was slowly degraded with a high percentage of B_3 protein escaping the rumen. Category C was bound true protein typically associated with lignin, tannin-protein complexes, and Maillard products and was not degraded in the rumen. Attachment is critical to proteolysis in the rumen; plant proteins often are encased in or associated with carbohydrate, and the structure of these complexes can affect proteolysis by interfering with microbial attachment to protein [66]. Treatments that protect feed proteins from ruminal degradation, such as heat, alter the structure of the feed protein, preventing attachment [66]. As feed intake increases, passage rate increases, which leads to a shorter retention time for digesta in the rumen. As a result, more protein escapes the rumen without being degraded by

ruminal microorganisms. Zinn *et al*. [67] evaluated ruminal degradation of different protein supplements at two different intake levels and observed higher degradation percentages at the lower intake level.

Fibrolytic bacteria are primarily associated with feed particles in the rumen. The major species include *Fibrobacter succinogenes* (Gram negative), *Ruminococcus albus*, (Gram variable), *Ruminococcus flavefaciens* (Gram positive), and *Prevotella ruminicola* (Gram negative) [68]. Fibrolytic bacteria are generally nonproteolytic and require ammonia as a source of nitrogen [69]. One or more branched-chain fatty acids also are required growth factors for fibrolytic bacteria. Fibrolytic bacteria produce several enzymes not produced by the animal that are required to break down cellulose and hemicelluloses in fibrous feed. Among fiber-fermenting bacteria, primary cellulolytic bacteria such as *Ruminococcus albus* and *Ruminococcus flavefaciens* are among the most restrictive ruminal microbes in terms of the niche they occupy [46]. They are restricted to fermenting disaccharides, trisaccharides, and oligosaccharides released during hydrolysis of holocellulose as sources of carbon and energy [46]. Cellulolytic bacteria often rely on other microbes to supply the nutrients they require for survival.

Ruminal bacterial are responsible for biohydrogenation of unsaturated lipids in the rumen. Unsaturated fatty acids are relatively toxic to some ruminal bacteria. Biohydrogenation converts unsaturated fatty acids to saturated fatty acids, which are less toxic. During biohydrogenation, free hydrogen ions are removed from the rumen. Major species involved in biohydrogenation include *Anaerovibrio lipolytica* [70,71], *Butyrivibrio fibrisolvens* [70,71], *Ruminococcus albus* [70], and *Treponema bryantii* [70].

Bacterial species in the ruminal ecosystem are highly interconnected, and their survival depends on other ruminal microorganisms. Because ruminal microflora are interdependent, changes that occur in the rumen that affect one species of microorganism will usually affect the entire ruminal microbial population. Competition for nutrients is vital for survival of ruminal microorganisms, and the ability to accrue limited nutrients such as ammonia, amino acids, and peptides dictates growth of a microbial species. In many instances, faster-growing bacterial species, such as starch fermenters, may have an advantage in using limited resources compared with slower growing organisms, such as fiber-fermenting bacteria.

Microbial Fermentation in the Rumen

Peyer discovered fermentation in the rumen in 1685 [70]. Since Peyer's discovery, the rumen has been recognized as an important microenvironment in the digestive tract of ruminants. Hungate [70] stated that the concentration of microorganisms in the rumen is as great as in any other natural habitat. The rumen is host to an assortment of microorganisms, notably bacteria and protozoa, that enable ruminants to effectively digest forages. Bacteria are the most abundant microorganism in the rumen and exist in a diverse population, but roughly 20 bacterial species dominate the population. These species are influenced by feedstuffs and additives consumed by ruminants. Ruminal bacteria are vital because they produce VFA from feedstuffs that are otherwise indigestible by the animal's digestive enzymes. The VFA are then absorbed as an energy source by the host animal. The microbial biomass produced from fermentation of feedstuffs is a source of protein for the host. Ruminal bacteria are sensitive to oxygen, pH, and nutrient availability. Altering conditions in the rumen can alter the population of microorganisms that are present to digest feedstuffs. Understanding ruminal microorganisms and their mechanisms for digesting feedstuffs has been the focus of ruminant nutrition research [70,72] for decades. Techniques for improving ruminant animal performance have focused on changes that occur in the rumen, and specifically the ruminal microflora, as a result of changes in the animal's diet. Manipulating microorganisms in the rumen is a means of improving fermentation to achieve more complete digestion of feedstuffs.

The rumen is a dynamic environment, and changes to the animal's diet, such as altering digestibility of feedstuffs, the forage-to-concentrate ratio, feed intake, and processing of the feedstuff, all can affect the microbial population [73-75]. The quantity of bacteria adherent to ruminal digesta can be altered by the previously mentioned dietary alterations as well as the presence of feed additives in the diet [76,77]. The impact of changing a component of the diet on ruminal fermentation has been of interest to many researchers. Hussein *et al*. [78] examined the influence of forage level on ruminal bacteria composition in ruminally cannulated beef steers fed corn-based diets with 30% or 70% corn silage (dry matter basis) for *ad libitum* intake and found an increase in organic matter, nitrogen, and amino acids in the mixed ruminal bacteria harvested from steers fed the diet with less forage. Sindt *et al*. [79] examined the impact of grain processing on ruminal fermentation and found that decreasing flake density from 360 or 310 g/L increased microbial efficiency ($P < 0.05$) and tended to increase microbial nitrogen flow to the duodenum ($P < 0.10$). Zinn *et al*. [80] studied the impact of grain processing and dry matter

intake on ruminal fermentation and found that steam-flaking corn increased ($P < 0.05$) ruminal digestion of organic matter and starch. Ruminal pH levels were lower and molar proportions of acetate were higher in steers with greater dry matter intake and for steers fed steam-flaked corn diets compared with steers fed dry-rolled corn ($P < 0.05$). Cooper *et al*. [81] examined the impact of grain processing on ruminal fermentation in six ruminally and duodenally cannulated steers fed high-moisture corn, steam-flaked corn, or dry-rolled corn and found that dry matter and organic matter intakes were approximately 15% higher for steers fed high-moisture corn than for steers fed dry-rolled corn or steam-flaked corn ($P < 0.05$). True ruminal organic matter digestibilities for steers fed high-moisture corn were 18% and 10% greater than those for steers fed dry-rolled corn and steam-flaked corn, respectively ($P < 0.05$), and ruminal starch digestibilities for steers fed high-moisture corn and steam-flaked corn were approximately 19% greater than those for steers fed dry-rolled corn ($P < 0.05$). Bacterial crude protein flow from the rumen in steers fed high-moisture corn was 29% greater ($P < 0.05$) than that in steers fed steam-flaked corn or dry-rolled corn. Cooper *et al*. [81] suggested that cattle fed high-moisture corn require more degradable intake protein than cattle fed dry-rolled corn or steam-flaked-corn. Calderon-Cortes and Zinn [82] examined the impact of forage particle size on ruminal digestion by feeding ruminally and duodenally cannulated steers sudangrass hay at 8% or 16% of diet dry matter. Increasing the concentration of forage tended to increase ruminal pH and decrease molar proportions of butyrate ($P < 0.10$). Theurer *et al*. [83] examined the impact of grain processing on ruminal digestion in steers fed dry-rolled sorghum or steam-flaked sorghum and found that starch digestion (as a percentage of intake) in the rumen was higher for steers fed steam-flaked sorghum than for steers fed dry-rolled sorghum (82% vs. 67%; $P < 0.05$). Theurer *et al*. [83] also tested the impact of degree of grain processing by feeding steers steam-flaked sorghum and steam-flaked corn flaked to bulk densities of 437 and 283 g/L, respectively. Decreasing flake density of steam-flaked sorghum and steam-flaked corn linearly increased starch digestion (as a percentage of intake) in the rumen ($P < 0.05$).

Any compound fed to ruminants can affect the ruminal microbial population and ruminal fermentation, and many feed components have been evaluated for their ability to do so. Ionophores directly affect Gram-positive bacteria [84], causing a shift in the proportions of VFA with little effect on total acid production [85,86]. Ionophores decrease methane production, proteolysis, and deamination in the rumen [87]. Antibiotics can alter ruminal microbial

population [88] and fermentation [89]. O'Connor *et al*. [90] observed that chlortetracycline, oxytetracycline, and dimetridazole reduced protozoal activity, which possibly alters the ruminal microflora population by reducing predation of bacteria by protozoa. O'Connor *et al*. [90] also examined changes to VFA in the presence of antibiotics and steroids. In general, antibiotics decreased total VFA production and increased the acetate-to-propionate ratio. Penicillin and spiramycin had the largest impact. Melengestrol acetate increased acetate concentration and total VFA, whereas diethylstilbestrol, desoxycorticosterone, hydrocortisone testosterone, methandrostenolone, and prednisolone had little impact. Dietary fat, predominantly unsaturated fatty acids, has been noted to be toxic to ruminal microbes; it decreases ruminal fermentation, particularly fiber digestion, when included at high levels. [91-94].

Protein

When energy or amino acids are limited, synthesis and breakdown of proteins are regulated to maintain cellular and tissue mass that contributes to critical metabolic needs of the animal [95]. Metabolic energy and amino acids are required for the continuous process of protein turnover in the body; these are provided in the diet and represent the primary input cost for meat animal production [96].

Suggested crude protein requirements for finishing cattle range from 12.5% to 13% of diet dry matter [97]. In a recent survey, most consulting feedlot nutritionists recommended a protein level of 13.5% of diet dry matter in finishing rations [98]. Nitrogen sources most commonly supplemented in feedlot rations include grain coproducts, soybean meal, cottonseed meal, and urea [98]. Urea is included in finishing cattle diets at up to 2% of dry matter, but it typically is included at 1.2% of dry matter [98]. Use of non-protein nitrogen by cattle involves the conversion to microbial protein by flora and fauna that colonize the rumen [99]. Studies on the nutritional requirements of ruminal bacteria, both in pure culture [100] and *in vivo* [101,102] have revealed that ammonia is a major nitrogen source for bacterial growth. Most nitrogen used by ruminal microorganisms is in the form of ammonia, and large amounts of nitrogen in feed are converted to ammonia by the microorganisms [99]. Hume *et al*. [103] found that microbial cell yields in the rumen are proportionate to dietary nitrogen. Nitrogen promotes microbial growth to the extent dictated by availability of fermentable carbohydrates [99]. Haskins *et al*. [104] and Bolsen *et al*. [105] found no difference in animal performance between concentrate diets with

soybean meal or urea as the nitrogen source. However, Braman *et al*. [106] found that steers supplemented with soybean meal had improved feed efficiency ($P < 0.05$) compared with steers fed urea as the only supplemental nitrogen source. There was a linear increase in gain and efficiency ($P < 0.05$) with increasing levels of true protein ranging from 10.8% to 18.4% crude protein, but there were no significant changes with equivalent nitrogen levels when nitrogen was provided as urea [106].

Dietary proteins ingested by ruminants are subjected to various rates and extents of digestion by ruminal microorganisms. The primary nitrogen-containing compounds in the ruminant diet are proteins, nucleic acids, and urea. Ruminal microorganism break down dietary protein to peptides, amino acids, or ammonia depending on the enzymes produced by the microbes present and the form of nitrogen they require. Protein available to the animal is a combination of dietary protein that has escaped the rumen and microbial crude protein from microbes that enter the small intestine. In finishing cattle, microbial crude protein output normally exceeds the animal's protein requirement [99].

Ruminal fermentation is a crucial factor to consider when determining the amount of metabolizable protein available to the animal [100]. There is a direct relationship between carbohydrate level in the diet and nitrogen required by ruminal microorganisms. As the amount of energy available to the microbes increases, so does their need for nitrogen. Therefore, the amount of microbial crude protein available to the animal is dependent on energy available in the rumen. Diets fed to finishing cattle typically are high in concentrate, which increases the microorganisms' requirement for nitrogen [99]. Peterson *et al*. [107] observed greater gains when dietary crude protein increased from 9% to 15% in high-concentrate diets. If the microbial requirement for nitrogen is increased by addition of starch and sugar in the diet, adding nitrogen in the form of non-protein nitrogen supports increased microbial synthesis and increases energy fermented in the rumen [99]. A response to additional non-protein nitrogen is indicative of a need for ammonia by microbes. Non-protein nitrogen is best utilized as a nitrogen source by ruminal microorganisms when diets are high in soluble carbohydrates, which is typical of diets fed to finishing cattle [99]. An estimated 80% of ruminal isolates can grow with ammonia as their sole nitrogen source [100]. Non-protein nitrogen is converted rapidly to ammonia by ruminal bacteria. If energy in the diet is not readily digested, ammonia will be absorbed through the rumen wall into the blood, where it will be converted to urea by the liver and potentially

excreted in the urine [99]. High-concentrate diets supply readily available energy, allowing ruminal microorganisms to efficiently use non-protein nitrogen. Peptides supply nitrogen for ruminal microorganisms with a more rapid fermentation rate and spare the cost of synthesizing amino acids. Amino acid uptake by bacteria is more efficient when amino acids are in the form of peptides [99]. Most amino acids are extensively degraded in the rumen to ammonia, carbon dioxide, VFA, and branched-chain fatty acids [46]. Amino acids can be degraded through decarboxylation to yield an amine and carbon dioxide, but this pathway is minor in the rumen and normally is associated with low ruminal pH and acidosis. More commonly, amino acids are degraded through nonoxidative deamination.

Beta-adrenergic Agonists and Protein

Walker and Drouillard's [39] *in vitro* results suggest that proteolysis may be directly affected by β-adrenergic agonists. Walker *et al*. [41] demonstrated that the response to ractopamine hydrochloride supplementation in finishing heifers could be improved by feeding ruminally degraded forms of nitrogen. They found an interaction between ractopamine hydrochloride and nitrogen source, noting that the ratio of degradable intake protein and undegradable intake protein provided to the ruminal microorganisms is important for maximizing response to ractopamine hydrochloride in feedlot heifers. Treatment diets were formulated to be isonitrogenous and had 13.7% crude protein. Expeller soybean meal, solvent soybean meal, and urea were used to achieve three levels of degradable intake protein in the diet (69.3%, 62.7%, and 57.3%). Observations from this experiment indicated that diets containing more ruminally degradable forms of protein yielded a greater response to ractopamine hydrochloride. This is in agreement with observations of Beermann *et al*. [42], who fed lambs diets with soybean meal plus fish meal or soybean meal alone. Lambs were supplemented with 0 or 10 ppm cimaterol for 5 or 10 weeks. Performance improved in lambs fed fish meal or cimaterol; however, there were no additive effects. Cimaterol was less effective at increasing the size of three foreleg muscles when fed in diets containing fish meal than when fed in diets containing only soybean meal. This difference was less pronounced in hindleg muscles.

Conclusion

Beta-adrenergic agonists improve gain and efficiency in ruminants during the final days before harvest. The response is primarily a result of repartitioning nutrients from adipose accretion to lean tissue accretion. However, effects of synthetic catecholamines on ruminal microflora have not been thoroughly researched. The effect of catecholamines on bacteria has been a focus of recent research in human health, and scientists have observed direct effects of catecholamines on bacteria. Natural catecholamines have been shown to increase bacterial growth, virulence factors, biofilm formation, and adhesion. There is potential for β-adrenergic agonists to directly affect ruminal microflora, thus altering digestive function within the ruminal ecosystem. Microbial species in the rumen are integrally connected, and cross-feeding in the rumen is important to microbes. Therefore, the effect of natural or synthetic catecholamines on a microorganism or group of microorganisms in the rumen could affect the entire population of ruminal microbes. The potential of β-adrenergic agonists to alter proteolysis could directly influence the type of protein that is considered ideal for diets fed with ractopamine hydrochloride. Understanding the interaction between catecholamines and microbes in the rumen will enable nutritionists to formulate diets capable of maximizing the response to the compound.

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CHAPTER 2 - Literature Review: Impacts of Ractopamine Hydrochloride on Ruminants

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Abstract

Beta-adrenergic agonists are phenethanolamine compounds that physically and pharmacologically resemble natural catecholamines. These compounds reparation energy from adipose tissue to muscle tissue, by binding to beta-adrenergic agonist receptors located on tissues and organs in the animal. Binding of a beta-adrenergic agonist to the receptor leads to the phosphorylation of key enzymes involved in various biological responses. Beta-adrenergic receptors are classified into three categories β1, β2, and β3; the response from the compound is a direct result of the β-adrenergic receptor type. Response to beta-adrenergic agonists is greater in ruminants than in simple stomached animals, and greater in older animals, where fat deposition is higher, than in younger animals that have a lower rate of fat deposition. Currently, ractopamine hydrochloride and zilpaterol hydrochloride are the two beta-adrenergic agonists approved by the Food and Drug Administration for use in cattle during the final 20 to 42 d before harvest. Ractopamine hydrochloride has been noted to increase rate of gain, improve feed efficiency and increase carcass lean when administered to cattle during the final 28 to 42 d before harvest. The response to ractopamine hydrochloride has varied among research studies, and could be dependent on the diet fed during supplementation, particularly in regards to the amount of rumen degradable protein available. Including non-protein nitrogen in the diet as a source of rapidly degraded nitrogen for the ruminal microorganisms has increased the response to beta-adrenergic agonists. Understanding the interaction between ractopamine hydrochloride and protein source could lead to improved performance response.

Introduction

Ractopamine hydrochloride (RAC), marketed under the commercial trade name Optaflexx (Elanco Animal Heath; Greenfield, IN), is a β-adrenergic agonist used to enhance performance in finishing cattle during the last 28 to 42 days before harvest. Ractopamine hydrochloride increases ADG and improves feed efficiency in swine, sheep, and cattle (Mersmann, 1998; Schroeder et al., 2003a; Schroeder et al., 2003b). Beta-adrenergic agonists (βAA) are orally active and have been noted for their ability to repartition energy from adipose tissue into lean tissue. Walker et al (2006) and Beermann et al (1986) have observed an increased response to βAA when fed in diets with increased levels of ruminally degradable protein source. Understanding how these compounds interact with the level of degradable protein in the diet can potentially enhance their use in livestock.

Beta-adrenergic Agonists

Beta-adrenergic agonists are phenethanolamine compounds that physically and pharmacologically resemble natural catecholamines (Bell et al, 1998). Critical activities impacted by natural catecholamines, and possibly by synthetic catecholamines such as RAC, include inotropic and chronotropic effects on heart contractions, vasoconstriction and dilation of blood vessels, contractions of and secretions by the gastrointestinal tract; secretion of insulin from the pancreas; and stimulation of lipolysis, glycogenolysis, and glycolysis (Beermann, 1993). Because of their involvement in heart contractions and vasoconstriction and dilation of blood vessels, βAA have been a research focus in human health to relieve asthma and alter cardiovascular function, leading to the development of synthetic compounds that bind to the β-adrenergic receptors. Beta-adrenergic receptors are located in the plasma membrane of almost all types of mammalian cells and are stimulated physiologically by catecholamines (Mersmann, 1998). The βAA binds to the receptor forming an agonist-receptor complex which activates the Gs protein. The α-subunit of the Gs protein then activates adenylate cyclase, and this enzyme, along with adenosine triphosphate, creates cyclic adenosine monophosphate. Cyclic adenosine monophosphate binds to the regulatory sub-unit of protein kinase A, causing its activation and leading to the phosphorylation of intracellular proteins. The phosphorylation activates some

intracellular proteins, while inactivating others; leading to increased muscle accretion and decreased adipose deposition (Mersmann, 1998). Effects of the binding of βAA receptors include stimulation of glycogen phosphorylase and inhibition of glycogen synthesis, resulting in production of glucose from glycogen stores; and stimulation of lipolysis, causing the release of free fatty acids from adipose tissue triglyceride stores (NRC, 1994). The receptors become desensitized over time, and response is maximized early in the administration of the βAA (Mersmann, 1998). The pharmacological and physiological responses to βAA are dependent on the tissue type, species of animal, and the particular mixture of β-adrenergic receptors on the cell (Mersmann, 1998). Adipose tissue appears to contain primarily β3-adrenergic receptors (Mersmann, 1996). Skeletal muscle has β1 and β2 adrenergic receptors, with a greater proportion being β2 receptors (Liggett et al., 1988). Mammalian and avian species respond differently to βAA, and in a dose dependent manner (Beermann, 1993). Responses to βAA seem to be greater in ruminants than in single-stomached animals (NRC, 1994). Mersmann (1998) suggested that species that had been intensively selected for growth may have less response to βAA because they are closer to their maximal growth potential. Also, βAA may not be as effective at targeting specific tissues in some species compared to others. Bell et al. (1998) found that maximum response to βAA is not achieved when used in conjunction with diets that are inadequate in total protein or amino acids. The response to βAA is less in young rapidly growing animals, in which muscle growth is rapid and lipid accretion is low. Response to βAA in adipose tissue appears to be driven by the tendency of finishing animals to deposit carcass fat at a higher rate than lean tissue (NRC, 1994).

Beta-adrenergic agonists have direct effects on muscle tissue in cattle (Byrem et al., 1998), binding to β2 and β1 receptors located on the tissue, thereby stimulating muscle accretion (Smith et al., 1989) and possibly decreasing protein degradation (Dawson et al., 1988). Byrem et al. (1998) observed increases in amino acid uptake by muscle tissue in steers treated with cimaterol, which is associated with the increased protein accretion. Wheeler and Koohmaraie (1992) observed a 27.1% lower fractional degradation rate (percentage/day) of skeletal muscle myofibrillar protein in steers fed $L_{644,969}$ for 3 wk ($P < 0.05$). The authors also observed a higher fractional accretion rate (percent/day) of skeletal muscle myofibrillar proteins in steers fed $L_{644,969}$ at wk 1, 3, 5, and 6 of a 6-wk study ($P < 0.05$). Beta-adrenergic agonists increase muscle size through hypertrophy (Beermann et al., 1987; Kim et al., 1987); however, response is

dependent on muscle types, and some muscle types are unaffected. Yang and McElligott (1989) noted that type II fibers (fast-contracting, mixed glycolytic-oxidative) account for the largest proportion of hypertrophy when compared to the type I (slow-contracting, oxidative) fibers. Ractopamine hydrochloride has a direct effect on individual muscle fibers, causing type-I slow-twitch muscle fibers to transition to type-II fast twitch muscle fibers (Aalhus et al., 1992; Gonzalez et al., 2009). Gonzalez et al. (2009) found that RAC changed the fiber type isoform in *longissimus lumborum*, *adductor*, *gracilis*, *vastus lateralis*, and *rectus femoris* ($P < 0.05$), but did not change fiber type isoform in semimembranosus ($P > 0.05$). The vastus lateralis and gracilis presented the greatest switch in fiber type with approximately 21% of type I fibers switching to type-II fibers.

Beta-adrenergic Agonists in Ruminants

Beta-adrenergic agonists are fed the last 20 to 42 d before harvest to increase muscle accretion and reduce fat deposition (Mersmann, 1998). Previous studies have observed that βAA improve ADG, efficiency, and carcass weight in cattle (Schroeder et al., 2003a; Laudert et al., 2004; Avendaño-Reyes et al., 2006). Kim et al. (1989) evaluated the response to βAA in lambs fed 10 ppm of cimaterol. The authors used fifty-two weaned lambs in a comparative slaughter experiment, plus six lambs in a digestibility experiment. The authors observed no differences in dry matter (DM) digestibility in lambs fed cimaterol. However, nitrogen retention was higher in lambs fed cimaterol ($P < 0.01$). This is similar to the observations of Rikhardsson, et al. (1991), where cimaterol again had no effect on digestibility, but increased nitrogen retention in sheep fed 0.5 mg cimaterol/kg $BW^{0.75}$.

Since its approval in 2003 by the Food and Drug administration (FDA) for use in feedlot cattle, RAC has been used by feedlots to improve growth performance. The effective dose in growing ruminants is higher for RAC than for other βAA, such as clenbuterol, cimaterol, or $L_{644,969}$. The reason for this difference is not well understood, but could be related to receptor type, pharmacokinetics, or desensitization of receptors (Beermann, 1993). Ractopamine hydrochloride has been noted to increase rate of gain and improve feed efficiency when fed the final 28 to 42 days before slaughter (Schroeder et al., 2003a; Schroeder et al., 2003b). Schroeder et al. (2003a) evaluated the effects of RAC on growth performance and carcass characteristics in steers, feeding RAC at 0, 100, 200, or 300 $mg \cdot steer^{-1} \cdot d^{-1}$ for the last 28 or 42 d before harvest in

five trials. Ractopamine hydrochloride did not affect DMI ($P = 0.38$); however, ADG and G:F were increased in steers for all levels of RAC compared to control steers ($P < 0.01$). Ractopamine hydrochloride increased HCW, dressing percentage, and LM area ($P < 0.05$). Twelfth rib fat, marbling, quality grade, percent KPH, carcass maturity, muscle color, meat firmness, meat texture, and incidence of dark cutters were not affected by RAC ($P > 0.15$). Calculated yield grade tended to improve in steers fed RAC ($P = 0.058$). Schroeder et al. (2003b) evaluated the effects of RAC on growth performance and carcass traits in heifers. Heifers were fed RAC at 0, 100, 200, or 300 mg·heifer^{-1}·d^{-1} for the last 28 or 42 d of finishing in five trials. Feed intake was not affected by RAC ($P > 0.19$). Average daily gain and G:F were increased for all RAC treatments compared to controls ($P < 0.05$). Hot carcass weight was increased in heifers fed 200 or 300 mg of RAC ($P < 0.01$), but dressing percentage in heifers was not affected ($P > 0.15$). Twelfth rib fat thickness and percent KPH also were not affected by RAC ($P > 0.50$), but RAC increased LM area in heifers fed 300 mg·heifer^{-1}·d^{-1} ($P < 0.05$) when compared to control animals. Calculated yield grade tended to improve in heifers fed 300 mg of RAC ($P = 0.09$), but RAC had no effect on marbling or quality grade ($P > 0.50$). Ractopamine hydrochloride decreased carcass maturity ($P = 0.03$) in heifers fed 300 mg·heifer^{-1}·d^{-1}, and tended to improve muscle color ($P < 0.06$) at all dose levels. Ractopamine hydrochloride did not affect muscle firmness and texture or the incidence of dark cutters ($P > 0.25$). Results of Schroeder et al. (2003a; 2003b) suggest response to RAC is more pronounced in steers than in heifers. Schroeder et al. (2003c) evaluated the effects of RAC on sensory properties of beef. Meat samples were collected post harvest from steers and heifers fed RAC at 0, 100, 200, or 300 mg·head^{-1}·d^{-1} for 28 or 42 d. Ractopamine hydrochloride had no effect on cooking loss or pH of fresh meat ($P > 0.05$). Tenderness, measured as Warner-Bratzler shear force, was not different among cattle fed 0, 100 or 200 mg RAC ($P > 0.45$), but cattle fed 300 mg RAC had increased Warner-Bratzler shear force values compared to cattle fed 0 mg RAC ($P < 0.05$). Sensory attributes were not different in cattle fed 0, 100, or 200 mg·head^{-1}·d^{-1}. However, initial and sustained tenderness were lower in cattle fed 300 mg of RAC ($P < 0.05$). There were no other differences in sensory attributes between cattle fed 0 or 300 mg/d RAC. Gruber et al. (2007) examined the effect of RAC on performance and carcass characteristics in feedlot steers with different biological types. In this experiment researchers utilized British, Continental crossbred, and Brahman cross-bred steers. They observed no interactions between RAC response and breed type for any of the traits

evaluated. Feeding RAC increased ADG ($P < 0.01$) and G:F ($P < 0.01$) without impacting DMI ($P = 0.48$). Dressing percent, adjusted fat thickness, KPH percentage, and yield grade were unaffected by RAC. However, steers fed RAC had heavier carcasses ($P = 0.01$), larger LM areas ($P = 0.046$), and tended to have poorer marbling scores ($P = 0.07$). The researchers concluded that feeding 200 mg/d RAC for 28 d elicited consistent responses in growth and carcass traits among the three diverse biological cattle types tested. Quinn et al. (2008) evaluated the effects of RAC in heifers implanted with Revalor-H (140 mg of trenbolone acetate, 14 mg of estradiol; Intervet Inc., Millsboro, DE) fed a steam-flaked corn diet. The researchers found that ADG and DMI were not different between heifers fed 0 or 200 mg/d RAC ($P > 0.17$); however, heifers fed 200 mg/d of RAC tended to have improved G:F ($P = 0.06$). Furthermore, heifers did not differ in final body weight, carcass characteristics, or meat quality ($P > 0.19$). Quinn et al. (2008) also evaluated different strategies for administrating RAC in order to determine if the duration and dose impacted the response to RAC in non-implanted heifers fed a steam-flaked corn diet. The experiment compared the performance and carcass traits of heifers fed 0 mg/d of RAC to heifers fed 200 mg/d RAC for 28 or 42 d, heifers fed 300 mg/d of ractopamine for 28 d, or an escalating dose consisting of 100 mg/d for 14 d, followed by 200 mg/d for 14 d, and 300 mg/d the final 14 d. There was tendency for increased efficiency of carcass gain in heifers fed RAC at 200 mg/d for 42 d and heifers fed the escalating dose ($P = 0.06$). Feeding RAC at 300 mg/d for 28 d reduced DMI compared to heifers fed RAC at 0 mg/d, 200 mg/d for 28 d, or 200 mg/d for 42 d ($P < 0.05$). The researchers concluded that RAC generally improves efficiency of carcass gain in finishing heifers with minimal effects on carcass characteristics, and that the benefits of RAC are greater in heifers fed RAC for 42 d. Winterholler et al. (2008) evaluated the effects of RAC in yearling steers in a conventional and natural feedlot management system in two experiments. Yearling steers in the conventional system were implanted with Revalor-S (120 mg of trenbolone acetate and 24 mg of estradiol-17β; Intervet Inc., Millsboro, DE) and fed 300 $mg \cdot steer^{-1} \cdot d^{-1}$ of monensin and 90 $mg \cdot steer^{-1} \cdot d^{-1}$ of tylosin. Steers in the conventional and natural management system (natural cattle received no antibiotic or growth promoting implants) were fed a steam-flaked corn based diet with either 0 or 200 mg/d RAC for 37 d in experiment 1 and for 28 d in experiment 2. In experiment 1, there was a management system by RAC interaction for DMI. Steers fed 200 mg/d had lower DMI in the natural system, compared to steers fed 0 or 200 mg/d of RAC in the conventional system ($P = 0.05$). Ractopamine hydrochloride increased average

daily gain during the last 37 d ($P = 0.05$) and increased overall G:F ($P = 0.02$). Ractopamine hydrochloride reduced marbling score ($P = 0.02$), improved yield grade ($P = 0.02$), and tended to increase *longissimus* muscle area ($P = 0.09$). In experiment 2, they observed an interaction between management system and RAC feeding for ADG, and G:F ($P < 0.05$), where feeding RAC improved performance in the conventional management system, but decreased performance in the natural system. The impact of RAC on HCW also was influenced by management system ($P < 0.01$), yielding heavier carcass weights in the conventional system with the addition of RAC, but lighter carcass weights for steers in the natural system. The researchers found no other differences in carcass traits measured in experiment 2. Gonzalez et al. (2009) evaluated the effect of RAC on shelf-life of steaks from the *longissimus lumborum*, *semimembranosus*, *adductor*, *gracilis*, *vastus lateralis*, and *rectus femoris* after being wet aged at 1 ± 2°C for 13 d. Steaks were evaluated subjectively by a trained panel over a 5-d simulated retail display study and objectively using a Hunter-Lab MiniScan XE. The researchers found that RAC had no effect on objective color measurements ($P > 0.05$); however, RAC tended to have a detrimental effect on subjective evaluations of lean color and surface discoloration scores of steaks from the vastus lateralis the last 3 d of the display ($P < 0.10$). Also, RAC deleteriously effected subjective measurements of surface discoloration of the rectus femoris on d 5 ($P < 0.05$).

Protein

Crude protein requirement of finishing cattle is suggested to range from 12.5 to 13% of dry matter (Duff, 2007). In a survey of feedlot nutritionists, most consultants recommended a protein level of 13.5% of diet DM in finishing rations (Vasconcelos and Galyean 2007). The source of nitrogen most commonly included in feedlot rations grain co-products, soybean meal, cottonseed meal, and urea (Vasconcelos and Galyean 2007). Urea is included in finishing cattle diets at up to 2% of DM, but more typically at 1.2% of DM (Vasconcelos and Galyean, 2007). Utilization of non protein nitrogen by cattle involves the conversion to microbial protein by flora and fauna that colonize the rumen (Van Soest, 1994)

With expansion of the ethanol industry, co-products of cereal conversion to ethanol have become a commonly used feedstuff for cattle. Co-products from the dry-milling industry have

replaced other plant based protein and energy feeds. Because of the increased concentration of protein in distiller's grains compared with that of corn, it historically was used primarily as a protein source (Klopfenstein et al., 1978). Distiller's grains are high in protein, and the majority (~70 %), is UIP. Lower ruminal ammonia levels in cattle fed DG have been observed by Santos et al. (1984) and Ham et al. (1994). While distiller's grains are a great protein source for ruminants, including DG in the diet can create a unique challenge of meeting the RDP required by the ruminal microorganisms without increasing N effluent.

Cooper et al. (2002a) individually fed 90 steers 90% concentrate diets consisting of dry-rolled corn (DRC), high-moisture corn (HMC), or steam-flaked corn (SFC) with increasing levels urea to provide increasing dietary DIP values. The results suggested that DIP requirements were 6.3%, 10.1%, and 8.3% for diets based on dry-rolled corn, high-moisture, and steam-flaked corn, respectively.

Conclusion

Ractopamine hydrochloride has been shown to improve gain and efficiency in ruminants during the final 28 to 42 d before harvest. By understanding the effects of protein source on response to RAC will enable nutritionist to formulate diets with the capability of optimizing response to the compound.

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CHAPTER 3 - Effects of Ractopamine Hydrochloride are not Confined to Mammalian Tissue: Evidence for Direct Effects of Ractopamine Hydrochloride Supplementation on Fermentation by Ruminal Microorganisms

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Abstract

Four experiments were conducted to investigate the effects of ractopamine hydrochloride (RAC) on ruminal fermentation and proteolysis. In Exp. 1, in vitro gas and VFA production was measured in flasks incubated with 0, 0.226, 2.26, 22.6, and 226.0 mg RAC/L buffered ruminal fluid. Ractopamine hydrochloride had a quadratic effect on in vitro gas production ($P < 0.05$; 177, 181, 185, 190, and 170 mL for 0, 0.226, 2.26, 22.6, and 226.0 mg, respectively). Total VFA production was not significantly changed with RAC ($P > 0.50$). In Exp. 2, IVDMD was measured with tubes incubated with 0, 0.226, 2.26, or 22.6 mg RAC/L of buffered ruminal fluid with 4 substrate combinations: corn, corn plus soybean meal, corn plus urea, and corn plus soybean meal plus urea. Dry matter disappearance was measured after 2, 4, 6, 8, or 12 h of fermentation. There was an interaction between RAC and substrate ($P < 0.01$), with more degradable forms of nitrogen eliciting greater IVDMD from RAC. Significant main effects also were detected for RAC, substrate, and hour ($P < 0.001$). In Exp. 3, AA and ammonia were measured in tubes treated with 0 or 2.26 mg RAC/L buffered ruminal fluid. Tubes were incubated for 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, or 240 min. There were decreases in ammonia and AA concentrations with RAC ($P < 0.001$). Experiment 4 utilized 16 ruminally fistulated Holstein steers in a 2 × 2 × 2 factorial arrangement of treatments. Factors consisted of grain processing method (steam-flaked or dry-rolled corn); concentration of dried distillers grain (DG) with solubles (0 or 25% DG, DM basis); and concentration of RAC (0 or 200 mg/d). Ruminal ammonia concentrations were less when RAC was fed in combination with dry-rolled corn but not when fed in conjunction with steam-flaked corn (grain processing × RAC, $P < 0.01$). Addition of RAC, steam-flaked corn, and DG all resulted in reduced ruminal ammonia concentrations ($P < 0.01$). Amino acid concentrations were decreased when RAC was added to diets with DG but were unchanged in diets without added DG (DG × RAC, $P < 0.05$). Changes in ruminal ammonia and AA concentration with RAC supplementation are dependent on grain processing and the addition of DG to finishing diets. Results from these studies suggest that RAC affects fermentation by ruminal microflora. Effects of the interactions between RAC and protein source, grain processing, and DG on proteolysis could have important implications when formulating diets for cattle supplemented with RAC.

Key words: cattle, ractopamine hydrochloride, rumen fermentation

Introduction

Ractopamine hydrochloride (RAC) is a β-adrenergic agonist (βAA) similar in structure to natural catecholamines. Beta-adrenergic agonists are fed to cattle the last 20 to 42 d before slaughter to increase muscle accretion and reduce fat deposition (Mersmann, 1998). Previous studies have shown that βAA improve ADG, feed efficiency, and carcass weight in cattle (Schroeder et al., 2003; Laudert et al., 2004; Avendaño-Reyes et al., 2006). However, little attention has been given to the potential effects of βAA on the rumen ecosystem. Naturally occurring catecholamines affect gut motility and secretory response (Ruckenbush, 1983; McIntyre and Thompson, 1992; Leek, 2001). Catecholamines also are known for their ability to affect populations of oral bacteria (Roberts et al., 2002). Lyte and Ernst (1992) observed that the addition of catecholamines dramatically increased gram-negative bacteria in vitro. Many of the ruminal bacteria that are vital to fermentation also are gram negative.

Walker et al. (2006) demonstrated that the response to RAC in finishing heifers could be improved by feeding ruminally degraded forms of nitrogen. They reported an interaction between RAC and nitrogen supply suggestive of a direct effect on microbial populations within the rumen. It is possible that RAC alters proteolysis in the rumen, resulting in less degradable intake protein available to ruminal microflora. The results of Walker et al. (2006) show that the ratio of degradable intake protein to undegradable intake protein provided to the ruminal microorganisms is important for maximizing response to RAC.

Direct effects of RAC on rumen fermentation could have important implications for diet formulation but have not been documented in cattle. The primary objective of this study was to determine the effects of RAC on the fermentative activity of ruminal microorganisms. A second objective was to determine the effect of protein source on ruminal fermentation and proteolysis when cattle are fed RAC.

Materials and Methods

All experiments were conducted with approval of the Kansas State University Institutional Animal Care and Use Committee.

For in vitro experiments, ruminal fluid was obtained on 6 separate days from 2 Holstein steers fitted with ruminal cannula (Bar Diamond Inc., Parma, ID; dorsal sac) and fed a 94%

concentrate diet formulated to contain 14% CP (Table 1). Steers were adapted to the diet no less than 60 d before sampling. Steers were housed in an enclosed barn within individual slatted, concrete-surfaced pens measuring 7 m^2 with individual access to feed and water. Steers were fed on an ad libitum basis daily at 0900 h. Ruminal contents were collected approximately 22 h post feeding from the ruminal cannula of 1 steer on each day the in vitro experiment was conducted. Whole ruminal contents were hand mixed in the rumen before sampling, strained through 4 layers of cheesecloth, and then immediately transported to the laboratory in a sealed, insulated container to maintain temperature and limit exposure to oxygen. Ruminal fluid was buffered with McDougall's artificial saliva (McDougall, 1948) adjusted to a pH of 6.8 with carbon dioxide immediately before mixing. Ractopamine hydrochloride (technical grade; Elanco Animal Health, Greenfield, IN) concentrations of 0, 0.226, 2.26, 22.6, and 226.0 mg RAC/L of buffered ruminal fluid were used in the in vitro experiments. The concentrations were selected by estimating a rumen volume of 100 L with RAC supplemented at 200 mg/d, represented by the 2.26 mg/L concentration. To evaluate a wide range of concentrations, we included concentrations above (22.6 and 226.0 mg/L) and below (0 and .226 mg/L) the assumed physiological concentration of RAC.

Exp. 1 - In Vitro Gas Production and VFA Assay

A titration experiment was designed to determine the concentration of RAC that would stimulate or impede fermentation. Each concentration of RAC (0, 0.226, 2.26, 22.6, and 226.0 mg RAC/L of buffered ruminal fluid) was evaluated in duplicate each day of the gas production assay. The assay was conducted on 4 separate days for a total of 8 replications per treatment. Strained ruminal fluid was mixed with McDougall's artificial saliva in a 1:2 ratio. Ractopamine hydrochloride was measured and applied directly to each flask immediately after adding buffered ruminal fluid. One gram of corn, ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen, was used as substrate for the microorganisms. After treatments were applied, flasks were immediately sealed with stoppers equipped with rubber tubing connected to flasks filled with water. Flasks containing water were sealed with stoppers equipped with rubber tubing that transferred water displaced by gas produced in the connected fermentation flask into graduated cylinders. Fermentation flasks were placed into a shaking water bath held at a constant temperature of 39°C. Gas production was quantified indirectly by

measuring water displacement throughout fermentation in a shaking water bath for 6 h. Initial and final pH were measured for each flask.

After fermentation, metaphosphoric acid was added to ruminal fluid in a 1:4 ratio and mixed by vortexing. Samples were centrifuged at 30,000 × g for 20 min at a temperature of 25°C, and a portion of the supernatant fluid was analyzed for VFA concentrations with gas chromatography (model 5890A, Hewlett-Packard, Palo Alto, CA; 183 × 0.635 cm column; Supelco column packing, Bellefonte, PA) with nitrogen as the carrier gas, a flow rate of 80 mL/min, and a column temperature of 130°C.

Exp. 2 – In Vitro DM Disappearance

The experiment was conducted as a completely randomized design with a 4 × 4 × 5 factorial arrangement of treatments to measure IVDMD. Concentrations of RAC (0, 0.226, 2.26, or 22.6 mg RAC/L of buffered ruminal fluid) were evaluated in combination with 4 substrates: corn, corn plus soybean meal, corn plus urea, and corn plus soybean meal plus urea. Samples were incubated for 2, 4, 6, 8, or 12 h. Corn, soybean meal, and urea were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen.

Combinations of RAC and substrate were evaluated in quadruplicate for each of the 5 incubation times. Blanks were included for each of the incubation times to correct for DM contained in the fermentation media. Treatments containing added protein source (soybean meal or urea) were formulated to be isonitrogenous (13% protein). Corn (7% protein) served as the control substrate in the experiment. Substrate combinations, (1 g, DM basis), were added to the tubes 24 to 48 h before the beginning of fermentation (Table 2). Fermentation was conducted in a batch fermentation system by using ruminal fluid buffered with McDougall's artificial saliva at a 1:2 ratio. Tubes were incubated at 39°C under anaerobic conditions, with only fermentative gases allowed to escape the tubes. Fermentation was terminated by adding 30 mL of 0°C 1.0 M acetate buffer in 2% saturated benzoic acid. Samples were held at 4°C and filtered (Whatman 541, 125 mm dia.) 24 to 48 h post fermentation. Samples were oven-dried for 12 h at 105°C. Samples were cooled in a dessicator and weighed to determine DM disappearance.

Exp. 3 – Effect of RAC on In Vitro Proteolysis

Experiment 3 evaluated the effect of RAC on the concentration of ammonia and AA in accordance with procedures described by Broderick (1987). Buffered ruminal fluid was mixed

with 60 m*M* hydrazine sulfate solution (25 mL/1,500 mL of buffered ruminal fluid), and chloramphenicol solution containing 1.80 mg/mL of chloramphenicol (25 mL/1,500 mL of buffered ruminal fluid) was added to the mixture. Hydrazine sulfate and chloramphenicol were included to reduce microbial uptake and metabolism of ammonia and AA. Two treatments were used to compare the effects of RAC on proteolysis: treatment 1 (control; 0 mg RAC/L of buffered ruminal fluid mixture) and treatment 2 (RAC; 2.26 mg RAC/L of buffered ruminal fluid mixture). Casein (20 mg/150 mL of buffered ruminal fluid mixture) was added to each tube as a source of readily degradable protein. Tubes were incubated at 39°C for 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, or 240 min in vented culture tubes. Treatments were evaluated in quadruplicate for each of the incubation times for a total of 96 tubes. Tubes were iced immediately after removal from the incubator to stop fermentation and then stored at 0°C.

Ammonia and AA were analyzed by allowing samples to thaw at room temperature and then centrifuging them for 15 min at 21,000 × g at a temperature of 25°C. The resulting supernatant was analyzed colorimetrically with an autoanalyzer (Technicon III Auto Analyzer, Seal Analytical, Mequon, WI) for concentrations of ammonia and AA following the procedure of Broderick and Kang (1980).

Exp. 4 – Effect of RAC on In Vivo Proteolysis

Effects of RAC supplementation on ruminal concentrations of ammonia and free AA were evaluated in a completely randomized complete block experiment with a 2 × 2 × 2 factorial arrangement of treatments for a total of 8 diets (Table 3). Factors consisted of grain processing method [steam-flaked (SFC) or dry-rolled corn (DRC)], concentration of dried distillers grain (DG) with solubles (0 or 25% DG, DM basis), and concentration of RAC (0 or 200 mg/d). Sixteen ruminally fistulated Holstein steers were randomly assigned to the 8 treatment combinations and adapted to their respective diets for 21 d before sampling ruminal fluid. Steers were housed in an enclosed barn in individual slatted, concrete-surfaced pens measuring 7 m^2 with individual access to feed and water. Steers were fed the diet for ad libitum intake once daily at 0800 h. Ruminal fluid was collected during a 3-d sampling period: d 1 at 0, 6, 12, 18 h post feeding; d 2 at 2, 8, 14, 20 h post feeding; and d 3 at 4, 10, 16, 22 h post feeding. Ruminal fluid was hand mixed in the rumen before sampling, sampled, strained through 4 layers of cheesecloth, mixed in a 4:1 ratio with 25% metaphosphoric acid solution, and immediately frozen at -20°C. Before analysis, samples were thawed, thoroughly homogenized by vortexing,

and then centrifuged at 21,000 × g for 15 min at a temperature of 25°C to remove particulate matter. Concentrations of AA and ammonia in the supernatant were measured colorimetrically with an autoanalyzer (Technicon III Auto Analyzer, Seal Analytical, Mequon, WI)) as described for Exp. 3.

Statistical Analyses

Experiment 1 was analyzed as randomized complete block designs with days of in vitro fermentation as the blocking factor. Data from Exp. 1 were analyzed with the MIXED procedure (SAS Inst. Inc., Cary, NC.). Model effects included concentration of RAC as a fixed effect, with sampling day as a random effect. Quadratic and linear effects of RAC on gas production in Exp. 1 were analyzed with the GLM procedure of SAS. Volatile fatty acid data for Exp. 1 were analyzed with the GLM procedure of SAS, with flask as the experimental unit and with model effects of treatment and block. Experiment 2 was analyzed as a completely randomized design with the GLM procedure of SAS. The model included substrate, RAC concentration, time, and all possible interactions. Experiment 3 was analyzed as a completely randomized design with the MIXED procedure of SAS. The model included concentration of RAC, time, and their interactions. Experiment 4 was a completely randomized design and was analyzed with the MIXED procedure of SAS with animal as the random effect and DG, grain processing, RAC, and hour as fixed effects. In all 4 experiments, significance was determined at $P < 0.05$, and trends were determined at $P < 0.10$.

Results and Discussion

Exp. 1 - In Vitro Gas Production and VFA Assay

There was a significant quadratic effect on gas production among the concentrations of RAC tested (Figure 1; $P < 0.05$). Rumen fermentation was stimulated with 0.226, 2.26, and 22.6 mg RAC/L of buffered ruminal fluid; the greatest concentration of RAC tested (226.0 mg RAC/L of buffered ruminal fluid) resulted in the least gas production but was not different from the control ($P = 0.19$). Gas production with 22.6 mg/L RAC was greater than that with the control ($P = 01$); however gas production with the smallest amount evaluated (0.226 mg/L) was numerically greater than that with the control, but not statically different ($P = 0.31$), and there was a tendency for an increase with the addition of 2.26 mg/L ($P = 0.08$). Changes in gas

production in our study were not due to pH changes. The initial pH for all treatments was 6.92, with a final pH of 6.85, 6.87, 6.85, 6.85, and 6.86 for RAC concentrations of 0.226, 2.26, 22.6, and 226.0 mg, respectively. Ractopamine hydrochloride did not affect pH in this 6-h in vitro fermentation study ($P = 0.76$). The increase in gas production in response to RAC concentrations up to 22.6 mg/L suggests that fermentative changes could also occur in the rumen of cattle fed RAC.

Mersmann (1998) stated that the effects of βAA differ among livestock species, suggesting that livestock species intensively selected for growth might respond less to βAA because they are closer to their maximal growth potential. In addition, βAA might not be effective at targeting specific tissues in all species. Results from Exp. 1 suggest another reason for differences in the effects of βAA among livestock species; differences could be due to the effect of βAA on ruminal microflora and the contributions of these microorganisms to digestive processes in ruminants. Although the 22.6 mg/L concentration significantly increased gas production, this will not exist in the rumen in vivo. However, the increase in gas production with the addition of a small quantity of RAC (0.226 mg/L), although only numerically different, suggests that fermentation was affected. Roberts et al. (2002), who studied bacteria common to the oral cavity of humans, reported that supplementing the bacteria with natural catecholamines produced by the human body resulted in increased growth in more than one-half the bacteria tested. They concluded that natural catecholamines had a direct effect on oral bacteria and that the response varied among the different bacterial species and catecholamines tested. Naturally occurring catecholamines increase growth of gram-negative bacteria (Lyte and Ernst 1992; Kinney et al., 2000). Bacterial species in the rumen responsible for the fermentation of starch and lactate, the biohydrogenation of fatty acids, and protein degradation are mostly gram negative. The potential effects of RAC supplementation on these ruminal bacteria populations and the changes to the rumen that could result have not been investigated previously. An increase in growth of gram-negative bacteria in the rumen could affect fermentation of the diet. The increase in fermentation measured by gas production in vitro suggests that microorganisms are being stimulated by RAC concentration up to 22.6 mg/L; however, whether all microorganisms respond the same to RAC is unknown. Belay and Sonnenfeld (2002) researched the effect of catecholamines on in vitro growth of pathogenic bacteria. Growth was enhanced by adding catecholamines but was dependent on the catecholamine and bacteria species. The results from

Exp.1, with smaller concentrations of RAC (0.226, 2.26 and 22.6 mg/L) increasing gas production but the greatest concentration (226.0 mg/L) decreasing gas production, suggest concentrations of RAC potentially present in the rumen affect fermentation. Belay et al. (2003) tested other pathogenic bacterial species and reported no enhanced growth with the addition of catecholamines, further illustrating that not all microorganisms respond in the same manner to catecholamines. The reason for different bacterial responses to catecholamines are not fully understood. One possibility is that catecholamines can increase the ability of bacteria to assimilate iron, an essential nutrient for bacterial growth (Kinney et al., 2000). Kinney et al. (2000) observed that catecholamines function as siderophores, chelating iron and giving an advantage to bacteria able to recognize and use the siderophores. Ractopamine hydrochloride might provide a competitive advantage in iron utilization and other unknown factors to specific ruminal microorganisms that are vital to fermentation. This advantage could have caused rapid growth of rumen bacterial populations present in the in vitro assay, thereby increasing gas production. Freestone et al. (2000) observed that norepinephrine stimulated *Escherichia coli* O127:H6 growth in nutrient-poor medium when transferrin or lactoferrin were present. Norepinephrine was able to facilitate the break between the bonds of iron and transferrin and iron and lactoferrin, yielding an available source of iron for the *E. coli* present. The use of iron use could cause a shift in the microbial population of the rumen, leading to changes in cattle performance. Although the mechanism for the increase in gas produced in the in vitro fermentation with increasing concentrations of RAC from 0.226 through 22.6 mg is unclear, results from this study indicate that microbial activity in the rumen is stimulated in the presence of RAC. However, the decrease in gas production in flasks incubated with the greatest quantity of RAC (226.0 mg/L) suggests that there is an upper limit to the concentration of RAC that stimulates microbial fermentation.

The increase in gas production provides evidence that the effects of administering RAC to cattle are not limited to mammalian tissue. Ractopamine hydrochloride has potential to affect microorganisms in the rumen, as well as other regions of the bovine digestive system. Beta-adrenergic agonist receptors are found along the digestive tract of animals. Binding of βAA to these receptors has been shown to directly affect motility and secretory functions of the gastrointestinal tract (Ruckebusch 1983; McIntyre and Thompson 1992). Rumen motility is an important factor influencing passage rate of ruminal fluid and particulate matter. Survival and

persistence of a specific microbe population depends on their growth rate and generation time relative to passage rate of ruminal contents (Van Soest, 1994). The effect of RAC on rumen motility might directly affect the microbial population influencing ruminal digestion.

Volatile fatty acid production from in vitro fermentations did not differ with the addition of RAC for acetate ($P = 0.38$), propionate ($P = 0.95$), butyrate ($P = 0.15$), and total VFA ($P = 0.54$; Table 4). Ractopamine hydrochloride had a quadratic effect on the concentrations of isobutyrate ($P = 0.01$), isovalerate ($P = 0.02$), and valerate ($P = 0.02$) produced during the 6-h fermentation (Table 4). Despite the increased gas production there was little difference in VFA production.

On the basis of our observation of increased gas production, the hypothesis was that VFA concentration also would increase in flasks with added RAC. The VFA results failed to support this hypothesis. The VFA concentration in Exp. 1 were small for a closed in vitro system, which may indicate poor fermentation in the flasks, and may have limited our ability to detect differences. Ractopamine hydrochloride had minimal effect on VFA production in vitro. Acetate, butyrate, and total VFA concentration numerically increased in RAC-treated flasks after 6 h of incubation, whereas propionate numerically decreased with addition of RAC. Significant changes were observed in isobutyrate, isovalerate, and valerate concentrations, for which RAC concentration had a quadratic effect ($P < 0.05$). Because we measured VFA concentration in the in vitro flasks only after 6 h of fermentation, it is unclear if the VFA profile differed among treatment within the 6-h fermentation. However, in the batch fermentation systems, VFA are not removed as would occur in vivo. Sampling ruminal fluid from ruminally cannulated cattle at several time points post-RAC feeding could better illustrate the effects on VFA profile and the rumen, and might be a subject for further research.

Another possible explanation for the increase in gas production without a significant increase in VFA concentration is an decrease in the amount of methane produced. *Methanobrevibacter ruminantium*, *Methanobacterium formicium*, and *Methanosarcina barkeri* are the common methanogens in the rumen, and it is conceivable that activity of methanogens might be altered in the presence of RAC; however, more research is needed.

Exp. 2 – In Vitro DM Disappearance

The 3-way interaction between, RAC concentration, substrate type, and time of fermentation was not significant ($P = 0.12$). There was a significant interaction for RAC

concentration and time of fermentation ($P < 0.01$; Table 5), with more DM disappearance in tubes treated with RAC. The response in IVDMD to RAC was greater at 2, 4 and 12 h than 6 or 8 h. The rate at which the microbes digested DM was affected by RAC. This is further evidence that RAC stimulates fermentation by ruminal microorganisms. There also was a significant interaction between RAC concentration and nitrogen source ($P < 0.01$; Table 6). This interaction suggests that more ruminally available forms of nitrogen increase the response to RAC. Inhibition of proteolysis may result in an insufficient supply of ammonia, which is essential to many ruminal bacteria. Significant main effects were detected for RAC concentration ($P < 0.01$), substrate type ($P < 0.01$), and time of fermentation ($P < 0.01$). The increase in response in DM disappearance to RAC supplementation may be a response to changes in protein and nitrogen utilization by ruminal microorganisms when RAC is included in the incubation media.

During the early time points, the response to RAC seemed to be greater at the greatest concentration; however, by the end of the IVDMD assay, all the RAC concentrations tested showed similar response in increased DM disappearance. It is unclear why there was no response to RAC at the time points of 6 and 8 h. The effect of protein source on DM disappearance was directly affected by the presence of the RAC. Urea, which is rapidly degraded by ruminal microorganisms, had the greatest stimulatory effect on utilization of DM with addition of RAC. This implies that ruminal degradability of protein in diets of cattle fed RAC may be an important consideration and that the response to RAC may be improved by nitrogen supply to ruminal microbes. Research investigating dietary protein in cattle supplemented with RAC has focused on the content of CP needed to optimize response of the βAA. Despite the increase in muscle accretion in animals fed RAC, there is no evidence that the dietary protein requirements of the animal increase with RAC supplementation (Mitchell et al., 1991; Xiao et al., 1999). Interactions between protein source and RAC in this experiment, in which treatments were formulated to be isonitrogenous, suggest that maintaining adequate concentrations of ruminal degradable protein may be necessary to achieve the optimal response to RAC. The amount of ruminally degraded protein appears to directly affect response to RAC in vitro. These results correspond to previous research conducted at Kansas State University, in which heifers were fed different protein sources with or without RAC supplementation (Walker et al., 2006). Treatment diets were formulated to be isonitrogenous with a CP concentration of 13.7%. Expeller soybean meal, soybean meal, and urea were used to achieve 3 concentrations of degradable intake protein in the

diet (69.3, 62.7, and 57.3%). The observations from this experiment indicate that diets containing more ruminally degradable forms of protein could improve response to RAC. The results of Exp. 2, combined with those of Walker et al. (2006), suggest that formulating finishing diets with increased amounts of ruminal degradable protein could potentiate the response to feeding RAC.

The increase in DM disappearance with increases in RAC concentration suggests microbial activity was stimulated. These results support the findings in Exp. 1, in which gas production increased with increased RAC concentrations up to 22.6 mg/L of buffered ruminal fluid. These experiments suggest that ruminal microorganisms are stimulated by RAC. Researchers have observed that natural catecholamines increase growth of gram-negative bacteria (Lyte and Ernst 1992; Kinney et al., 2000; Roberts et al., 2002). It remains to be shown what effect RAC may have on specific populations of ruminal bacteria. Changes in the population of ruminal bacteria could affect nutrient digestion within the rumen.

Exp. 3 – Effects of RAC on Proteolysis

In Exp. 3, the interaction between time and RAC tended to be significant for ammonia concentration ($P < 0.10$): and, the time × RAC interaction was significant for AA concentrations ($P < 0.05$). There was a decreased concentration of ammonia when RAC was added to fermentation tubes ($P < 0.001$; Figure 2). In practical feeding in vivo for optimal protein concentrations, ammonia might become limiting when cattle are fed RAC. Decreased availability of ammonia for ruminal microorganisms could explain the results reported in Exp. 2, in which treatments with increased concentrations of degradable protein in the form of urea resulted in more IVDMD. This could also explain the results seen by Walker et al. (2006), in which heifers fed RAC had numerically greater BW gains with increased concentrations of dietary urea. Amino acid concentration also was less when RAC was added to the fermentation tube ($P < 0.001$; Figure 3). Amino acids are an intermediate product in the breakdown of true protein to ammonia. The changes observed in AA and ammonia in Exp. 3 suggest that proteolysis and deamination might be decreased by RAC. Ractopamine hydrochloride may have a direct effect on the microorganisms needed to break down AA into ammonia. Cross-feeding is a vital part of the rumen ecosystem, and its effect on some microorganisms may affect the functions they perform and may have a domino-like effect. Results suggest that decreased ammonia concentrations in cattle fed RAC are an effect of the conversion of true protein to ammonia.

Consequently, it may be beneficial to increase NPN as a source of ammonia in cattle fed RAC, as opposed to feeding additional true protein in the diet.

Exp. 4 – Effects of RAC on AA and Ammonia In Vivo

The 4-way interaction between RAC, grain processing, DG, and hour for ammonia (P = 0.28) or AA (P = 0.99) concentrations was not significant. The only 3-way interaction that affected ruminal ammonia concentrations was between RAC, DG, and hour (P = 0.01; Figure 4). The DG × RAC interaction was not significant for ruminal ammonia (P > 0.10). Ruminal ammonia concentrations were less when RAC was fed in combination with DRC, but were unchanged when RAC was fed in conjunction with SFC (grain processing × RAC, P < 0.01; Figure 5). Addition of RAC (P < 0.01), SFC (P < 0.01), and DG (P < 0.01) all resulted in decreased ruminal ammonia concentrations compared with DRC diets without RAC and DG. Decreased ruminal ammonia concentrations with RAC reported in this experiment were similar to the results in Exp. 3. The decreased ruminal ammonia concentrations in cattle fed DG in this experiment are in agreement with previous findings of Santos et al. (1984) and Ham et al. (1994). Cooper et al (2002b) reported reduced ruminal ammonia concentrations in cattle fed SFC compared with DRC, which corresponded to the results observed in our experiment. Cooper et al. (2002a) reported a quadratic response in DMI and ADG in steers fed different concentrations of urea (0, 0.5, 1.0, or 2.0% of dietary DM) in a SFC diet. These results suggest that steam-flaked diets could benefit from additional NPN. There were no significant 3-way interactions for ruminal AA concentrations in the present study (P = 0.34). Amino acid concentrations were less when RAC was added to diets with DG but were unchanged in diets without DG (DG × RAC, P < 0.05; Figure 6). Ruminal AA concentrations were less in cattle fed SFC than in cattle fed DRC (P < 0.01; Figure 7). Changes in ruminal ammonia and AA concentration with RAC supplementation are dependent on processing method of the grain and the addition of DG to finishing diets. In Exp. 4, RAC significantly decreased AA concentration in vitro; however, the effect of RAC on proteolysis in vivo was dependent on the protein source in the diet. Results of this experiment suggest that RAC may affect ruminal degradation of dietary protein, which could have important implications for diet formulation. Steam flaking and dry rolling are common grain processing methods used for enhancing starch utilization in finishing cattle diets, with corn being the primary cereal grain included in the diet (Vasconcelos and Galyean, 2007). Results from this experiment suggest that ammonia concentrations are more affected by RAC

supplementation in diets containing DRC than in diets containing SFC. This is supported by the in vitro results reported in Exp. 3, in which addition of RAC reduced ruminal ammonia concentrations, and by the conclusion of Exp. 2 and 3 that suggests deamination could be a limiting factor in RAC response. We speculate that ruminal ammonia concentrations were not decreased as much by the presence of RAC in cattle fed SFC diets because the concentrations was already reduced.

Because of increased ethanol production, coproducts are commonly included in feedlot diets (Vasconcelos and Galyean, 2007). Distillers grains contain large amounts of protein, with 60% of the protein undegradable intake protein (NRC, 1996). It might be necessary to add additional NPN to these diets to achieve optimal ruminal fermentation, particularly when cattle are supplemented with RAC, but this also may exacerbate problems associated with nitrogen waste excretion from feedlots.

Results from these studies illustrate that RAC effects are not confined to mammalian tissues. Ractopamine hydrochloride affects ruminal metabolism of nutrients in cattle. These results suggest it may be prudent to alter diet composition to fully exploit the benefits of βAA.

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Table 3.1 Diet Composition (% of DM) Fed to Cannulated Cattle Used in Exp. 1 - 3

Ingredient	% of DM
Steam-flaked corn	81.20
Alfalfa hay	7.00
Corn steep liquor	6.72
Vitamin/trace mineral premix[1]	2.23
Chemical concentrations analyzed	
DM	79.55
CP	13.50

[1]Vitamin-trace mineral premix provided monensin (Rumensin, Elanco Animal Health, Greenfield, IN) and tylosin (Tylan, Elanco Animal Health, Greenfield, IN) at 300 and 90 mg/animal daily in a ground corn carrier.

Table 3.2 Substrate Combinations Used for IVDMD Assay in Exp. 2

Treatment combination	Corn	Corn + SBM[1]	Corn + urea	Corn + SBM + urea
Corn, g	1.0	0.85	0.9778	0.9249
SBM, g	-	0.15	-	0.064
Urea, mg	-	-	22.2	11.1

[1]SBM = Soybean meal.

Table 3.3 Diet Composition (% of DM) Fed to Cattle Used in Exp. 4

	Dry-rolled corn		Steam-flaked corn	
Ingredient	0% DGGS[1]	25% DDGS	0% DDGS	25% DDGS
Steam-flaked corn	-	-	73.85	56.44
Dry-rolled corn	73.82	56.40	-	-
DDGS	-	25.24	-	25.21
Corn silage	13.88	13.84	13.83	13.83
Vegetable oil	2.18	-	2.18	-
Soybean meal	4.45	-	4.45	-
Urea	1.17	0.14	1.19	0.14
Limestone	1.62	1.65	1.62	1.65
Vitamin/trace mineral premix[2]	0.69	0.53	0.69	0.53
Feed additive[3]	2.19	2.20	2.19	2.20

[1]Dried distiller's grains with solubles.

[2]Formulated to provide 2,650 IU/kg vitamin A, 0.15 mg Co, 10 mg Cu, 0.5 mg I, 50 mg Mn, and 50 mg Zn per kg DM.

[3]Provided 300 mg of monensin (Elanco Animal Health, Greenfield, IN) and 90 mg of tylosin (Tylosin, Elanco Animal Health, Greenfield, IN) per animal daily in a ground corn carrier Ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN) was included in the diet at 0 or 200 mg per animal daily.

Table 3.4 Effects of Ractopamine Hydrochoride[1] on In Vitro VFA Profiles Following a 6-h Incubation in Exp. 1

	Ractopamine-HCl, mg/L buffered ruminal fluid						*P*-value		
VFA concentration, mM	0	0.226	2.26	22.6	226.0	SEM	Treatment	Linear	Quadratic
Acetate	28.41	28.35	29.05	29.26	28.74	0.38	0.38	0.93	0.11
Propionate	13.21	13.08	13.06	13.15	13.17	0.15	0.95	0.77	0.92
Butyrate	7.07	7.35	7.66	7.57	7.37	0.17	0.15	0.92	0.20
Isobutyrate	0.63	0.64	0.64	0.67	0.67	0.01	0.01	0.01	0.01
Isovalerate	1.62	1.66	1.67	1.71	1.68	0.02	0.07	0.29	0.02
Valerate	1.04	1.08	1.05	1.10	1.10	0.01	0.002	0.02	0.02
Total	51.96	52.17	53.12	53.45	52.72	0.71	0.54	0.85	0.17

[1]Elanco Animal Health (Greenfield, IN)

Table 3.5 Effects of Ractopamine Hydrochloride (RAC) on IVDMD[1] in Exp. 2

	Disappearance,[3] %				
RAC[2], mg/L	2-h fermentation	4- h fermentation	6-h fermentation	8-h fermentation	12-h fermentation
0	35.8	37.5	48.2	51.1	54.2
0.226	34.0	44.4	47.7	48.8	57.7
2.26	34.9	47.3	48.5	55.7	58.2
22.6	40.1	42.0	48.1	52.0	59.5

[1]Effect of time, $P < 0.01$; SEM 0.007, linear effect $P < 0.001$; quadratic effect $P < 0.10$.
[2]Effect of RAC (Elanco Animal Health, Greenfield, IN), $P < 0.01$; SEM 0.007; linear effect, $P < 0.05$; quadratic effect, $P < 0.001$.
[3]Time × RAC interaction, $P < 0.01$.

Table 3.6 Effects of Nitrogen Source[1] and Ractopamine Hydrochloride (RAC) on IVDMD in Exp. 2

RAC[2], mg/L	Disappearance,[3] %			
	Corn only	Corn + SBM[4]	Corn + SBM + Urea	Corn + Urea
	Disappearance[4], %			
0	44.2	44.5	46.1	46.6
0.226	45.7	42.1	51.2	47.3
2.26	45.5	49.7	48.6	51.8
22.6	45.0	47.8	48.6	51.9

[1]Effect of substrate, $P < 0.01$; SEM 0.007.
[2]Effect of RAC (Elanco Animal Health, Greenfield, IN), $P < 0.01$; SEM 0.007; linear effect, $P < 0.05$; quadratic effect, $P < 0.001$.
[3]Substrate × ractopamine interaction, $P < 0.01$.
[4]Soybean meal

Figure 3.1 Amount of Fermentative Gas Produced by Ruminal Microbes During a 6-h In Vitro Fermentation with 5 Concentrations of Ractopamine Hydrochloride (Elanco Animal Health, Greenfield, IN).

[a-d]Means without a common superscript letter tend to differ ($P < 0.10$). SEM = 4.80. Ractopamine-HCl had a quadratic effect on gas production ($P < 0.05$).

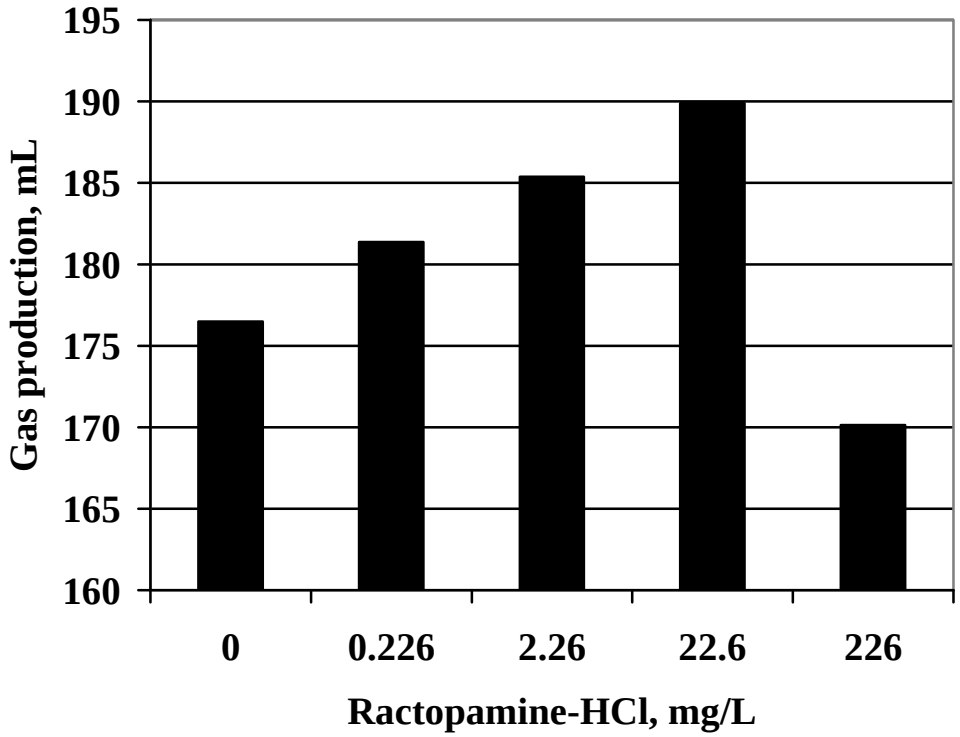


Figure 3.2 Molar Concentration of Ruminal Ammonia in Tubes Incubated with 2.26 mg of Ractopamine Hydrochloride (Elanco Animal Health, Greenfield, IN) per Liter (○) or 0 mg of Ractopamine Hydrochloride per Liter (□).

Ractopamine hydrochloride × time effect, $P < 0.10$; SEM = 0.77. Ractopamine hydrochloride effect, $P < 0.001$; SEM = 0.22. Time effect, $P < 0.01$; SEM = 0.61**.**

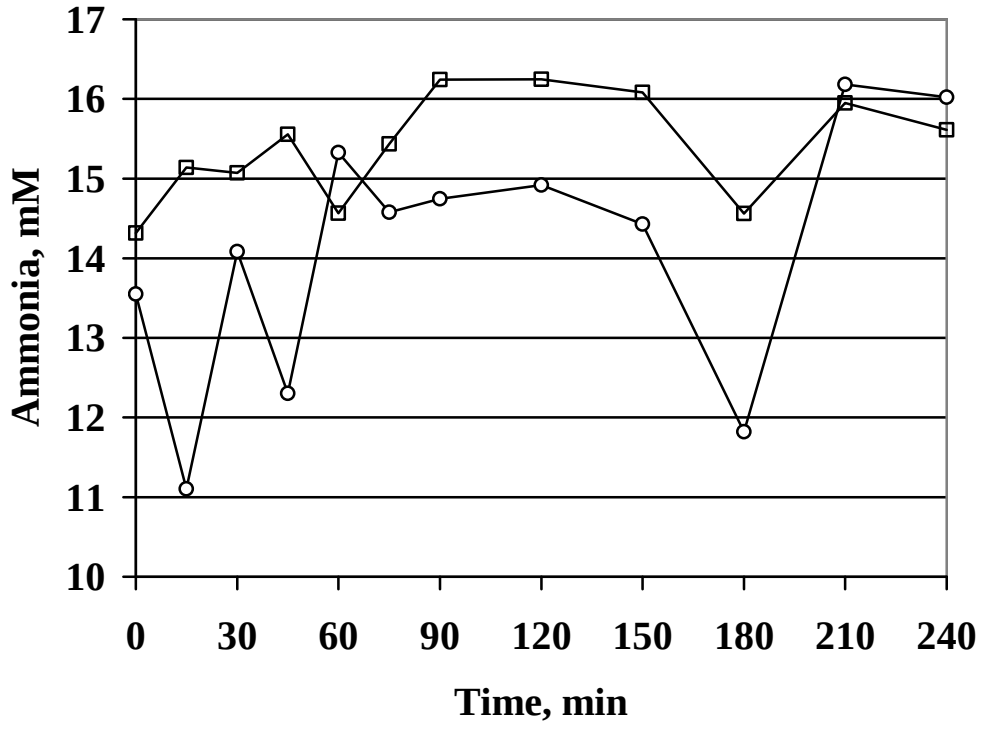


Figure 3.3 Molar Concentration of Ruminal AA in Tubes Incubated with 2.26 mg of Ractopamine Hydrochloride (Elanco Animal Health, Greenfield, IN) per Liter (○) or 0 mg of Ractopamine Hydrochloride per Liter (□).

Ractopamine hydrochloride × time interaction, $P < 0.05$; 0.15. Ractopamine hydrochloride effect, $P < 0.001$; SEM = 0.04. Time effect, $P < 0.01$; SEM = 0.10.

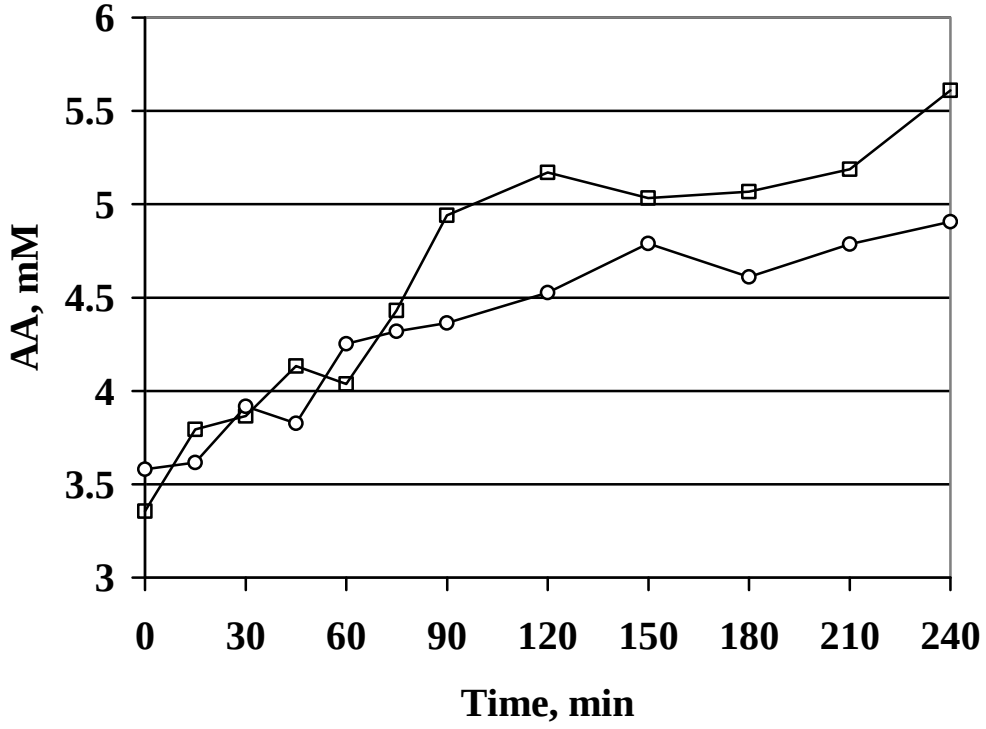


Figure 3.4 Effect of Ractopamine Hydrochloride (RAC; Elanco Animal Health, Greenfield, IN) and Distillers Grains (DG) on Concentrations of Ruminal Ammonia in Cattle Fed Diets with 25% DG with 0 mg RAC per Day (□), 0% DG with 0 mg RAC per Day (■), 25% DG with 200 mg RAC per Day (○), or 0% DG with 200 mg RAC per Day (●).
Ractopamine hydrochloride × DG× hour, $P = 0.01$. Ractopamine hydrochloride × DG, $P > 0.10$. Ractopamine hydrochloride, $P < 0.01$. Distiller's grains, $P < 0.01$. SEM = 0.72.

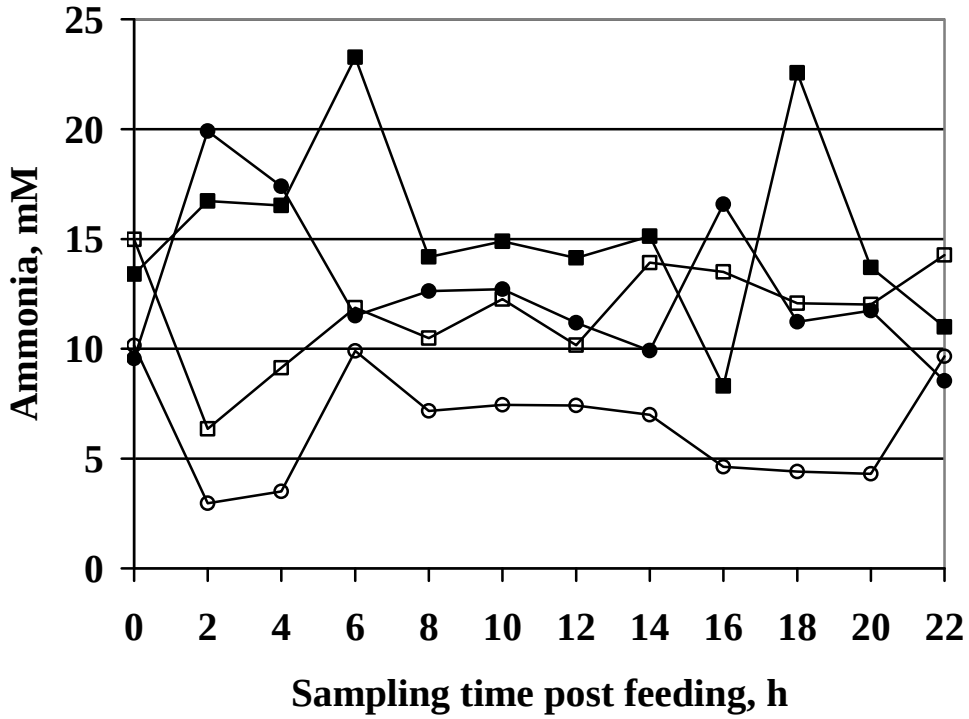


Figure 3.5 Effect of Ractopamine Hydrochloride (RAC; Elanco Animal Health, Greenfield, IN) and Grain Processing on Concentrations of Ruminal Ammonia in Cattle Fed Diets of Dry-rolled Corn with 0 mg RAC per Day (□), Steam-flaked Corn with 0 mg RAC per Day(■), Dry-rolled Corn with 200 mg RAC per Day (○), or Steam-flaked Corn with 200 mg RAC per Day (●). Ractopamine hydrochloride × grain processing, $P < 0.01$. Ractopamine hydrochloride, $P < 0.01$. Grain processing, $P < 0.01$. SEM = 0.72.

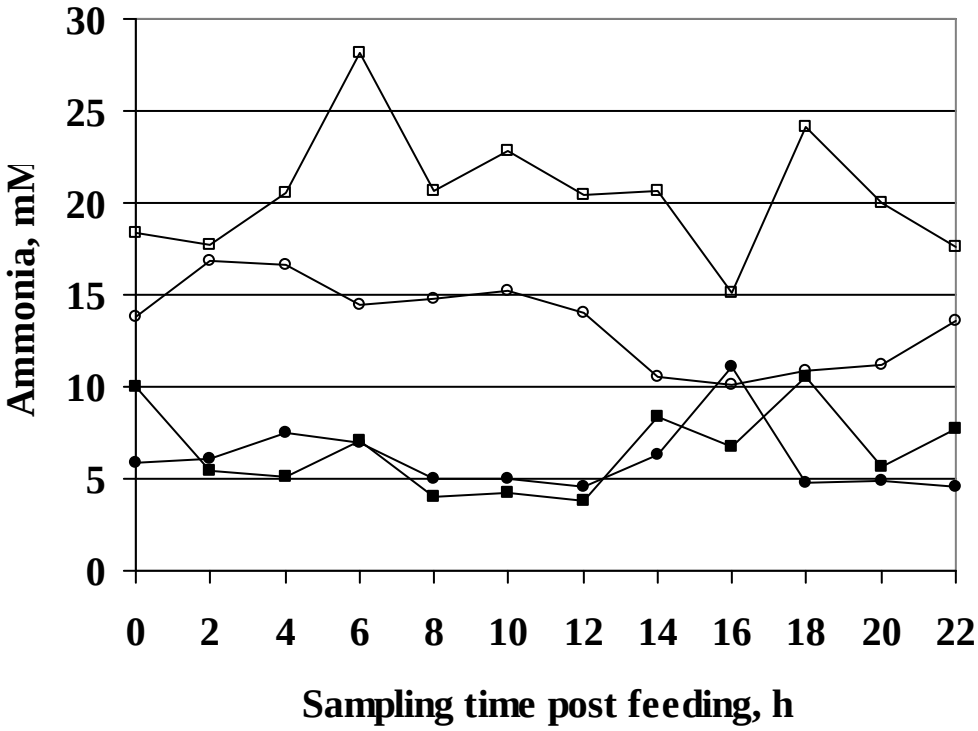


Figure 3.6 Effect of Ractopamine Hydrochloride (RAC; Elanco Animal Health, Greenfield, IN) and Distillers Grains (DG) on Concentrations of Ruminal AA in Cattle Fed Diets of 25% DG with 0 mg of RAC (□), 0% DG with 0 mg of RAC (■), 25% DG with 200 mg of RAC (○), or 0% DG with 200 mg of RAC (●).

Ractopamine hydrochloride X DG, $P < 0.05$. Ractopamine hydrochloride, $P > 0.10$. Distiller's grains, $P > 0.40$. SEM = 1.67.

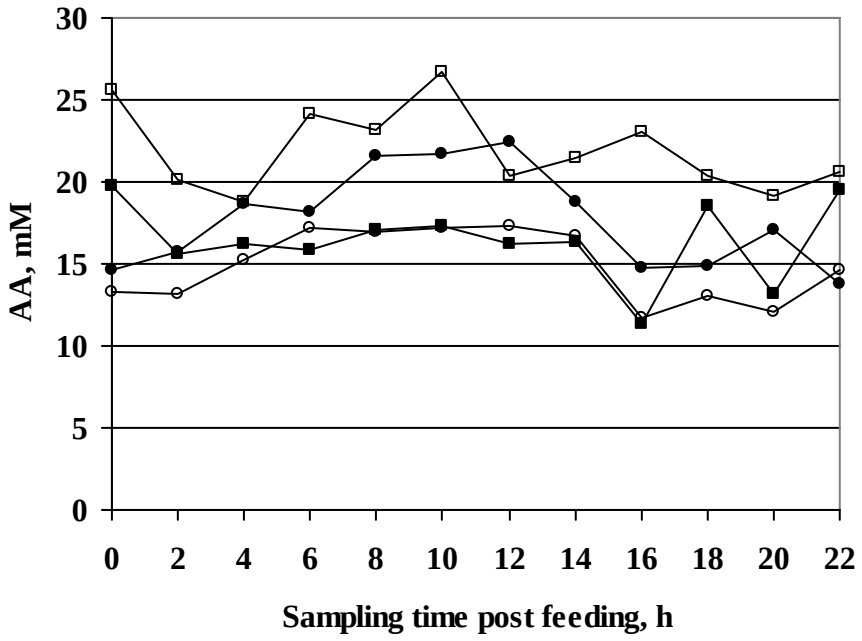
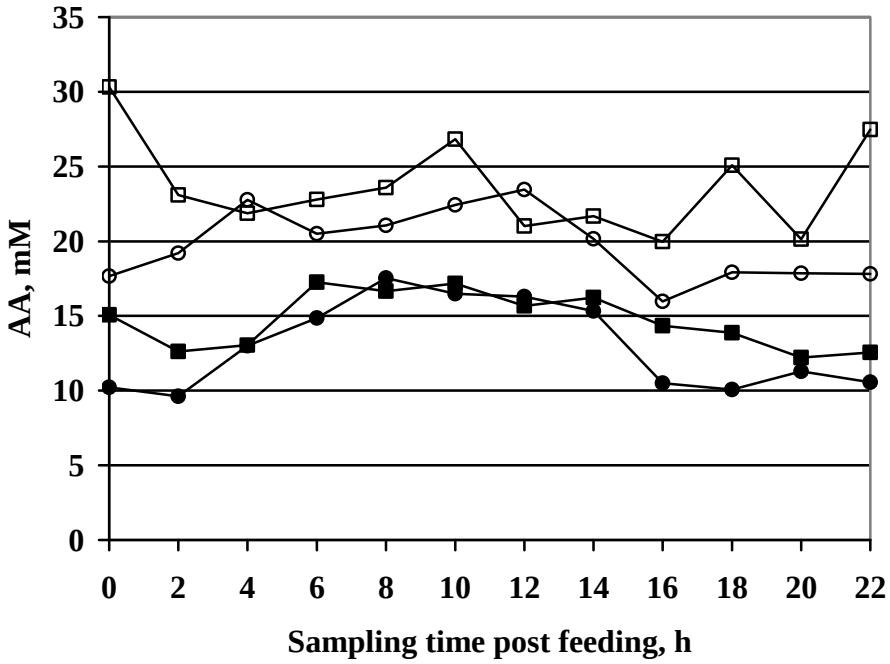


Figure 3.7 Effect of Ractopamine Hydrochloride (RAC; Elanco Animal Health, Greenfield, IN) and Grain Processing on Concentrations of Ruminal AA in Cattle Fed Diets of Dry-rolled Corn with 0 mg of RAC (□), Steam-flaked Corn with 0 mg of RAC (■), Dry-rolled Corn with 200 mg of RAC (○), or Steam-flaked Corn with 200 mg of RAC (●).
Ractopamine hydrochloride × grain processing, $P > 0.50$. Ractopamine hydrochloride, $P > 0.10$. Grain processing, $P < 0.01$. SEM = 1.67.



CHAPTER 4 - Ractopamine Hydrochloride Does Not Affect Growth or Fermentation of Ruminal Bacteria in Pure Culture

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Abstract

Catecholamines have been observed to enhance pure culture bacterial growth *in vitro*. In this research, effects of the synthetic catecholamine ractopamine hydrochloride (RAC) on bacterial growth were evaluated *in vitro*. Bacterial species common in the rumen of high-concentrate fed cattle, particularly species involved in proteolysis were evaluated. Pure cultures of *Butyrivibrio fibrisolvens, Clostridium aminophilum*, *Clostridium sticklandii, Fusobacterium necrophorum*, *Megasphaera elsdenii, Prevotella ruminicola, Selenomonas ruminantium,* and *Streptococcus bovis* were obtained from the American Type Culture Collection. Cultures were grown in Hobson M2 media and incubated at 38°C. Treatments were 0 mg (Control) or 2.26 mg RAC/L, which was applied to culture tubes immediately before inoculation with bacterial cultures. Bacterial growth was evaluated as changes in optical density at 650 nm using a spectrophotometer. The change in pH and redox potential for each tube was determined after incubation was complete. Cultures were then centrifuged and the pellet dried at 105°C to determine impact of RAC on bacterial cell yield. To measure the impact of RAC on fermentation, concentrations of VFA and lactate were analyzed using gas chromatography, and ammonia and α-amino N were analyzed colorimetrically by an auto analyzer. Ractopamine hydrochloride did not affect pure culture growth of the ruminal bacterial species evaluated ($P > 0.10$). There were no treatment differences for pH, redox potential or bacterial cell yield ($P > 0.10$). Ractopamine hydrochloride did not affect the concentrations of VFA, lactate, ammonia or amino acid *in vitro* ($P > 0.10$). Incubating ruminal bacteria with RAC did not impact growth or fermentation end products when grown in pure cultures.

Keywords: Ractopamine hydrochloride, ruminal bacteria, ruminal fermentation

Introduction

Ractopamine hydrochloride (RAC), a synthetic catecholamine, has been noted for its ability to accelerate gain and improve efficiency when administered to cattle during the final 4 to 6 weeks of finishing (Schroeder et al., 2003; Laudert et al., 2004). These effects are presumed to be a direct result of RAC's activity as a β-adrenergic agonist (βAA), repartitioning energy from adipose deposition towards lean tissue deposition. Natural catecholamines have been noted to increase growth of Gram negative bacteria (Lyte and Ernst, 1992; Kinney et al., 2000; Belay and Sonnenfeld, 2002). Catecholamines previously have been noted for their ability to influence populations of oral bacteria (Roberts et al., 2002). The increase in bacterial growth is dependent on the type and concentration of catecholamine to which the bacteria are exposed (Lyte and Ernest, 1992; Freestone et al., 1999). It is possible that RAC could directly affect microorganisms in the rumen. The addition of RAC to rumen fluid *in vitro* increased gas production and IVDMD (Walker and Drouillard, 2009). The IVDMD response to RAC was dependent on the nitrogen (N) source used (Walker and Drouillard, 2009). *In vivo* response to RAC has been observed to depend on N source of the diet (Beermann et al., 1986; Walker et al., 2006). Beermann et al. (1986) observed a greater response to cimaterol when fed in diets with soybean meal alone than soybean meal plus fishmeal. Walker et al. (2006) demonstrated that response to RAC supplementation could be improved by feeding ruminally degraded forms of N. The variation in response to βAA with different N source in the diet suggests that rumen fermentation is altered. The effects of RAC on specific populations of ruminal microorganisms could alter fermentation. The objective of this study was to evaluate the direct effect of RAC on individual ruminal bacterial species *in vitro* to provide insight into the ability of the compound to alter ruminal fermentation.

Materials and Methods

The study was conducted with approval of the Kansas State University Institutional Animal Care and Use Committee. Ruminal bacterial species evaluated were obtained from American Type Culture Collection (ATCC) and included: *Butyrivibrio fibrisolvens* (ATCC 19171), *Clostridium aminophilum* (ATCC 49005), *Clostridium sticklandii* (ATCC 49006), *Fusobacterium necrophorum* (ATCC 25286), *Megasphaera elsdenii* (ATCC 17753), *Prevotella*

ruminicola (ATCC 19189), *Selenomonas ruminantium* (ATCC 19205), and *Streptococcus bovis* (ATCC 35034). Pure cultures of bacteria identified above were first grown in sterile peptone yeast media (Table 1) to establish growth from the freeze dried culture, then transferred to Hobson M2 (Hobson, 1969; Table 2) media and grown to a concentration of approximately 10^8 cfu/mL. These cultures were then used to inoculate culture tubes containing fresh Hobson M2 media for the experiment.

Ruminal digesta for preparation of the Hobson M2 media was collected from each of two ruminally fistulated steers fed a high-concentrate diet (Table 3) and on two separate days. Four separate batches of Hobson M2 media were made with the combinations of steer and day; steer 1, d 1; steer 1 d 2; steer 2, d 1; steer 2 d 2. Technical grade RAC (Elanco Animal Health, Greenfield, IN) was added to glass culture tubes at the rates of 0 (CON) or 7.87 X 10^{-11} M per tube (RAC). There was 1 tube per treatment (CON or RAC) for each Hobson M2 media combination, for a total of four observations per treatment. Each tube received 8.9 mL of Hobson M2 media before the start of the trial. Each day the trial was conducted, a stock solution was made by adding RAC to sterile distilled water and vortexing for 1 min to assure homogenous mixing. Each tube received 100 μL of either the RAC solution or sterile distilled water to bring total volume of media in the tube to 9 mL. Bacterial cultures were diluted 1:10 by adding 1 mL of the bacterium culture into tubes containing 9 ml of media. Tubes were mixed by vortexing 1 min each, and then incubated at 39˚C for 24-40 h.

Growth Analysis

The effect of RAC on growth of ruminal bacteria was evaluated using an *in vitro* batch fermentation system following the procedures of McIntosh et al. (2003). Briefly, bacterial growth was measured by reading optical density at 650 nm hourly for 12 h beginning at 0 h, then again at 23 and 24 h using a spectrophotometer (Spectronic 20D+; Thermo Electron Corporation, Waltham, MA). *Clostridium aminophilum* and *Clostridium sticklandii* grew slower, and as a consequence optical density was measured through 36 and 40 h, respectively. For each reading, tubes were removed from the incubator and vortexed 60 sec to homogenize the contents. The exterior was cleaned of dust and fingerprints with a Kimwipe (Kimberly-Clark Global Sales, Inc., Roswell, GA). The first optical density reading was taken immediately after inoculating with bacteria to obtain a baseline value. After absorbance was read, pH and redox potential were

measured (Orion 420A, Thermo Fisher Scientific, Waltham, MA). Tubes were then centrifuged at 14,000 X g for 20 min. The supernatant was removed and the pellet was dried at 100˚ C for 10 h and weighed to measure dry cell yield.

Fermentative End-Product Analysis

The effect of RAC on bacterial fermentation was analyzed by measuring concentration of fermentative end products. Culture tubes were fermented in an incubator at 39˚C for 24 h for all bacteria except *Clostridium aminophilum*, which was incubated for 36 h, and *Clostridium sticklandii*, which incubated for 40 h. After incubation, 4 mL of culture were added to 1 mL of metaphosphoric acid and vortexed. Samples were centrifuged at 30,000 × *g* for 20 min, and a portion of the supernatant was analyzed for VFA concentrations by gas chromatography (Model 5890, Agilent Technologies, Santa Clara, CA) with a flame ionization detector (compressed air flow was set at 200 mL/min and hydrogen flow was set at 20 mL/min). The chromograph was fitted with a 183 cm × 0.635 cm column (B-DA 80/120 4% CW20M; Supelco #11889, Bellefonte, PA) and maintained at 130°C, and the detector and injector were maintained at 250°C. The carrier gas was nitrogen, with a flow rate of 80 mL/min and a run time of 20 min. A portion of the supernatant was analyzed for ammonia and α-amino N colorimetrically with an autoanalyzer (Technicon III Auto Analyzer, Seal Analytical, Mequon, WI) following the procedure of Broderick and Kang (1980).

Statistics

Data were analyzed using the mixed procedure of SAS. Day and steer were blocking factors, and culture tube was the experimental unit. Model for growth analysis included the effects of treatment, incubation time, and the interaction between treatment and time. The model for measurements of pH, redox potential, cell yield, VFA, lactate, ammonia, and amino acid included treatment as a fixed effect. Differences were declared significant at $P < 0.05$, and tendencies declared at $P < 0.10$.

Results and Discussion

Growth Analysis

Ractopamine hydrochloride did not impact absorbance reading for *Butyrivibrio fibrisolvens* ($P > 0.60$; Figure 1); *Clostridium aminophilum* ($P > 0.80$; Figure 2); *Clostridium sticklandii* ($P > 0.70$; Figure 3); *Fusobacterium necrophorum* ($P > 0.10$; Figure 4); *Megasphaera elsdenii* ($P > 0.20$; Figure 5); *Prevotella ruminicola* ($P > 0.20$; Figure 6); *Selenomonas ruminantium* ($P > 0.35$; Figure 7); or *Streptococcus bovis* ($P > 0.10$; Figure 8). There were no differences for pH ($P > 0.10$; Table 4), redox potential ($P > 0.10$; Table 5), or cell yield ($P > 0.50$; Table 6) between RAC and control treatments for any of the bacterial species evaluated. Ractopamine hydrochloride did not affect *in vitro* growth of ruminal bacterial species evaluated in pure culture. These observations are similar to the results observed by Edrington et al. (2006), who examined the effects of RAC on *Escherichia coli* O157:H7 and *Salmonella in vitro* in pure culture and *in vivo* on intestinal populations and fecal shedding in experimentally inoculated sheep and pigs. Edrington et al. (2006) observed an increase in fecal shedding of *E. coli* O157:H7 in sheep administered RAC before and after inoculation ($P < 0.05$); while RAC decreased fecal shedding of Salmonella in pigs fed RAC before and after inoculation ($P < 0.05$). There was a tendency for RAC to increase cecal populations of the inoculated strain of *E. coli* O157:H7 in sheep. Edrington et al. (2006) then examined the potential for a direct effect of RAC on pure culture growth of *E. coli* O157:H7 strains used to inoculate the sheep and the *Salmonella* strain used to inoculate the pigs, but observed no differences in growth rate for either of the bacteria when incubated with increasing concentrations of RAC. This agrees with our observations where RAC increased *in vitro* fermentation (Walker et al. 2009), but had no direct effect on bacterial species evaluated in pure culture in the current research. These results differ from research with natural catecholamines and bacteria (Lyte and Ernest 1992; Freestone et al., 1999; Roberts et al., 2002). Lyte and Ernest (1992) evaluated various catecholamines, including norepinephrine, epinephrine, dopamine, and dopa, for their potential to directly influence growth of Gram negative bacteria, including *Escherichia coli*, *Yersinia enterocolitica*, and *Pseudomonas aeruginosa*. Results indicated that catecholamines directly increased growth of the Gram negative bacteria. Freestone et al. (1999) evaluated a much broader range of bacterial species and observed that the response to catecholamines was widespread among Gram negative and Gram positive bacteria. Roberts et al. (2002) concluded that natural catecholamines have a direct effect on the *in vitro* growth of oral bacteria and that the response varies among different bacterial

species and the catecholamines tested. Kinney et al. (2000) and Freestone et al. (2007) observed that catecholamines function as siderophores, chelating iron, thus giving an advantage to bacteria that are able to recognize and utilize the siderophores. The Hobson M2 media utilized in our experiment was presumably sufficiently supplemented with all required nutrients. In the absence of competition from other species, it is possible that effects of RAC are not realized. Belay et al (2003) observed no increase in growth with the addition of epinephrine or norepinephrine among *Porphyromonas gingivalis*, *Bacteriodes fragilis*, *Shigella boydii*, *Shigella sonnie*, *Enterobacter Sp*, and *Salmonella choleraesuis*. Belay and Sonnenfeld (2002) observed no enhanced growth of *Pseudomonas aeruginosa* or *Staphylococcus aureus* with the addition of norepinephrine. O'Donnell et al. (2006) evaluated the effect of norepinephrine on growth of bacterial species previously reported to be unaffected by norepinephrine (Belay et al., 2003; Belay and Sonnenfeld, 2002) and observed norepinephrine to increase growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella sonnie*, and *Enterobacter cloacae* when small inoculums of the bacteria were used in the *in vitro* experiment. Lyte and Ernest (1992) observed a greater growth in response to norephenephrine when added to cultures with low initial bacterial concentration. The observations of O'Donnell et al. (2006) and Lyte and Ernest (1992) could explain the results in the present study, where there were no differences in growth with the addition of RAC in any of the bacterial species examined; perhaps a lower inoculation concentration would have yielded different results.

Fermentative End-Product Evaluation

Ractopamine hydrochloride did not alter the VFA concentration among any of the bacterial species evaluated in pure culture ($P > 0.10$, data not shown). Lactate concentration was also unaffected by the addition of RAC in pure culture *in vitro* fermentation ($P > 0.10$, data not shown). This is in agreement with research conducted by Walker and Drouillard (2009) where RAC had a quadratic effect on *in vitro* gas production in ruminal fluid ($P < 0.05$); however, there were minimal changes to VFA concentrations ($P < 0.10$). Walker and Drouillard (2009) observed an increase in IVDMD with the addition of RAC suggesting that fermentation was enhanced by the addition of RAC ($P < 0.05$). The media and thus the substrates available for the bacteria in the present study were different which could lead to different results. McIntosh et al. (2003) examined the impact of essential oils on ruminal bacterial growth in pure culture using

Hobson M2 media, and similar techniques utilized in our experiment. McIntosh et al. (2003) observed a decrease in growth in the presence of essential oils; however the sensitivity to essential oils varied among the ruminal bacterial species evaluated. It appears Hobson M2 media and the technique used in the current experiment can be utilized to evaluate impact of a compound on growth of ruminal bacteria. In the current study bacterial species common to the rumen were grown in pure cultures, as a result there was no competition or cross feeding among microorganisms which could also lead to a different outcome. The result from this pure culture experiment suggests that VFA and lactate concentration produced by ruminal bacteria are not directly affected by RAC.

Ractopamine hydrochloride did not impact ammonia concentration for *Butyriovibrio fibrisolvens* ($P > 0.10$; Table 4.4) *Clostridium aminophilum* ($P > 0.40$; Table 4.4), *Clostridium sticklandii* ($P > 0.10$; Table 4.4) *Fusobacterium necrophorum* ($P > 0.40$; Table 4.4), *Megasphaera elsdenii* ($P > 0.50$; Table 4.4), *Prevotella ruminicola* ($P > 0.60$; Table 4.4), *Selenomonas ruminantium* ($P > 0.30$; Table 4.4), or *Streptococcus bovis* ($P > 0.60$; Table 4.4). Ractopamine hydrochloride did not effect amino acid concentrations for any of the bacterial species evaluated: *Butyriovibrio fibrisolvens* ($P > 0.10$; Table 4.4); *Clostridium aminophilum* ($P > 0.50$; Table 4.4); *Clostridium sticklandii* ($P > 0.90$; Table 4.4); *Fusobacterium necrophorum* ($P > 0.40$; Table 4.4); *Megasphaera elsdenii* ($P > 0.10$; Table 4.4); *Prevotella ruminicola* ($P > 0.20$; Table 4.4); *Selenomonas ruminantium* ($P > 0.10$; Table 4.4); *Streptococcus bovis* ($P > 0.40$; Table 4.4). This differs from previous research that suggested RAC impacted proteolysis, decreasing the concentration of ammonia and amino acids in vitro with buffered rumen fluid as the media ($P < 0.001$; Walker and Drouillard, 2009). Walker and Drouillard (2009) evaluated the potential impact of RAC on in vitro fermentation using buffered ruminal fluid, along with 4 substrate combinations: corn, corn + soybean meal, corn + urea, and corn + soybean meal + urea. There was an interaction between RAC concentration and nitrogen source ($P < 0.01$), with more degradable forms of nitrogen eliciting greater responses from RAC, suggesting that RAC impacts proteolysis. However, in both these in vitro experiments the impact of RAC was evaluated in mixed cultures of ruminal microbes where there was likely competition for nutrients as well as cross feeding among organisms. Edward et al. (2005) examined the influence of flavomycin on ruminal microbial population; they observed that Hobson M2 gave a higher specific activity of ammonia formation than a simpler medium. Perhaps there was no difference

in end product formation because the media provided ample substrate for the bacteria. Also it was not possible to obtain and evaluate each microorganism in the rumen in pure culture, and thus we could have failed to test the microbial species impacted by RAC. Beermann et al. (1986) evaluated the effects of cimaterol in lambs fed diets with soybean meal plus fishmeal or soybean meal alone. Lambs were supplemented with 0 or 10 ppm cimaterol for 5 or 10 wks. Performance was improved in lambs fed fishmeal or cimaterol; however the were no additive effects. Cimaterol was less effective in increasing the size of three foreleg muscles when fed with diets containing fishmeal than those fed only soybean meal. Walker et al. (2006) evaluated the response to RAC supplementation in finishing heifers fed different sources of ruminally degraded nitrogen. Treatment diets were formulated to be isonitrogenous with CP level of 13.7%; expeller soybean meal, soybean meal, and urea were used to achieve 3 levels of degradable intake protein in the diet (69.3, 62.7, and 57.3%). Observations from this experiment indicate that diets containing more ruminally degradable forms of protein elicited a greater response to RAC. The previous studies conducted in our laboratory (Walker and Drouillard, 2009); along with the study of Walker et al. (2006), and Beermann et al. (1987), indicate that RAC directly influences proteolysis in the rumen. The results of this study suggest that the response is either the result of a change to a microorganism not evaluated in pure culture or that RAC could have an in direct effect on ruminal bacteria.

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Table 4.1 Peptone Yeast Extract Media

Ingredient	Amount
Peptone, g	0.5
Tryptone, g	0.5
Yeast Extract, g	1.0
Indigo carmine, ml	0.4
Salt solution[a], ml	4.0
DI water, mL	100.0
Hemin solution[b], ml	1.0
Vitamin K, mg	0.02
Cysteine $HCl \cdot H_2O$, g	0.05

Vitamin K1, Hemin Solution, and cysteine are dispensed after autoclaving.

[a]Salt Solution; 0.2 g, $CaCl_2$; 0.48g, $MgSO_4.7H_2O$; 1.0g, K_2HPO_4; 1.0g, KH_2PO_4; 10.0g, $NaHCO_3$; 2.0g, NaCl

[b]Hemin solution: 50mg hemin in 1 mL 1N NaOH; make up to 100ml with DI water.

Table 4.2 Composition of Hobson M2 Media.

Ingredient	Amount
Bacto Casitone, g	1.0
Bacto yeast extract, g	0.25
Mineral solution A, mL [a]	15
Mineral solution B, mL [b]	15
Clarified (centrifuged) rumen fluid, mL	20
Resazurin, g	0.0001
Sodium lactate (70% w/v), g	1.0
Glucose, g	0.2
Maltose, g	0.2
Cellobiose, g	0.2
Cysteine HCl, g	0.05
Sodium bicarbonate	0.4
Distilled water	to 100 mL

[a]Contains (per 1000 mL) 3.0 g K_2HPO_4

[b]Contains (per 1000 mL) 3.0 g KH_2PO_4; 6.0 g NaCl; 0.6 g $MgSO_4 \cdot 7H_2O$; and 0.6 g $CaCl_2$.

Table 4.3 Composition (DM basis) of Diet Fed to Fistulated Steers Used as Donors of Ruminal Fluid for Pure Culture Experiments.

Ingredient	% of DM
Steam-flaked corn	82.59
Alfalfa hay	6.00
Corn steep liquor	5.75
Urea	1.20
Limestone	1.56
Potassium chloride	0.25
Sodium chloride	0.30
Vitamin/trace mineral premix[a]	0.12
Feed additive premix[b]	2.23
Chemical composition	
DM, analyzed	79.55
CP, analyzed	13.50

[a]Vitamin/trace mineral premix provides 2,400 IU/kg vitamin A; 10 mg/kg Cu; 0.5 mg/kg I; 0.15 mg/kg Co; 0.3 mg/kg Se; 60 mg/kg Zn, and 60 mg/kg Mn.
[b]Feed additive premix provided 300 mg monensin sodium and 90 mg tylosin per animal daily using a ground corn carrier.

Table 4.4 Effect of Ractopamine Hydrochloride on α-amino acids and ammonia concentration in Pure Cultures of Bacteria Post-fermentation.

	α-amino acids				Ammonia			
Bacterial species	CON[1]	RAC[2]	SEM	*P* -value	CON	RAC	SEM	*P*-value
Butyriovibrio fibrisolvens	28.46	28.79	0.13	0.12	8.76	8.62	0.06	0.12
Clostridium aminophilum	22.48	22.52	0.04	0.52	7.20	7.17	0.02	0.41
Clostridium sticklandii	22.28	22.30	0.11	0.91	11.97	11.96	0.07	0.94
Fusobacterium necrophorum	24.40	24.46	0.05	0.41	21.30	21.23	0.06	0.43
Megasphaera elsdenii	25.40	25.99	0.24	0.12	14.56	14.60	0.04	0.53
Prevotella ruminicola	27.07	27.01	0.03	0.21	5.45	5.45	0.01	0.64
Selenomonas ruminantium	32.78	32.69	0.04	0.19	10.04	10.01	0.02	0.33
Streptococcus bovis	31.39	31.62	0.19	0.41	7.22	7.19	0.04	0.63

[1]Control
[2]Ractopamine hydrochloride

Table 4.5 Effect of Ractopamine Hydrochloride on pH of Pure Cultures of Bacteria Post-fermentation.

Bacterial species	Control	RAC[1]	SEM	*P*-value
Butyriovibrio fibrisolvens	6.69	6.61	0.05	0.24
Clostridium aminophilum	6.08	6.08	0.03	0.85
Clostridium sticklandii	6.55	6.51	0.24	0.91
Fusobacterium necrophorum	6.24	6.19	0.11	0.79
Megasphaera elsdenii	6.26	6.27	0.01	0.65
Prevotella ruminicola	6.24	6.19	0.11	0.79
Selenomonas ruminantium	6.53	6.63	0.06	0.27
Streptococcus bovis	5.59	5.64	0.02	0.11

[1]Ractopamine hydrochloride

Table 4.6. Effect of Ractopamine Hydrochloride on Redox Potential of *In Vitro* Bacterial Culture Post-fermentation.

Bacteria	Control	RAC[1]	SEM	*P*-value
Butyriovibrio fibrisolvens	47.0	51.2	2.53	0.28
Clostridium aminophilum	44.2	45.0	1.23	0.66
Clostridium sticklandii	23.0	24.8	12.69	0.92
Fusobacterium necrophorum	38.0	38.4	5.30	0.96
Megasphaera elsdenii	36.8	36.0	0.68	0.46
Prevotella ruminicola	38.0	38.4	5.30	0.96
Selenomonas ruminantium	46.9	49.7	3.76	0.62
Streptococcus bovis	105.3	101.9	1.25	0.10

[1]Ractopamine hydrochloride

Table 4.7. Effect of Ractopamine Hydrochloride on Bacterial Cell Yield (mg) of Bacterial Culture *In Vitro*

Bacteria	Control	RAC[1]	SEM	*P*-value
Butyriovibrio fibrisolvens	3.0	2.9	0.2	0.60
Clostridium aminophilum	26.5	30.1	8.5	0.77
Clostridium sticklandii	13.6	13.9	0.4	0.69
Fusobacterium necrophorum	55.4	54.8	10.0	0.95
Megasphaera elsdenii	5.5	5.7	0.6	0.82
Prevotella ruminicola	36.7	38.7	8.5	0.87
Selenomonas ruminantium	3.8	4.0	0.001	0.73
Streptococcus bovis	8.7	8.8	0.42	0.87

[1]Ractopamine hydrochloride

Figure 4.1 Growth of *Butyrivibrio fibrisolvens* (plate 1; $P > 0.10$, SEM = 0.0122), *Clostridium aminophilum* (plate 2; $P > 0.10$, SEM 0.0022), *Clostridium sticklandii* (plate 3; $P > 0.10$, SEM 0.0026), *Fusobacterium necrophorum* (plate 4; P > 0.10, SEM 0.0057), *Megasphaera elsdenii* (plate 5; $P > 0.10$; 0.0169), *Prevotella ruminicola* (plate 6; $P > 0.10$, SEM 0.0042), *Selenomonas ruminantium* (plate 7; $P > 0.10$, SEM 0.0101), and *Streptococcus bovis* (plate 8; $P > 0.10$, SEM .0087) with RAC (x) or without RAC (□).

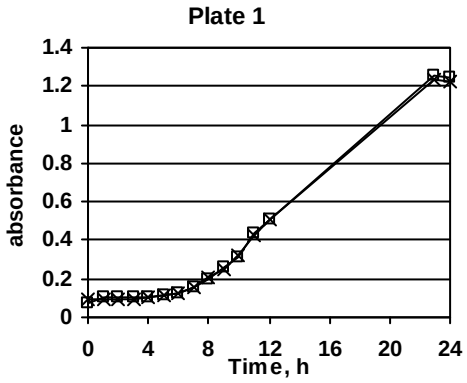
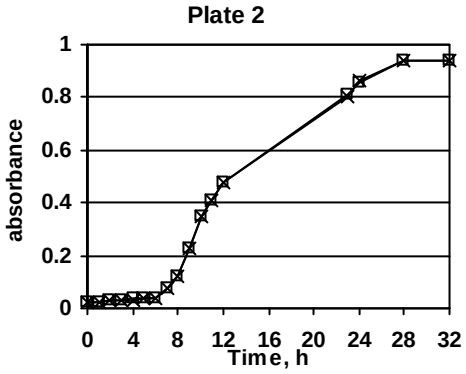


Plate 1
absorbance
Time, h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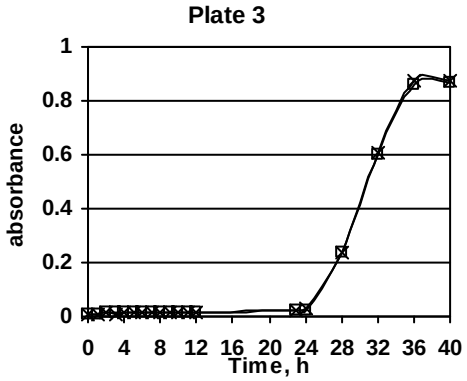
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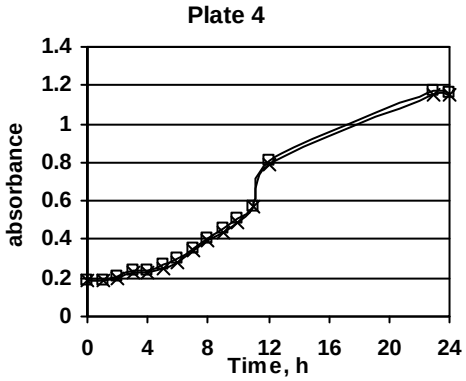
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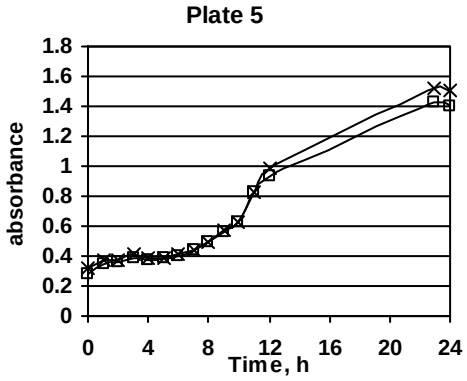
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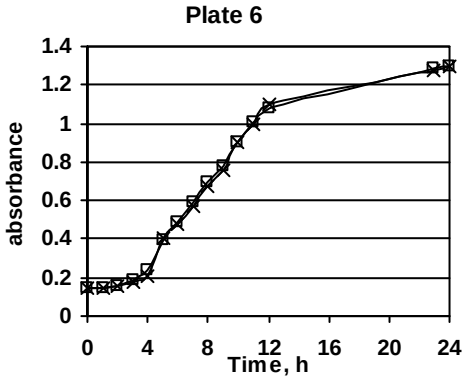
absorbance
Time, h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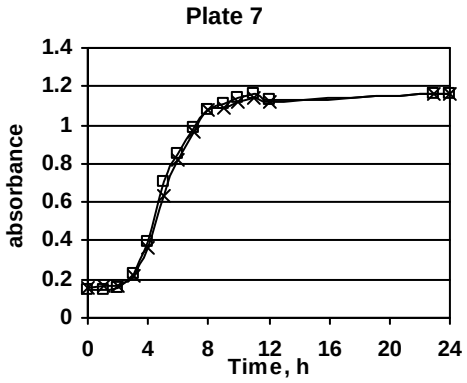
absorbance
Time, h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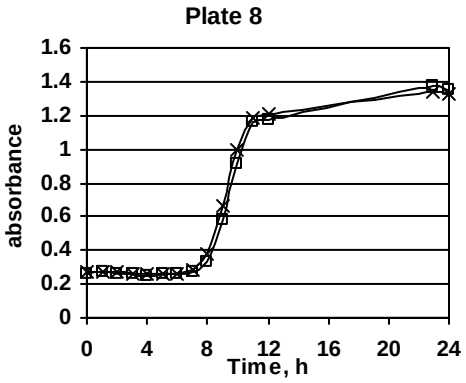
Plate 8
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CHAPTER 5 - The Impact of Urea Concentration and Monensin Concentration on Response to Ractopamine Hydrochloride in Steers Fed Wet Sorghum Distiller's Grains

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Abstract

Seven-hundred-twenty crossbred steers (initial BW = 453 kg) were utilized to evaluate the effect of nitrogen source on changes in growth performance and carcass traits in response to ractopamine hydrochloride (RAC; Optaflexx; Elanco Animal Health, Greenfield, IN). The experimental design was a randomized complete block with a 2 × 3 × 2 factorial treatment arrangement. Factor 1 consisted of 33 or 44 mg/kg monensin (MON; Rumensin, Elanco Animal Health, Greenfield, IN); factor 2 was urea concentration in the diet (0.0, 0.35, or 0.70% of DM); and factor 3 was 0 or 200 $mg \cdot hd^{-1} \cdot d^{-1}$ RAC supplemented the final 42 d. Steers were housed in dirt-surfaced pens with 15 steers per pen, and 4 pens per treatment combination. Weight of each pen was determined at the beginning of the experiment, at the beginning of RAC administration, and before shipping to a commercial abattoir for harvest. A 3-way interaction was noted for final weight and ADG for d 0 to 103 ($P < 0.05$). There was a 2-way interaction between urea and RAC for final BW and ADG d 0 to 103 ($P < 0.05$); however, urea concentration did not impact the response to RAC d 61 to 103 ($P > 0.05$). Final BW, ADG, DMI, and feed efficiency were not different with the addition of RAC ($P > 0.05$). The additions of RAC, did not impact HCW, incidence or severity of liver abscesses, KPH, or LM area ($P > 0.05$); however, feeding RAC decreased yield grade ($P < 0.05$). Ractopamine hydrochloride decreased marbling and the percent of steers grading USDA Choice, thus resulting in an increase in the percent of steers grading USDA Select ($P < 0.05$). Ractopamine hydrochloride supplementation decreased 12^{th} rib fat thickness ($P < 0.01$). Increasing dietary urea concentrations had a no impact on HCW, incidence or severity of liver abscesses, KPH, LM area, 12^{th} rib fat thickness, or yield grade, ($P > 0.05$). Increased concentration of urea in the diet increased the number of steers grading USDA Choice, and decreased the number of steers grading USDA Select ($P < 0.05$). Increasing MON from 33 to 44 mg/kg did not impact ADG or feed efficiency; however, feeding 44 mg/kg MON decreased DMI from d 0 to 103 ($P < 0.05$). Steers fed 44 mg/kg MON had lower yield grades than steer fed 33 mg/kg MON ($P < 0.05$). Increasing the concentration of MON did not impact other carcass traits measured ($P > 0.05$). Increasing the amount of ruminally available nitrogen did not impact the response to RAC during the supplementation period (RAC x Urea interaction, $P > 0.10$).

Introduction

Ractopamine hydrochloride (RAC) is marketed commercially under the trade name Optaflexx (Elanco Animal Health, Greenfield, IN), and has been noted for its ability to accelerate gain and improve efficiency when administered to cattle during the final 4 to 6 wk of finishing (Schroeder et al., 2003; Laudert et al., 2004). Researchers have observed an increase in the magnitude of response to β-adrenergic agonists in cattle fed diets with more degradable forms of nitrogen (Beermann et al., 1986; Walker et al., 2006) and *in vitro* fermentation by cultures of mixed ruminal microorganisms increased in response to ractopamine hydrochloride, but was greater when increased concentrations of degradable nitrogen were included in the substrate (Walker and Drouillard, 2009). Ractopamine hydrochloride has been observed to decrease ammonia and amino acid concentrations when evaluated *in vitro* and *in vivo* (Walker and Drouillard, 2009). The results of these studies suggest that β-adrenergic agonists may impact proteolysis in the rumen. Consequently, degradable intake protein concentration in the diet could influence the performance response in cattle supplemented with β-adrenergic agonists.

Monensin (MON; Rumensin, Elanco Animal Health, Greenfield, IN) is a polyether ionophore that improves energetic efficiency of ruminal fermentation (Haney and Hoehn, 1967), and has been noted for it ability to spare protein from degradation in the rumen (Goodrich et al., 1984). Chen and Russell (1991) observed decreased ruminal ammonia and an increased amount of peptides that could not be degraded by ruminal microorganisms with the addition of monensin *in vitro*. Decreasing protein degradation in the rumen by including monensin at higher concentration in the diet could impact the response to RAC.

Materials and Methods

The experiment and all procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Crossbred steers (n =720, 453 kg initial BW) were used in a randomized complete block experiment with 3 factors. Factor 1 was 33 or 44 mg/kg MON (Rumensin, Elanco Animal Health, Greenfield, IN); factor 2 was 0, 0.35, or 0.70% dietary urea (DM basis); factor 3 was 0 or 200 $mg \cdot hd^{-1} \cdot d^{-1}$ RAC (Optaflexx, Elanco Animal Health, Greenfield, IN). Upon arrival to the Kansas State Beef Cattle Research Center, steers were allowed *ad libitum* access to alfalfa hay and water before processing. After arrival of all the cattle for the experiment, steers were

processed as follows: individual weights were recorded, steers were assigned an individual ear tag number, cattle were vaccinated with Ultrabac 7 (Pfizer Animal Health, Exton, PA) and Bovisheild Gold (Pfizer Animal Health, Exton, PA), and implanted with Revalor-S (120 mg trenbolone acetate, 24 mg estradiol; Intervet, Millsboro, DE). Steers were stratified by initial BW and randomly allocated, within strata, to treatments and dispersed among 48 dirt-surfaced pens (10.4 × 26.8 m) containing 15 steers each. Steers were blocked by weight with 24 pens per block. Finishing diets were fed twice daily at approximately 0900 and 1500 h. During the transition period to the high-concentrate diet, steers were supplement with either 33 or 44 mg/kg MON using a series of step-up diets that contained no urea. On d 50 after initial processing, the study was initiated and steers were fed diets with 0, 0.35, or 0.70% urea according to their treatment assignment. Pen weights were recorded before feeding urea on d 0, before supplementing RAC on d 61, and immediately before shipping to a commercial abattoir on d 103. Thirteen steers were removed form the trial prior to completion due to death (n = 3), injury (n = 9), and laminitis (n =1). Average daily gain, DMI, and efficiency were determined for each pen. Carcass data, including HCW, incidence and severity of liver abscesses, and dressing percentage were obtained on the day of harvest. After a 36-h chill, carcasses were evaluated for subcutaneous fat thickness over the 12th rib, KPH, and LM area. Marbling score, quality grade, and yield grade were determined by USDA graders and recorded for each carcass.

Statistical Analysis

The experiment was designed as a randomized complete block with a 2 × 3 × 2 factorial arrangement, with pen as the experimental unit. The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used to analyze performance and carcass data. The model included fixed effects of MON, RAC, and urea. The model assessed linear and quadratic effects of urea, and all 2- and 3-way interactions. Day 0 weight was used as a covariate. For performance parameters, block was included as a random effect. For analysis of carcass data, block and pen within treatment were included as a random effect. Treatment effects were declared significant at $P < 0.05$.

Results and Discussion

Growth Performance

There was a 3-way interaction for final weight and ADG from d 0 to 103 ($P < 0.05$). Adding RAC to diets with 0.35% urea improved performance, and adding RAC to diets with 0.70% urea decreased performance. However, adding RAC to diets with 0% urea and 33 mg/kg MON decreased performance, while adding RAC to diets with 0% urea and 44 mg/kg MON improved performance.

There was a 2-way interaction between urea concentration and RAC for ADG from d 0 to 103 and for final BW ($P < 0.05$); this could be a result of an interaction between urea concentration and RAC for BW at the start of RAC supplementation ($P < 0.05$). There was a difference in ADG between the cattle fed 0.70% urea in the 0 and 200 mg of RAC treatment pens before the administration of RAC. Before administration of RAC, steers fed 0.70% urea assigned to the 200 mg RAC treatment had lower DMI than steers fed 0.70% urea assigned to the 0 mg RAC treatment. It is unclear what caused the difference since these pens were on the same treatment before administration of RAC.

Ractopamine hydrochloride did not influence DMI in the present experiment ($P > 0.05$). This agrees with observations of Schroeder et al. (2003), who observed no difference in DMI between steers fed either 0, 200, or 300 mg·head^{-1}·day^{-1} for either 28 or 42 d before harvest ($P > 0.10$). Gruber et al. (2007) also reported that RAC supplementation did not influence DMI in steers differing in breed type ($P > 0.10$). Quinn et al. (2008) observed no difference in DMI between heifers supplemented with 0 mg·head^{-1}·d^{-1}, 200 mg·head^{-1}·d^{-1} for 28 d, or 200 mg·head^{-1}·d^{-1} for 42 d ($P > 0.10$); however, heifers supplemented with RAC at 300 mg·head^{-1}·d^{-1} for 28 d had lower DMI than the control or either 200 mg·head^{-1}·d^{-1} treatment ($P < 0.05$). This evidence suggests that RAC supplemented at 200 mg·head^{-1}·d^{-1} does not influence DMI of cattle.

In the present study ADG was not influenced by RAC ($P > 0.10$); however there was a tendency for RAC to improve feed efficiency ($P = 0.08$). This agrees with observations of Quinn et al. (2008), who observed no difference in ADG between heifers supplemented with RAC for 28 d and control heifers fed 0 mg RAC ($P > 0.10$). These researchers observed only a tendency for RAC to improve G:F ($P = 0.06$). However, RAC has previously been noted to increase ADG and improve feed efficiency in cattle (Schroeder et al., 2003, Laudert et al., 2004; Gruber et al., 2007). The response to RAC in the current experiment appears to be smaller than previously reported.

Average daily gain and feed efficiency did not differ between steers fed 33 and 44 mg/kg MON ($P > 0.10$); however, increasing MON decreased DMI ($P < 0.05$). Boling et al. (1977) evaluated the effects of monensin supplemented at 4 concentrations (0, 25, 50, or 100 mg·head^{-1}·d^{-1}) and observed a tendency for feed intake to decrease with increased concentrations of MON. These results are contradictory to Erickson et al. (2003), who observed no difference in carcass-adjusted performance between cattle fed 28.6 or 36.3 mg/kg MON. Stock et al. (1995) also evaluated the effect of increasing monensin concentration (22 or 33 mg/kg) on DMI intake in steers, and observed no additional impact on DMI by increasing the amount of monensin in the diet ($P > 0.10$). In a review, monensin was observed to decrease feed intake and improve rate of gain resulting in improved efficiency compared to cattle not supplemented with monensin (Goodrich et al., 1998).

The addition of dietary urea did not impact ADG, DMI, or feed efficiency ($P > 0.05$). These results differ from observations of Shain et al. (1998), who evaluated supplemental urea in finishing diets at concentrations of 0, 0.88, 1.34, and 1.96% (DM basis), and observed increased BW gain and improved efficiency in steers fed diets containing urea compared to steers not fed urea ($P < 0.01$). Their diet containing 0% urea was 9.7% CP, this is much lower than the 14.2% CP used in the 0% urea diet in the current experiment. Other scientific investigators have observed that supplementing urea above the concentration needed by the ruminal microorganisms may enhance growth and performance of cattle (Zinn et al., 1994; Milton et al., 1997). Zinn et al. (2003) evaluated the impact of dietary urea concentration (0, 0.4, 0.8 and 1.2 %, DM basis), and observed a linear increase in ADG and DMI with greater concentration of urea in the diet ($P = 0.01$). Because ADG and DMI increased with greater concentrations of dietary urea, there was no difference in efficiency of gain between any of the urea concentrations tested (Zinn et al., 2003). Zinn et al. (2003) evaluated urea concentrations in diets with lower CP than the current experiment (10.5, 11.5, 12.5, and 13.5% CP for 0, 0.4, 0.8, and 1.2% urea, respectively). The increased CP in our experiment (14.2, 15.4, and 16.5% CP for 0, 0.35, and 0.7% urea, respectively) could explain the lack of response in performance with additional urea observed in this experiment.

Carcass Analyses

There were no 3-way or 2-way interactions for any of the carcass traits measured ($P > 0.05$).

Ractopamine hydrochloride did not impact HCW or LM area ($P > 0.10$). These results coincide with observations of Quinn et al. (2008), who reported that supplementation of RAC to heifers did not impact HCW or LM area ($P > 0.10$). In contrast, Schroeder et al. (2003) and Gruber et al. (2007) observed improvements in HCW and LM area in steers supplemented with RAC ($P > 0.05$). In the current experiment, RAC decreased marbling, thus resulting in a decreased percentage of carcasses grading USDA Choice and an increased percentage of carcasses grading USDA Select ($P < 0.05$). Gruber et al. (2007) observed RAC to have similar effects on marbling, while RAC did not impact marbling or quality grade in research conducted by Quinn et al. (2008) or Schroeder et al. (2003). In this experiment, RAC supplementation decreased 12th rib fat thickness and yield grade ($P < 0.01$). These results, along with decreased marbling, would imply that RAC decreased adipose deposition; however, because REA and HCW did not increase nutrients apparently were not repartitioned to lean tissue. Previously, RAC has been observed to have minimal effects on 12th rib fat thickness and yield grade (Schroeder et al., 2003; Gruber et al., 2007; Quinn et al., 2008). Ractopamine hydrochloride did not effect KPH or incidence or severity of liver abscesses ($P > 0.10$), this is consistent with previous research in cattle supplemented RAC (Schroeder et al., 2003; Gruber et al., 2007; Quinn et al., 2008).

Urea concentration did not impact HCW, LM area, 12th rib fat, yield grade, or KPH ($P > 0.10$), but increased ($P < 0.05$) the number of steers grading USDA Choice, and decreased ($P < 0.05$) the number of steers grading USDA Select. Gleghorn et al. (2004) observed a linear increase in HCW and LM area with increasing concentrations of urea, substituting urea for cotton seed meal ($P < 0.05$), but no difference in other carcass attributes were detected ($P > 0.10$). Shain et al. (1998) observed similar carcass traits ($P > 0.10$) between cattle fed differing urea concentrations (0, 0.88, 1.34, and 1.96%, DM basis). Milton et al. (1997) evaluated the effect of urea concentration (0, 0.5, 1.0, and 1.5%, DM basis) on carcass characteristics, and observed a linear increase in 12th rib fat thickness and yield grade with increased urea concentration ($P < 0.10$). The CP in the 0% urea diet was greater than the CP in the 0% urea

diets used in the previous research (Gleghorn et al., 2004; Shain et al., 1998; Milton et al., 1997), resulting in minimal response when additional N was added to the diet.

Within the current experiment, increasing MON from 33 to 44 mg/kg minimally affected carcass characteristics measured. Yield grade was decreased in steers fed a higher concentration of MON ($P < 0.05$). Monensin concentration did not influence any other carcass traits measured ($P > 0.05$). In a review of 228 trials, Goodrich et al. (1984) noted that monensin had minimal effects on carcass traits. Similarly, Erickson et al. (2003) evaluated the effects of monensin concentration (28.6 and 36.3 mg/kg) on carcass characteristics, and observed no effects of monensin concentration on carcass measurements.

Response to RAC in cattle was not influenced by concentration of urea in the diet. This is contradictory to previous research evaluating responses to β-adrenergic agonist with different sources of nitrogen. Research conducted by Walker et al. (2006) demonstrated that response to RAC supplementation in finishing heifers could be improved by feeding ruminally degraded forms of nitrogen, most notably urea. Walker et al. (2006) included urea at a concentration of 1.5% (DM basis), higher than the concentrations included in the current experiment (0.35 and 0.70%, DM basis). It is possible that urea concentrations in the current study were too small to elicit an increased response to RAC. In addition, Walker et al. (2006) included alfalfa hay in the diet which has a higher concentration of degradable intake protein when compared to the wheat straw used in the present study. This evidence suggests that more degradable intake protein is required to evoke a response. Similarly, Beermann et al. (1986) fed lambs diets with soybean meal or soybean meal plus fishmeal, in combination with 0 or 10 ppm cimaterol for 5 or 10 wk. Cimaterol was less effective at increasing the size of muscles evaluated when added to diets containing fishmeal, compared to when it was added to diets containing only soybean meal. The studies of Beermann et al. (1986) and Walker et al. (2006) suggest that adequate degradable intake protein is necessary to maximize response to βAA. Walker and Drouillard (2009) evaluated the impact of nitrogen source on response to RAC in an IVDMD experiment, and observed greater DM disappearance in tubes containing RAC with increased concentrations of ruminally degradable nitrogen. Ractopamine hydrochloride reduced ruminal ammonia and AA concentrations *in vitro* ($P < 0.05$; Walker and Drouillard, 2009). They interpreted their studies to suggest that β-adrenergic agonists may affect protein degradation within the rumens of cattle fed

these compounds. This concept is not supported by the results of the current research, where the CP level is higher than the requirements of the rumen and the host.

The diets fed in the current research included 30% wet sorghum distiller's grains. Researchers have observed decreased ruminal ammonia concentrations in cattle fed distiller's grains (Santos et al., 1984; Ham et al., 1994; Walker and Drouillard, 2009). It is possible that the current experimental diets consisting 14.2, 15.4, or 16.5% CP provide adequate ruminal nitrogen which reduced the likelihood of seeing an improvement in performance or carcass attributes when additional nitrogen was added. Walker and Drouillard (2009) observed decreased ruminal ammonia concentration in cannulated steers with the addition of RAC or distiller's grains in the diet ($P < 0.05$). This decrease could be a result of decreased proteolysis in the rumen, decreased recycling of urea into the rumen, or both. Nitrogen retention is increased in cattle fed β-adrenergic agonists (Beermann et al., 1993; Ricke et a., 1999), which could lead to less N available for urea recycling. In the current diet with 14.2% CP in the 0% urea diet there many have been more N available for recycling compared to the diets of Beermann et al. (1986) or Walker et al. (2006). Walker and Drouillard (2009) observed an interaction between RAC and distiller's grains on α-amino N concentration; α-amino N concentrations decreased when RAC was added to diets with distiller's grains, but were unchanged in diets without distiller's grains ($P < 0.05$). It is possible that the diets in the current study did not provide adequate ruminally available AA for the rumen microorganisms, resulting in minimal response to RAC.

Increasing the concentration of MON in the diet did not influence the response to RAC in this experiment. Because there was no treatment with no MON it is unclear if the presence of MON in the diet influences the response to RAC. Monensin has been noted to have a protein sparing effect (Goodrich et al., 1984). This is supported by findings of Chen and Russell (1991), who incubated mixed ruminal bacteria *in vitro* with degraded casein and soluble soy protein and observed decreased ($P < 0.05$) ammonia production with MON and increased ($P < 0.05$) non-ammonia non-protein nitrogen. This suggests that ruminal microorganisms were unable to degrade substantial quantities of peptide nitrogen. Results of this experiment suggest the response to RAC is not enhanced through provision of ruminally degraded nitrogen in the form of urea.

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Table 5.1 Composition of Final Finishing Diets (DM basis).

	Urea Concentration, % DM		
Ingredient, % DM	0	0.35	0.70
Steam-flaked corn	58.1	57.7	57.3
Wet sorghum distiller's grains	30.0	30.0	30.0
Wheat straw	7.0	7.0	7.0
Feed additive premix[a]	2.38	2.38	2.38
Limestone	1.81	1.80	1.80
Urea	-	0.35	0.70
Supplement[b]	0.71	0.72	0.72
Analyzed nutrient composition, %			
DM	57.5	57.5	57.5
CP	14.2	15.4	16.5
NDF	19.2	19.2	19.1
Ca	0.75	0.75	0.75
P	0.42	0.42	0.42

[a]Feed additive premix provided 33 or 44 mg/kg monensin sodium (Elanco Animal Health, Greenfield, IN) and 11 mg tylosin (Elanco Animal Health, Greenfield, IN) per animal daily using a ground corn carrier.

[b]Supplement provided 2,650 IU vitamin A; 10 mg Cu; 0.5 mg I; 0.15 mg Co; 50 mg Zn; and 50 mg Mn per kg diet DM. Ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN) included in the diet at 0 or 200 mg per animal daily the last 42 d before harvest.

Table 5.2 Least Squares Means for Performance Traits of Steers Fed Finishing Diets with 0, 0.35, or 0.70% Urea Supplemented with 0 or 200 mg of Ractopamine Hydrochloride for 42 d Before Harvest.

	0 mg RAC			200 mg RAC				*P*-value		
	0%	0.35%	0.70%	0%	0.35%	0.70%	SEM	Urea	RAC	Urea*RAC
n	117	119	114	119	118	120	-	-	-	-
Weight, kg										
d -50	366	366	366	366	366	366	23.09	0.36	0.32	0.44
d 0	453	454	453	454	453	449	23.09	0.44	0.52	0.72
d 61	552	562	565	559	563	551	13.29	0.12	0.47	< 0.01
d 103	622	631	638	634	635	623	28.38	0.87	0.45	< 0.01
DMI, kg										
d 0 - 60	10.92	11.28	11.60	11.26	11.14	10.76	0.16	0.75	0.12	< 0.01
d 61 - 103	10.86	11.06	10.89	10.99	10.78	10.53	0.77	0.56	0.36	0.51
d 0 - 103	10.89	10.16	11.24	11.12	10.95	10.66	0.33	0.83	0.20	0.07
ADG, kg										
d 0 - 60	1.60	1.75	1.81	1.71	1.77	1.58	0.21	0.12	0.47	< 0.01
d 61 - 103	1.67	1.62	1.75	1.76	1.73	1.78	0.07	0.44	0.18	0.94
d 0 - 103	1.64	1.72	1.79	1.75	1.77	1.68	0.15	0.39	0.64	< 0.01
Gain:Feed										
d 0 - 60	0.147	0.156	0.157	0.152	0.160	0.147	0.019	0.14	0.98	0.16
d 61 - 103	0.154	0.147	0.159	0.160	0.159	0.168	0.003	0.23	0.08	0.90
d 0 - 103	0.151	0.154	0.160	0.157	0.161	0.158	0.007	0.14	0.06	0.07

Table 5.3 Least Squares Means for Performance Traits of Steers Fed Finishing Diets with 33 or 44 mg/kg Monensin, with 0, 0.35, or 0.70% Urea, and Supplemented with 0 or 200 mg of Ractopamine Hydrochloride for 42 d Before Harvest.

	33 mg/kg monensin						44 mg/kg monensin										
	0 %[1]		0.35 %		0.70 %		0 %		0.35 %		0.70 %		P- value[3]				
	0[2]	200	0	200	0	200	0	200	0	200	0	200	SEM	U	R	M	U*M*R
n	57	59	60	59	56	60	60	60	59	59	58	60					
Weight, kg																	
d -50	366	365	366	366	365	366	366	366	366	366	366	366	22.3	0.36	0.32	0.02	0.86
d 0	455	451	456	455	456	445	452	456	453	452	450	453	23.2	0.52	0.44	0.84	0.37
d 61	558	555	563	560	565	557	545	563	560	565	565	545	13.7	0.12	0.47	0.39	0.05
d 103	632	630	632	635	633	627	612	636	627	634	642	624	15.7	0.39	0.66	0.44	0.04
DMI, kg																	
d 0 - 60	11.1	11.4	11.4	11.3	11.6	10.9	10.8	11.1	11.2	11.0	11.6	10.7	0.23	0.75	0.12	0.13	0.94
d 61- 103	11.6	11.0	11.3	11.1	10.9	10.5	10.1	10.9	10.9	10.5	10.9	10.5	0.80	0.56	0.36	0.03	0.18
d 0- 103	11.3	11.2	11.3	11.2	11.2	10.7	10.5	11.0	11.0	10.7	11.3	10.6	0.38	0.80	0.20	0.03	0.39
AGD, kg																	
d 0- 60	1.70	1.65	1.77	1.73	1.81	1.68	1.50	1.77	1.73	1.82	1.81	1.49	0.22	0.13	0.47	0.39	0.05
d 61 - 103	1.75	1.78	1.64	1.79	1.63	1.68	1.59	1.75	1.60	1.63	1.84	1.88	0.10	0.44	0.18	0.98	0.67
d 0 - 103	1.74	1.72	1.74	1.77	1.75	1.69	1.55	1.78	1.70	1.76	1.84	1.66	0.15	0.39	0.64	0.45	0.04
Gain:Feed																	
d 0 - 60	0.154	0.146	0.156	0.154	0.157	0.155	0.139	0.159	0.155	0.165	0.157	0.140	0.019	0.14	0.98	0.75	0.05
d 61 - 103	0.152	0.159	0.147	0.162	0.151	0.158	0.156	0.161	0.148	0.156	0.167	0.178	0.003	0.23	0.08	0.22	0.90
d 0 - 103	0.154	0.153	0.154	0.159	0.156	0.159	0.148	0.161	0.154	0.164	0.164	0.157	0.007	0.14	0.06	0.28	0.07

[1]Urea; [2]Ractopamine hydrochloride mg·head^{-1}·day^{-1}; [3]U= Urea; R = ractopamine hydrochloride; M= monensin. Monensin did not interact with ractopamine, or urea ($P > 0.05$).

Table 5.4 Least Squares Means of Overall Carcass Traits of Steers Fed Finishing Diets with 0, 0.35, or 0.70% Urea Supplemented with 0 or 200 mg Ractopamine Hydrochloride for 42 d Before Harvest.

	0 mg/d RAC			200 mg/d RAC				*P*-value		
Item	0% Urea	0.35% Urea	0.70% Urea	0% Urea	0.35% Urea	0.70% Urea	SEM	Urea	RAC	U x R
No. of steers	117	119	114	119	118	120	-	-	-	-
HCW, kg	372.9	376.8	378.0	378.1	381.1	374.2	15.4	0.40	0.40	0.21
Dressing percent, %	62.3	62.3	61.8	62.1	62.6	62.5	0.003	0.72	0.34	0.22
KPH, %	2.29	2.26	2.25	2.17	2.24	2.26	0.03	0.69	0.13	0.06
12^{th} rib fat thickness, cm	1.00	1.10	1.00	0.90	1.00	1.00	0.02	0.20	0.02	0.69
longissimus muscle area, cm^2	90.2	85.3	88.2	90.2	88.0	88.7	0.46	0.12	0.46	0.71
Yield grade	2.44	2.68	2.45	2.13	2.31	2.28	0.11	0.17	<0.01	0.67
Marbling[1]	406	421	418	392	404	397	7.1	0.10	<0.01	0.84
Prime, %	0.0	0.0	0.9	0.0	0.0	0.9	0.54	0.13	0.95	1.0
Choice, %	48.3	58.4	53.4	35.8	48.2	49.6	5.4	0.04	0.02	0.63
Premium Choice, %	6.7	12.7	13.3	4.2	6.8	0.8	3.2	0.30	<0.01	0.21
Select, %	50.0	39.9	43.8	62.5	51.8	47.0	5.5	0.04	0.02	0.53
Low grade, %	1.7	1.7	1.8	1.7	0	2.5	1.2	0.55	0.73	0.57
Liver abscesses, %[3]	6.0	5.9	4.4	5.0	2.6	3.4	2.2	0.73	0.31	0.82
A^+, %	0.9	0	0	0	0	0	0.4	0.37	0.32	0.37
A^o, %	0	0	0.9	0.8	0	0.8	0.6	0.38	0.58	0.72
A^-, %	5.1	5.9	3.6	4.2	2.6	2.5	1.9	0.66	0.24	0.76

[1]Marbling score $Slight^0$ = 300; $Small^0$ = 400.

[2]Liver abscess scores: A^- = one or two minor abscesses; A° = two to four well-established abscesses; and A^+ = large, active abscesses, may contain inflammation on the abscess periphery (Liver Abscess Technical Information AI 6288, Elanco Animal Health, Greenfield, IN).

Appendix A -Permission Letter Journal of Animal Science

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