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Effects of Catecholamines on Gut Microflora and Potential for Beta-Adrenergic Agonists to Impact Ruminal Fermentation[†]

C.E. Walker and J.S. Drouillard[*]

Department of Animal Sciences and Industry, Kansas State University, Manhattan 66506, USA

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 prior to harvest. Responses to beta-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 beta-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 beta-adrenergic agonists, threreby increasing the growth performance response in ruminants.

Keywords: Catecholamines, ruminal bacteria, beta-adrenergic agonists.

1. 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 prior to 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.

2. 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.

3. 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

*Address correspondence to this author at the Department of Animal Sciences and Industry, Kansas State University, Manhattan 66506, USA; Tel: (785)532-1204; Fax: (785)532-5681; E-mail: jdrouill@ksu.edu

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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, creates cyclic adenosine monophosphate. 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 there few compounds bind almost 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, Indianapolis, IN) and zilpaterol hydrochloride (Zilmax®, Intervet Inc., Millsboro, DE). Ractopamine hydrochloride and zilpaterol hydrochloride 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.

4. 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 revealed that various catecholamines directly increased growth of Gram-negative bacteria, including norepinephrine, epinephrine, dopamine, and dopa, to directly influence 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]. Norepinephrine induced growth in small inoculation of bacteria previously reported to be unaffected by the catecholamine. O'Donnell *et al*., [14] found cultures of *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Enterbacter cloacae*, *Shigella sonnei*, and *Staphylococcus aureus* grown using low initial inoculums density had shorter lag times and increased bacterial growth (CFU/ml) in the presence of norepinephrine. The results from O'Donnell *et al*., [14] and Freestone *et al.,* [13] suggest that the response of bacteria to catecholamines is influenced by the combination of bacteria species and catecholamine, and the initial inoculums density of the bacteria. The results of O'Donnell *et al*., [14] agree with previous observations reported by Lyte [11] where infectious dose of Clostridium perfringens was lowered more than four logs in the presence of adrenaline. Freestone *et al*., [15] observed that norepinephrine and dopamine were more potent at inducing growth of *Escherichia 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 level of noradrenaline, and found that the neurophysiologic conditions of the host can lead to major shifts in microflora in the gastro-intestinal 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. 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 and initial inoculum density. 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 microbial and speculate the role of catecholamines in microbial cell 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 *Escherichia 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 as well as 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. Researchers have observed that catecholamines produce a novel autoinducer of growth referred to as Norepinephrine-induced autoinducer (NE-AI) [13, 28]. Freestone *et al.,* [13] observed similar increase in bacteria growth in the presence of NE-AI and norepinephrine. Sperandio *et al.,* [29] observed another autoinducer-like activity (AI-3) involved in the increases of growth enteric bacteria in the presence of catecholamines. These results suggest bacteria perceive catecholamines as a host environmental cue, suggesting that catecholamines are involved in quorum-sensing which is a mechanism for bacteria to communicate [30]. Freestone *et al*., [26] evaluated specific catecholamine receptor agonists to determine if the increase in bacterial growth was a result of the 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.

5. 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 glycolgenolysis and gluconeogenesis in the liver [1].

Naturally occurring catecholamines, such as epinephrine and norepinephrine, affect gut motility and secretory responses in mammals [31-33]. 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 [31, 32, 34]. 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*., [35] observed increased mortality in steers fed zilpaterol hydrochloride compared with steers fed no zilpaterol hydrochloride ($P < 0.01$); six mortalities among the steers fed zilpaterol hydrochloride were due to digestive bloat compared with one among steers not fed zilpaterol hydrochloride. Research also suggests that β-adrenergic agonists increase absorption in the digestive tract [33, 36, 37]. Aschenbach *et al*., [37] found that β_2 adrenergic agonists increased glucose uptake via sodium-glucose-linked transporter. Glucose typically is found at low levels in the

rumen; however, levels 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*., [37] did not observe the same increase with the 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.*, [38, 39], Poletto *et al*., [40], and Walker and Drouillard [41]. Edrington *et al*., [38, 39] examined the effects of ractopamine hydrochloride on *Escherichia coli* O157:H7 and *Salmonella* in experimentally inoculated sheep and swine [38] and feedlot cattle [39]. Edrington *et al*., [38] observed that sheep administered ractopamine hydrochloride before and after oral inoculation of *Escherichia 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*., [38] found a different result when examining the effect of ractopamine hydrochloride in pigs experimentally inoculated with *Salmonella*. Pigs fed ractopamine hydrochloride 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*., [39] found that cattle administered ractopamine shed less *Escherichia 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 *Escherichia coli* O157 [28, 42, 15], more research needs to be conducted to determine the potential impact of β-adrenergic agonists on pathogenic bacteria. Walker and Drouillard [41] 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 [41] 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 of proteolytic activity and degradation of dietary nitrogen sources. Walker and Drouillard [41] 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 [41]. This could explain results of Walker [43] and Beermann [44], in which β-adrenergic agonists elicited a greater response in ruminants fed protein sources that were more readily degraded by ruminal microbes.

6. RUMINAL BACTERIA

The ecosystem of the rumen is diverse, and bacteria play the dominant role in ruminal fermentation. Ruminal bacteria numbers have been reported to be 10^{10} cells per gram of contents [45]. 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 metabolized from succinate, which is decarboxylated to propionate by organisms such as *Selenomonas ruminantium* [46, 47]. Methanogens use hydrogen and carbon dioxide produced by other microorganisms to generate methane as an end product. This benefits the methanogens and enables the rumen to remain anaerobic, thus ensuring survival of ruminal microorganisms. The ability of microorganisms to interact in the rumen leads to improved digestion of complex feeds [48]. 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 [49]. 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 [48].

Ruminal bacteria are the main starch-fermenting microorganisms in the rumen [49]. Amylolytic and dextrinolytic microbial species vary the greatest in number because of the variation in starch content and solubility of diets [48], 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*., [50] 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 monosaccharides requires coordination among bacteria species. Cotta [51] 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.

Protein degradation in the rumen is initiated by attachment of microorganisms to feed particles, after which, cell-bound microbial proteases are activated [52]. An estimated 70 to 80% of ruminal microorganisms are attached to feed particles [53], and 30 to 50% of the attached microorganisms have proteolytic activity [54]. 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 [48]. Russell *et al*., [55] found *Streptococcus bovis* to be very proteolytic. Fulghum and Moore [56] identified *Butyrivibrio sp*., *Succinivibrio sp*., *Selenomonas ruminantium*, *Borrelia sp*., and *Bacteroides sp*. as major proteolytic bacteria. Atwood *et al*., [57] 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*., [58] 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 [59] 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 [60] 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 [61-63]. Hino and Russell [64] evaluated the relative contributions of ruminal bacteria and protozoa in degradation of protein in an *in vitro* experiment. They observed that more 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*., [65] 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 [66].

Factors that effect 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*., [67] 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 [68]. Treatments that protect feed proteins from ruminal degradation, such as heat, alter the structure of the feed protein, preventing attachment [69]. 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*., [69] 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) [70]. Fibrolytic bacteria are generally nonproteolytic and require ammonia as a source of nitrogen [71]. 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 [48]. They are restricted to fermenting disaccharides, trisaccharides, and oligosaccharides released during hydrolysis of holocellulose as sources of carbon and energy [48]. 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* [72, 73], *Butyrivibrio fibrisolvens* [72, 73], *Ruminococcus albus* [72], and *Treponema bryantii* [72].

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 longevity of a microbial species. In many instances, faster growing bacteria species, such as starch fermenters, may have an advantage in using limited resources compared with slower growing organisms, such as fiber-fermenting bacteria.

7. MICROBIAL FERMENTATION IN THE RUMEN

Peyer discovered fermentation in the rumen in 1685 [72]. Since Peyer's discovery, the rumen has been recognized as an important microenvironment in the digestive tract of ruminants. Hungate [72] 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 [72, 74] 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 [75-77]. 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 [78, 79]. The impact of changing a component of the diet on ruminal fermentation has been of interest to many researchers. Hussein *et al*., [80] 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) *ad libitum* 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*., [81] 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*., [82] 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*., [83] 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*., [83] 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 [84] 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 level of forage tended to increase ruminal pH and decrease molar proportions of butyrate ($P < 0.10$). Theurer *et al*., [85] 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*., [85] 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 [86], causing a shift in the proportions of VFA with little effect on total acid production [87,88]. Ionophores decrease methane production, proteolysis, and deamination in the rumen [89]. Antibiotics can alter ruminal microbial population [90] and fermentation [91]. O'Connor *et al*., [92] 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*., [92] 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. [93-96].

8. PROTEIN

Suggested 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 perform-ance 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 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 [48]. 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.

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 [108]. 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 [109].

9. BETA-ADRENERGIC AGONISTS AND DIETARY PROTEIN

Walker and Drouillard's [41] *in vitro* results suggest that proteolysis may be directly affected by β-adrenergic agonists. Walker *et al*., [43] demonstrated that the response to ractopamine hydrochloride supplementation in finishing heifers could be attenuated 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, 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*., [44], 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 prior to 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 resent 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 agonist 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.

CONFLICT OF INTEREST

None declared.

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None declared.

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