Curr. Issues Intest. Microbiol. (2003) 4: 43-51.

Ionophores: Their Use as Ruminant Growth Promotants and Impact on Food Safety

T. R. Callaway[1], T. S. Edrington[1], J. L. Rychlik[2], K. J. Genovese[1], T. L. Poole[1], Y. S. Jung[1], K. M. Bischoff[1], R. C. Anderson[1], and David J. Nisbet[1]

[1]Food and Feed Safety Research Unit, Southern Plains Agricultural Research Center, 2881 F and B Rd., Agricultural Research Service, USDA, College Station, TX 77845, USA
[2]Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR, USA

Abstract

Ionophores (such as monensin, lasalocid, laidlomycin, salinomycin and narasin) are antimicrobial compounds that are commonly fed to ruminant animals to improve feed efficiency. These antimicrobials specifically target the ruminal bacterial population and alter the microbial ecology of the intestinal microbial consortium, resulting in increased carbon and nitrogen retention by the animal, increasing production efficiency. Ionophores transport ions across cell membranes of susceptible bacteria, dissipating ion gradients and uncoupling energy expenditures from growth, killing these bacteria. Not all bacteria are susceptible to ionophores, and several species have been shown to develop several mechanisms of ionophore resistance. The prophylactic use of antimicrobials as growth promotants in food animals has fallen under greater scrutiny due to fears of the spread of antibiotic resistance. Because of the complexity and high degree of specificity of ionophore resistance, it appears that ionophores do not contribute to the development of antibiotic resistance to important human drugs. Therefore it appears that ionophores will continue to play a significant role in improving the efficiency of animal production in the future.

Introduction

The relationship between the ruminant animal and its resident ruminal microbial population is clearly symbiotic and allows ruminants to utilize fibrous plant material via a microbial fermentation (Hungate, 1966). However, the ruminal fermentation is inherently inefficient. Up to 12% of dietary carbon and energy can be converted to methane and heat, end-products that are unusable by the animal (Blaxter, 1962), and up to 50% of dietary protein can be degraded to ammonia and lost in the urine.

Carbohydrates in ruminant diets are fermented to produce volatile fatty acids (VFA) and microbial biomass (Hungate, 1966) which provide much of the energy and over 50% of the protein requirements of the animal, including most of the dietary essential amino acids (Owens, 1988; Stewart and Bryant, 1988). Dietary protein is degraded by ruminal microorganisms to peptides and amino acids which can then be fermented to provide carbon skeletons (branched chain VFA) and ammonia for the rest of the microbial population (Hungate, 1966). When carbohydrate availability is limited, protein fermentation can produce excess ruminal ammonia that is not used by the animal and is excreted as urinary urea (Chalupa, 1976).

The ruminant animal does not efficiently convert feedstuffs into meat or milk since it takes more than five pounds of feed (grain) to produce one pound of beef. As a result, several strategies have been used to improve ruminant feed efficiency, such as heat-treating (to alter protein structure) or coating diets with inert ingredients (*e.g.*, oils) to make them unavailable to the ruminal microorganisms, allowing nutrients to by-pass the ruminal fermentation. Another technique to improve the efficiency of the fermentation has included the addition of antimicrobial compounds in the diet to alter the ruminal microbial ecosystem.

Today, the antimicrobials most commonly used in beef cattle production are ionophores. Lasalocid (Bovatec™), monensin (Rumensin™) and laidlomycin propionate (Cattlyst™) are ionophores with combined yearly sales of more than $150 million. The cost to benefit ratio of ionophore usage has been estimated to save the cattle industry approximately $1 billion per year. The most commonly used, and most well-understood ionophore in the United States is monensin, therefore this review will discuss monensin at length, however the effects and mechanisms of other feed-grade ionophores are generally similar.

Why are Ionophores Used in Cattle Rations?

Ionophores cause cattle to grow more efficiently (Russell and Strobel, 1989) but were originally used to control intestinal parasites (coccidiostat) in poultry (Bergen and Bates, 1984). Monensin has been marketed for cattle as a methane inhibitor and propionate (the most efficiently utilized [gluconeogenic] VFA) enhancer (Dinius*, et al.*, 1976; Richardson*, et al.*, 1976; Russell and Strobel, 1989). Additional benefits of monensin usage include a reduction of dietary protein deamination, resulting in less ammonia urinary excretion (Russell and Strobel, 1989), and a decrease in lactic acid production (Dennis*, et al.*, 1981) which results in a reduction in ruminal acidosis (Russell and Strobel, 1989) and liver abscesses (Nagaraja and

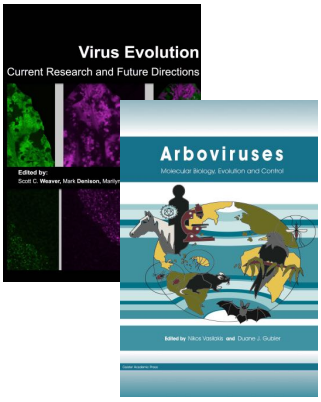
"Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product, and exclusion of others that may be suitable."

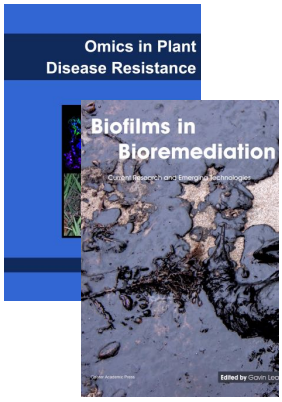
*For correspondence. Email callaway@ffsru.tamu.edu.

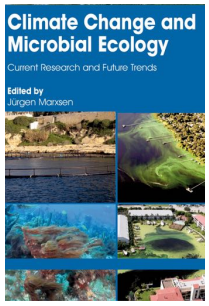
Further Reading

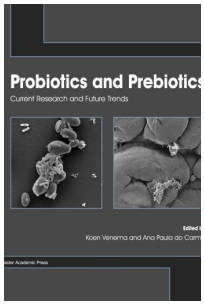
Caister Academic Press is a leading academic publisher of advanced texts in microbiology, molecular biology and medical research. Full details of all our publications at **caister.com**

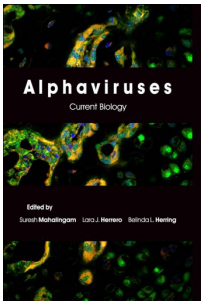
- **MALDI-TOF Mass Spectrometry in Microbiology**
  Edited by: M Kostrzewa, S Schubert (2016)
  www.caister.com/malditof
- ***Aspergillus* and *Penicillium* in the Post-genomic Era**
  Edited by: RP Vries, IB Gelber, MR Andersen (2016)
  www.caister.com/aspergillus2
- **The Bacteriocins: Current Knowledge and Future Prospects**
  Edited by: RL Dorit, SM Roy, MA Riley (2016)
  www.caister.com/bacteriocins
- **Omics in Plant Disease Resistance**
  Edited by: V Bhadauria (2016)
  www.caister.com/opdr
- **Acidophiles: Life in Extremely Acidic Environments**
  Edited by: R Quatrini, DB Johnson (2016)
  www.caister.com/acidophiles
- **Climate Change and Microbial Ecology: Current Research and Future Trends**
  Edited by: J Marxsen (2016)
  www.caister.com/climate
- **Biofilms in Bioremediation: Current Research and Emerging Technologies**
  Edited by: G Lear (2016)
  www.caister.com/biorem
- **Microalgae: Current Research and Applications**
  Edited by: MN Tsaloglou (2016)
  www.caister.com/microalgae
- **Gas Plasma Sterilization in Microbiology: Theory, Applications, Pitfalls and New Perspectives**
  Edited by: H Shintani, A Sakudo (2016)
  www.caister.com/gasplasma
- **Virus Evolution: Current Research and Future Directions**
  Edited by: SC Weaver, M Denison, M Roossinck, et al. (2016)
  www.caister.com/virusevol
- **Arboviruses: Molecular Biology, Evolution and Control**
  Edited by: N Vasilakis, DJ Gubler (2016)
  www.caister.com/arbo
- ***Shigella*: Molecular and Cellular Biology**
  Edited by: WD Picking, WL Picking (2016)
  www.caister.com/shigella
- **Aquatic Biofilms: Ecology, Water Quality and Wastewater Treatment**
  Edited by: AM Romaní, H Guasch, MD Balaguer (2016)
  www.caister.com/aquaticbiofilms
- **Alphaviruses: Current Biology**
  Edited by: S Mahalingam, L Herrero, B Herring (2016)
  www.caister.com/alpha
- **Thermophilic Microorganisms**
  Edited by: F Li (2015)
  www.caister.com/thermophile

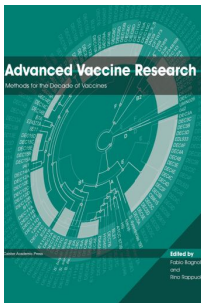


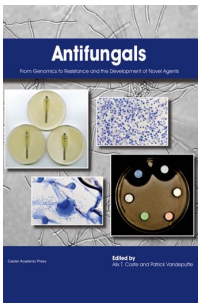












- **Flow Cytometry in Microbiology: Technology and Applications**
  Edited by: MG Wilkinson (2015)
  www.caister.com/flow
- **Probiotics and Prebiotics: Current Research and Future Trends**
  Edited by: K Venema, AP Carmo (2015)
  www.caister.com/probiotics
- **Epigenetics: Current Research and Emerging Trends**
  Edited by: BP Chadwick (2015)
  www.caister.com/epigenetics2015
- ***Corynebacterium glutamicum*: From Systems Biology to Biotechnological Applications**
  Edited by: A Burkovski (2015)
  www.caister.com/cory2
- **Advanced Vaccine Research Methods for the Decade of Vaccines**
  Edited by: F Bagnoli, R Rappuoli (2015)
  www.caister.com/vaccines
- **Antifungals: From Genomics to Resistance and the Development of Novel Agents**
  Edited by: AT Coste, P Vandeputte (2015)
  www.caister.com/antifungals
- **Bacteria-Plant Interactions: Advanced Research and Future Trends**
  Edited by: J Murillo, BA Vinatzer, RW Jackson, et al. (2015)
  www.caister.com/bacteria-plant
- ***Aeromonas***
  Edited by: J Graf (2015)
  www.caister.com/aeromonas
- **Antibiotics: Current Innovations and Future Trends**
  Edited by: S Sánchez, AL Demain (2015)
  www.caister.com/antibiotics
- ***Leishmania*: Current Biology and Control**
  Edited by: S Adak, R Datta (2015)
  www.caister.com/leish2
- ***Acanthamoeba*: Biology and Pathogenesis (2nd edition)**
  Author: NA Khan (2015)
  www.caister.com/acanthamoeba2
- **Microarrays: Current Technology, Innovations and Applications**
  Edited by: Z He (2014)
  www.caister.com/microarrays2
- **Metagenomics of the Microbial Nitrogen Cycle: Theory, Methods and Applications**
  Edited by: D Marco (2014)
  www.caister.com/n2

Order from caister.com/order

Chengappa, 1998). The increases in energy availability and nitrogen retention improve the efficiency of feed utilization by the ruminant animal, and thus improve animal productivity and production profitability (Potter*, et al.*, 1976b; Russell and Strobel, 1989).

Ruminal methane production is decreased 30% by monensin treatment (Wedegaertner and Johnson, 1983; Russell and Strobel, 1989; Johnson and Johnson, 1995). However, ruminal methanogens are not directly inhibited by monensin, rather, the bacteria responsible for cross-feeding nutrients (H_2) to methanogens are inhibited (Van Nevel and Demeyer, 1977; Dellinger and Ferry, 1984). The rate of ruminal methane production is directly correlated with dissolved H_2 concentrations, thus a reduction in (dissolved) H_2 results in reduced methanogenesis (Czerkawski*, et al.*, 1972). Many of the ruminal acetate producing bacteria (*e.g.*, ruminococci, *Eubacterium*) are monensin-sensitive; and because acetate production is linked to the disposal of reducing equivalents via methanogenesis, a decrease in acetate production further reduces methane production (Hegarty, 1999). Moreover, some monensin-sensitive, acetate-producing bacteria also produce formate and H_2, which serve as additional substrates for ruminal methanogenesis (Chen and Wolin, 1979; Slyter, 1979; Russell and Strobel, 1989). Formate degradation by monensin-sensitive species via formate lyase to CO_2 and H_2 is also reduced by monensin treatment, further limiting the substrates available for methanogenesis (Van Nevel and Demeyer, 1977).

Methane and VFA production are closely linked, thus decreases in methane production requires reducing equivalents to be disposed of via alternative electron sinks. Monensin treatment increases production of the most reduced VFA, propionate (Dinius*, et al.*, 1976; Richardson*, et al.*, 1976; Van Nevel and Demeyer, 1977). Many propionate-producing ruminal bacteria (*e.g.*, *Selenomonas ruminantium*, *Megasphaera elsdenii*) are not inhibited by monensin (Callaway*, et al.*, 1999). Alterations in the ruminal population result in a decrease in the acetate to propionate ratio (A:P), a hallmark of increased energy availability to the animal (Russell and Strobel, 1989). Propionate is the gluconeogenic VFA and is more efficiently utilized by the animal than other VFA, and the decrease in the A:P ratio caused by monensin increases gross energy (GE) available to the animal by 5.6% (Richardson*, et al.*, 1976). Despite the observed changes in the ratios of the VFA, total VFA production is not altered by monensin treatment (Russell and Strobel, 1989).

An additional benefit of monensin supplementation is the profound impact on ruminal nitrogen retention (Russell and Strobel, 1989). Ruminal amino acid degradation and resulting ammonia accumulation are decreased 50% by monensin and this phenomenon has been described as a "protein sparing" effect (Dinius*, et al.*, 1976; Russell and Strobel, 1989; Yang and Russell, 1993b). Amino acids in the rumen are fermented by several species of ruminal bacteria (*e.g.*, *M. elsdenii*, prevotellas); however the highest specific activities of ammonia production belong to a group of ruminal bacteria (*i.e.*, *Peptostreptococcus anerobius*, *Clostridium aminophilum*, *C. sticklandii*) that obligately ferment amino acids (Chen and Russell, 1989; Krause and Russell, 1996). Because of their high specific activities of ammonia production, the obligate amino acid-fermenting bacteria are thought to be capable of deaminating over 25% of the protein in feeds (Krause and Russell, 1996). These obligate amino acid-fermenting bacteria are very sensitive to monensin (Callaway*, et al.*, 1997) and their numbers can be reduced 10-fold by monensin treatment (Yang and Russell, 1993a; Krause and Russell, 1996). Much of the decrease in ruminal ammonia production caused by monensin can be specifically attributed to the inhibitory effects on the obligate amino acid-fermenting bacteria.

Monensin treatment reduces morbidity and mortality among feedlot animals by reducing the incidence of acute and sub-acute ruminal acidosis, bloat, and bovine emphysema (Galyean and Owens, 1988). Dietary carbohydrates are rapidly fermented in the rumen which can result in an accumulation of lactic acid, resulting in a lowered ruminal pH, ruminal dysfunction and acidosis (Nagaraja*, et al.*, 1982; Burrin and Britton, 1986; Russell and Rychlik, 2001). Ruminal acidosis is associated with reduced feed intake, lowered feed efficiency and cyclic feeding, as well as the death of some animals. Monensin reduces acidosis by directly inhibiting the lactate-producing bacteria (*e.g.*, *Streptococcus bovis*, lactobacilli) (Dennis*, et al.*, 1981). Bloat is a serious animal production problem that occurs when fermentation gases are retained in the ruminal fluid rather than eructated, often due to an increase in the viscosity of the ruminal fluids, resulting in an accumulation of gas that causes a "swelling" of the rumen that can asphyxiate the animal (Bartley*, et al.*, 1985; Katz*, et al.*, 1986). The anti-bloat effects of monensin are mediated by a direct inhibition of encapsulated ("slime-producing") bacteria, as well as a decrease in overall ruminal gas production (Galyean and Owens, 1988). Bovine emphysema is another costly problem to the cattle industry that is caused by the eructation of 3-methylindole (skatole), a byproduct of L-tryptophan fermentation, which can be inhaled by the animal, causing asphyxiation (Honeyfield*, et al.*, 1985). Monensin directly inhibits skatole-producing lactobacilli (Honeyfield*, et al.*, 1985).

Because of the above changes in the ruminal fermentation, the feed efficiency and overall health of feedlot cattle is improved by monensin inclusion (Goodrich*, et al.*, 1984). Feed intake is decreased in a dose response fashion by monensin, but average daily gain remains unchanged (Raun*, et al.*, 1976; Goodrich*, et al.*, 1984) which results in an increase in feed efficiency of 10-17% (Potter*, et al.*, 1976a; Potter*, et al.*, 1976b). Fortunately, monensin does not affect carcass measurements or quality (Potter*, et al.*, 1976b); but carcasses from steers treated with monensin tend to be heavier and fatter, with higher marbling scores and yield grades (Steen*, et al.*, 1978).

How do Ionophores Work?

All improvements in animal productivity caused by ionophore treatment represent a secondary effect caused by the disruption of normal bacterial membrane physiology (Bergen and Bates, 1984). Ionophores are moderate molecular weight compounds (~700 MW) that are mobile

ion carriers (Pressman, 1976). Because ionophores are highly lipophilic, they rapidly dissolve into bacterial cell membranes (Pressman, 1976). Ionophores bind ions, shield the ionic charges and translocate ions across the bacterial membrane, disrupting crucial ion gradients (Pressman, 1976).

Bacterial membranes are relatively impermeable to ions, allowing ionic gradients to be utilized as a driving force for nutrient uptake (Mitchell, 1961; Rosen, 1986). Ruminal bacteria maintain high intracellular potassium and low intracellular sodium concentrations (Chow and Russell, 1992) and conversely, the ruminal environment contains high sodium and low potassium concentrations. Thus, ruminal bacteria rely heavily upon ion gradients (both K^+ and Na^+ gradients) to take up nutrients and to establish a proton motive force (PMF) (Rosen, 1986; Dawson and Boling, 1987; Van Kessel and Russell, 1992). Ruminal pH is somewhat acidic due to VFA concentrations, however the intracellular pH of many ruminal bacteria is near neutral, thus creating an inwardly directed proton gradient (Russell and Strobel, 1989).

Monensin, is a metal/proton antiporter that can exchange H^+ for either Na^+ or K^+ (Pressman, 1976; Russell and Strobel, 1989). Once inserted in the membrane, monensin exchanges intracellular potassium ions for extracellular protons, or extracellular sodium for intracellular protons (Russell, 1987) (Figure 1). Because the potassium gradient is greater than the sodium gradient, protons accumulate inside the bacterium (Chow*, et al.*, 1994). The bacterium reacts to this cytoplasmic acidification by activating a reversible ATPase to pump these protons out of the cell (Booth, 1985). Additionally, other ATP-utilizing primary pumps for Na^+ removal and K^+ uptake are activated to reestablish ion gradients; resulting in the uncoupling of ATP hydrolysis from growth, thereby decreasing intracellular ATP pools, leading to cellular death (Russell, 1987; Russell and Strobel, 1989).

Which Bacteria are Sensitive to Ionophores?

Because ionophores are lipophilic compounds that exert their effects at the membrane level, they are most effective against gram-positive bacteria. The peptidoglycan layer that surrounds gram-positive bacteria is porous, and allows small molecules to pass through, reaching the cytoplasmic membrane where the lipophilic ionophore rapidly dissolves into the membrane. Conversely, gram-negative bacteria, are separated from the environment and antimicrobial agents by a lipopolysaccharide layer, outer membrane and periplasmic space. Monensin is bound by both gram-positive and –negative bacteria (Chow, *et al.*, 1994); however the cascade of monensin-catalyzed effects has only been shown to occur in gram-positive species (Russell, 1987; Chow and Russell, 1990; Kajikawa and Russell, 1992). *Escherichia coli*, a gram-negative bacterium of significant importance to food safety and human health, is insensitive to ionophore addition (Buchko*, et al.*, 2000; Edrington*, et al.*, 2003b) unless the outer membrane is removed (Ahmed and Booth, 1981). Thus, it has been generally believed that monensin treatment provides gram-negative bacteria (ionophore-insensitive) a competitive advantage by specifically inhibiting ionophore-sensitive gram-positive species (Chen and Wolin, 1979; Russell and Strobel, 1989; Newbold*, et al.*, 1993). Phylogenetically-based hybridization probes have proven useful in determining the effect of monensin on the microbial ecology

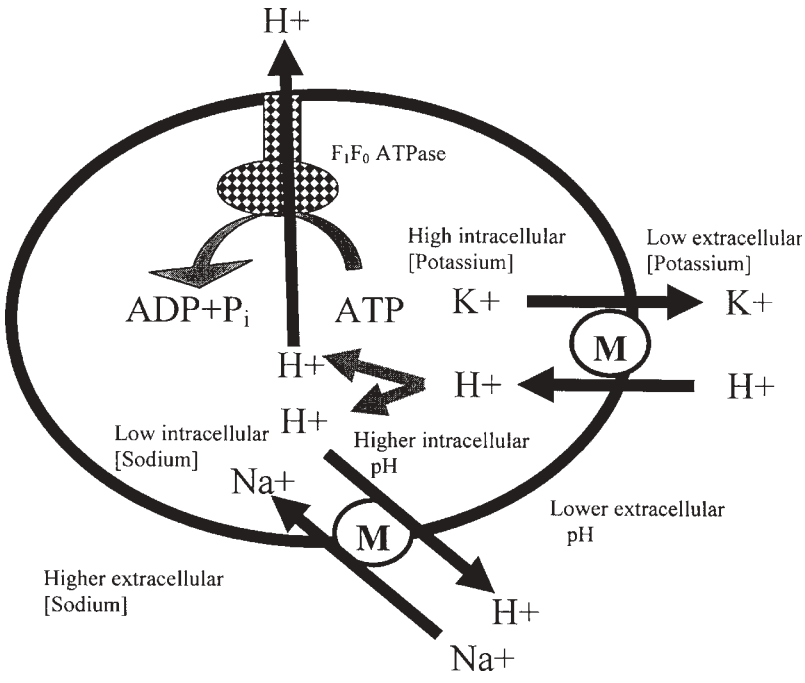


Figure 1. Gradient dissipating mechanism of monensin.

of the rumen (Stahl*, et al.*, 1988). Preliminary 16S rRNA studies demonstrated, however, that no dramatic microbial population shift occurred in the rumen following the initiation of monensin treatment (Stahl*, et al.*, 1988).

Recently, an alternative theory of the effects of monensin on the ruminal microbial ecosystem has emerged following the development of potassium depletion assays to assess ionophore sensitivity (Lana and Russell, 1996; Callaway*, et al.*, 1999). When bacteria are treated with various concentrations of ionophores, potassium is depleted from the cells in a dose-dependent fashion exhibiting Michaelis-Menten kinetics (Lana and Russell, 1996). The growth of pure cultures of gram-positive bacteria was always reduced by monensin treatment, and potassium was depleted from several gram-positive ruminal species (Callaway*, et al.*, 1999). When the gram-negative *Escherichia coli* O157:H7 or K12 (ionophore-insensitive) were treated with monensin, growth was not inhibited (Buchko*, et al.*, 2000), nor was potassium depleted (Callaway*, et al.*, 1999); however, this is not the case for all gram-negative bacteria (Callaway and Russell, 2000). Some gram-negative ruminal bacteria are initially as sensitive to monensin treatment as some gram-positive species; however the development of monensin resistance occurs in both gram-positive and negative species (Callaway and Russell, 2000; Rychlik and Russell, 2002). Because of the ability of ruminal bacterial species to become more resistant to ionophores, it appears that use of monensin in the animal could alter the ruminal microbial ecosystem by selecting for ionophore-resistant members of the microbial population.

What Mechanisms of Ionophore Resistance Exist?

Though some ruminal bacteria are insensitive to ionophores, some ionophore-sensitive bacteria are able to utilize specialized membrane-associated uncoupler translocases to transport uncouplers (*e.g.*, ionophores) from the cell membrane across the periplasm and outer membrane (Lewis*, et al.*, 1994). A rapid translocation of the monensin molecule could be a feasible mechanism for increasing monensin resistance in some gram-negative species. The need for the translocase system to be induced by exposure to the ionophore could explain the initial sensitivity and rapid appearance of resistance seen in some ruminal bacterial species. However, the presence of a monensin translocase in ruminal bacteria has yet to be demonstrated.

Prevotella ruminicola is a gram-negative ruminal bacteria that is initially monensin sensitive, but quickly becomes monensin-resistant (Chen and Wolin, 1979; Newbold and Wallace, 1989; Newbold*, et al.*, 1993). Research found that ionophore-adapted cells did not bind as much ionophore as did unadapted controls (Newbold and Wallace, 1989). Ionophore-adapted cultures could ferment dipeptides, but lost the ability to ferment tripeptides (Newbold and Wallace, 1989). Therefore, it was hypothesized that *P. ruminicola* excluded ionophores from the cell membrane by reducing porin size to prevent ionophores reaching the inner membrane (Newbold and Wallace, 1989; Newbold*, et al.*, 1993). *Prevotella bryantii* and other closely related ruminal *Prevotella* strains can quickly adapt to grow in the presence of ionophores by selecting for a highly resistant subpopulation with altered outer membrane characteristics (Callaway and Russell, 1999).

Although gram-positive ruminal bacteria are initially quite monensin-sensitive they can also increase their monensin-resistance (Callaway*, et al.*, 1999). Some low G+C gram-positive ruminal bacteria (*i.e.*, *Streptococcus bovis*, *Clostridium aminophilum* and *S. ruminantium*) were highly sensitive to monensin upon first exposure, but were able to significantly increase their resistance to monensin following repeated exposures (Callaway*, et al.*, 1999). The resistance acquired by *Clostridium aminophilum* appeared to be mediated by an increase in extracellular polysaccharide that prevented binding of monensin to the cell wall (Rychlik and Russell, 2002). The mechanisms by which resistance is increased in other gram-positive species have not been clearly elucidated. Thus it appears that several mechanisms of action can be used by ruminal bacteria to increase their resistance to ionophores. Yet, the observed changes in the rumen appear to be a result of the selection of intrinsically resistant organisms as opposed to the emergence of novel traits; however, further work is needed to investigate the physiological mechanisms of monensin resistance.

Does Ionophore Resistance Actually Occur in the Animal?

In spite of the evidence that some ruminal bacteria can become resistant to monensin *in vitro* and that ionophore-insensitive species could be selected for by long-term monensin treatment, the monensin-catalyzed improvements in feed efficiency have persisted for 30 years following the introduction of monensin into the diets of cattle being grown out in feedlots (Vogel, 1996; Rogers*, et al.*, 1997). Some researchers have demonstrated that during long-term supplementation the beneficial effects of monensin on the ruminal fermentation have been shown to decrease (Poos*, et al.*, 1979; Perry*, et al.*, 1983; Rumpler*, et al.*, 1986; Lana*, et al.*, 1997). However other research has demonstrated that the beneficial effects of monensin can persist for over 400 d (Hanke*, et al.*, 1985; Rogers*, et al.*, 1997). Interestingly, a daily or weekly ionophore rotation scheme has been shown to improve feed efficiency more than feeding a single ionophore (Galyean and Owens, 1988; Galyean and Hubbert, 1989), and the benefits of alternating ionophores have been hypothesized to be due to a reduced adaptation to ionophores (Galyean and Owens, 1988).

In an effort to determine if ruminal bacteria increased their *in vivo* ionophore-resistance, potassium depletion was used to estimate sensitivity. Mixed ruminal bacteria from cattle fed feedlot rations quickly lose potassium when treated with ionophores (Lana and Russell, 1996). When cattle were fed monensin, the resistance of the mixed ruminal bacterial population to monensin (as measured by potassium depletion) was significantly greater within 3 d of initiation of monensin feeding (Lana and Russell, 1996). Yet in spite of the widespread and indiscriminant use of

ionophores (as growth promotants in ruminants and monogastrics in the United States and in Europe), highly ionophore-resistant isolates have been only rarely isolated from animals (Aarestrup*, et al.*, 1998; Aarestrup*, et al.*, 2000). Taken collectively, these results suggest that the increase observed in *in vivo* monensin-resistance as measured by potassium depletion can be explained by an increase in the physiological resistance of many bacterial species, but that this increase in resistance does not necessarily lead to complete monensin insensitivity of the ruminal microbial population.

Does Ionophore Treatment Affect Antibiotic Resistance?

The use of antimicrobials as growth promotants in food animals has come under increased fire in recent years (APUA, 1999). Concerns have been raised not only about the development of resistance of antibiotics used in animal medicine, but about the development and dissemination of cross-resistance to entire classes of antibiotics. Additionally, the role of commensal bacteria in harboring and disseminating transferable resistance elements has become more of a cause for serious concern (APUA, 1999). Antibiotic resistance has become even more important in modern times due to the increasing number of immunocompromised, young and elderly population. Antibiotic resistance has increased in recent years as confirmed by gene transfer studies (Shoemaker*, et al.*, 2001), yet when assessing resistant organisms, it needs to be determined if the resistance is a result of the selection of intrinsically resistant populations or the emergence of novel resistant populations. Moreover, whether these genes are transmissible or not needs to be addressed as well.

The antibiotic avoparcin was used as a growth promoter in Norway from 1986 until it was banned in 1997. Avoparcin is an analog of the theraputic antibiotic vancomycin, and its ban was due to an increase in vancomycin resistant enterococci isolated from sewage, animal feces and raw meat (Woodford, 1998). Vancomycin resistance is a stable phenotype and vancomycin resistant organisms were still isolated three years after its ban (Borgen*, et al.*, 2001). Avoparcin was never used as a growth promoter in the United States (Woodford, 1998)), and changes in antibiotic usage in U.S. hospitals have appeared to decrease the prevalence of vancomycin resistance (Fridkin*, et al.*, 2002).

Cells have developed several mechanisms to combat the lethal effects of antibiotics. The vancomycin-resistant bacteria mentioned above have altered peptidoglycan which prevents the antibiotic from binding (Woodford, 1998). Fluroquinone-resistant gram-positive bacteria have not only mutated target enzymes (DNA gyrase, topoisomerase IV), but also activated native pumps to extrude the antibiotic (Hooper, 2002). Some of the pumps are highly specific and others have a broad specificity (Paulsen*, et al.*, 1996; Schnappinger and Hillen, 1996). It appears that many organisms have numerous pumps and the *E. coli* genome encodes for 29 multi-drug export systems (Saier*, et al.*, 1998). These pumps extrude metals and ions as well as antibiotics, biocides and drugs, moreover, they have not arose recently, but have been encoded in the genomes for millions of years (Saier*, et al.*, 1998). All of the pumps are energy dependent, (ie., driven by proton motive force (multi-drug efflux proteins) or by ATP hydrolysis (pump P glycoprotein)) (Paulsen*, et al.*, 1996). Dutch researchers demonstrated that the efflux of toxic molecules by *Lactococcus lactis* was inhibited when the PMF and intracellular ATP was depleted by treatment with nigericin (Bolhuis*, et al.*, 1994). Since monensin also dissipates ionic gradients and depletes intracellular ATP levels, it follows that multidrug resistant pumps would be inactive in monensin sensitive cells; however, this hypothesis has yet to be confirmed. In a recent *in vivo* study, ionophore treatment did not affect the antimicrobial susceptibility of *E. coli* O157:H7 or *Salmonella enteritidis* Typhimurium isolates from the gastrointestinal tract of sheep fed several different feed-grade antimicrobials (Edrington*, et al.*, 2003a).

Other researchers (Dealy and Moeller, 1977) have reported that feeding the ionophore-like antimicrobial bambermycin decreased the percentage of *E. coli* resistant to streptomycin and oxytetracycline and the percentage of *E. coli* that were resistant to multiple antibiotics. Feeding low levels of bambermycin to swine has been shown to reduce the numbers of tetracycline-resistant *E. coli* (Sokol*, et al.*, 1973). Further experiments showed that bambermycin treatment reduced the number of *E. coli* resistant to streptomycin and sulfonamides (Federic and Sokol, 1973). Recent studies (Edrington*, et al.*, 2003a) have reported the number of *E. coli* isolates resistant to streptomycin appeared to be less in bambermycin treated sheep as compared with control treatments and *E. coli* isolates showed resistance to fewer antibiotics compared with controls.

Do Ionophores Affect Pathogenic Bacterial Populations?

The effect of ionophores on pathogenic bacterial populations has attracted interest, largely due to the temporal relationship between the introduction of ionophore use in the U.S. cattle industry and an increase in recognized *E. coli* O157:H7 cases (Griffin and Tauxe, 1991; Rasmussen*, et al.*, 1999). Early surveys and experiments in cattle yielded conflicting results as to the effect of ionophores on pathogen levels and shedding. Some studies have reported no association between fecal shedding of *E. coli* O157:H7 and ionophore use in dairy calves (Garber*, et al.*, 1995) or in feedlot cattle (Dargatz*, et al.*, 1997). In a survey of 100 United States feedlots no difference was found in the number of fecal samples positive for *Salmonella* when cattle were fed ionophores (Losinger*, et al.*, 1997). However, in a contradictory study, the prevalence of *E. coli* O157 was higher in dairy herds that used monensin or lasalocid in their heifer rations compared with herds not using these ionophores (Herriott*, et al.*, 1998). Bambermycin (an ionophore-like feed grade antimicrobial) supplemented calves contained similar intestinal *E. coli* populations compared with control calves (Dealy and Moeller, 1977). Bambermycin supplementation reduced the duration and prevalence of *Salmonella*

Typhimurium shedding in experimentally infected calves and swine (Dealy and Moeller, 1976;1977).

Until recently, there have been very few studies directed at understanding the microbial ecology of food borne pathogens in the gut of food animals. A recent series of experiments was performed to evaluate the effects of the ionophores monensin, lasalocid, laidlomycin propionate and the ionophore-like antimicrobial, bambermycin on four *Salmonella* serotypes and two strains of *E. coli* O157:H7 in both pure and mixed ruminal fluid culture (Edrington*, et al.*, 2003b). Overall, they reported no effects of ionophore treatment on *E. coli* O157:H7 or *Salmonella* with respect to growth rates in pure culture or populations in *in vitro* mixed ruminal fluid incubations (Edrington*, et al.*, 2003b). Likewise, short-term ionophore feeding in growing lambs had no effect on fecal shedding or gastrointestinal concentrations of *E. coli* O157:H7 or *Salmonella* (Edrington*, et al.*, 2003a). These authors also reported that bambermycin feeding did not affect fecal shedding of *Salmonella* or *E. coli* O157:H7 in lambs (Edrington*, et al.*, 2003a).

The lack of direct ionophore effects on these pathogens of interest may be attributable to the physiological characteristics of the double membrane of gram-negative bacteria (Ahmed and Booth, 1981). Ionophore exposure may have inhibited gram-positive species in the above studies, however ionophores did not appear to benefit *Salmonella* or *E. coli* populations in the gastrointestinal microbial ecosystem.

Conclusions

Ionophores are potent antimicrobial compounds that improve production efficiency and health in cattle by altering the composition of the ruminal microbial ecosystem, thereby reducing the incidence of illnesses related to the ruminal fermentation (*e.g.*, bloat, bovine emphysema). However, like all other antimicrobial compounds, concerns have been raised about the development of antimicrobial resistance and the potential for the transfer of cross-resistance to antibiotics used in human medicine. It appears that ionophores do not promote the development or dissemination of antibiotic resistance, likely due to the complex nature and high degree of specificity of ionophore resistance mechanisms. Therefore, it appears that the use of ionophores will continue to be an important tool in improving animal production efficiency.

References

Aarestrup, F.M., Bager, F., Jensen, N.E., Madsen, M., Meyling, A., and Wegener, H.C. 1998. Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. APMIS. 106: 606-622.

Aarestrup, F.M., Kruse, H., Tast, E., Hammerum, A.M., and Jensen, L.B. 2000. Associations between the use of antimicrobial agents for growth promotion and the occurence of resistance among *Enterococcus faecium* from broilers and pigs in Denmark, Finland, and Norway. Microb. Drug. Resis. 6: 63-70.

Ahmed, S., and Booth, I.R. 1981. Quantitative measurements of the proton-motive force and its relation to steady state lactose accumulation in *Escherichia coli*. Biochem. J. 200: 573-581.

APUA. 1999. Facts about antibiotics in animals and their impact on resistance. Alliance for the Prudent Use of Antibiotics. 23 October 2002. Available at: http: // www.tufts.edu/med/apua/Ecology/faair.html.

Bartley, E.E., Nagaraja, T.G., Pressman, E.S., Dayton, A.D., Katz, M.P., and Fina, L.R. 1985. Effects of lasalocid or monensin on legume or grain (feedlot) bloat. J. Anim. Sci. 56: 1400-1406.

Bergen, W.G., and Bates, D.B. 1984. Ionophores: Their effect on production efficiency and mode of action. J. Anim. Sci. 58: 1465-1483.

Blaxter, K. 1962. The energy metabolism of ruminants. In: The energy metabolism of ruminants. K. Blaxter, ed. Charles C. Thomas, Springfield, IL. p. 197-200.

Bolhuis, H., Molenaar, D., Poelarends, G., van Veen, H.W., Poolman, B., Driessen, A.J., and Konings, W.N. 1994. Proton motive force-driven and ATP-dependent drug extrusion systems in multidrug-resistant *Lactococcus lactis*. J. Bacteriol. 176: 6957-6964.

Booth, I.R. 1985. Regulation of cytoplasmic pH in bacteria. Microbiol. Rev. 49: 359-378.

Borgen, K., Sorum, M., Wasteson, Y., and Kruse, H. 2001. VanA-type vancomycin-resistant Enterococci (VRE) remain prevalent in poultry carcasses 3 years after avoparcin was banned. Int. J. Food Microbiol. 64: 89-94.

Buchko, S.J., Holley, R.A., McAllister, T.A., McMahon, L.R., and Veira, D.M. 2000. Effect of monensin on *E. coli* O157: H7 and *E. coli* ATCC 25922. Can. J. Anim. Sci. 80: 777.

Burrin, D.G., and Britton, R.A. 1986. Response to monensin in cattle during subacute acidosis in cattle. J. Anim. Sci. 63: 888-893.

Callaway, T.R., Adams, K.A., and Russell, J.B. 1999. The ability of "low G+C gram-positive" ruminal bacteria to resist monensin and counteract potassium depletion. Curr. Microbiol. 39: 226-230.

Callaway, T.R., Carneiro De Melo, A.M.S., and Russell, J.B. 1997. The effect of nisin and monensin on ruminal fermentation *in vitro*. Curr. Microbiol. 35: 90-96.

Callaway, T.R., and Russell, J.B. 1999. Selection of a highly monensin-resistant *Prevotella bryantii* subpopulation with altered outer membrane characteristics. Appl. and Environ. Microbiol. 65: 4753-4759.

Callaway, T.R., and Russell, J.B. 2000. Variations in the ability of ruminal gram-negative *Prevotella* species to resist monensin. Curr. Microbiol. 40: 185-189.

Chalupa, W. 1976. Degradation of amino acids by the mixed rumen microbial population. J. Anim. Sci. 43: 828-834.

Chen, G., and Russell, J.B. 1989. More monensin-sensitive, ammonia-producing bacteria from the rumen. Appl. Environ. Microbiol. 55: 1052-1057.

Chen, M., and Wolin, M.J. 1979. Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria. Appl. Enviro. Microbiol. 38: 72-77.

Chow, J.M., and Russell, J.B. 1990. Effect of ionophores and pH on growth of *Streptococcus bovis* in batch and

continuous culture. Appl. Environ. Microbiol. 56: 1588-1593.
Chow, J.M., and Russell, J.B. 1992. Effect of pH and monensin on glucose transport by *Fibrobacter succinogenes*, a cellulolytic ruminal bacterium. Appl. Environ. Microbiol. 58: 1115-1120.
Chow, J.M., Van Kessel, J.A.S., and Russell, J.B. 1994. Binding of radiolabeled monensin and lasalocid to ruminal microorganisms and feed. J. Anim. Sci. 72: 1630-1635.
Czerkawski, J.W., Harfoot, C.G., and Breckenridge, G. 1972. The relationship between methane production and concentrations of hydrogen in the aqueous and gaseous phases during rumen fermentation *in vitro*. J. Appl. Bacteriol. 35: 537-551.
Dargatz, D.A., Wells, S.J., Thomas, L.A., Hancock, D.D., and Garber, L.P. 1997. Factors associated with the presence of *Escherichia coli* O157 in feces of feedlot cattle. J. Food Prot. 60: 466-470.
Dawson, K.A., and Boling, J.A. 1987. Effects of potassium ion concentrations on the antimicrobial activities of ionophores against ruminal anaerobes. Appl. Environ. Microbiol. 53: 2363-2367.
Dealy, J., and Moeller, M.W. 1976. Influence of bambermycins on *Salmonella* infection and antibiotic resistance in swine. J. Anim. Sci. 42: 1331-1336.
Dealy, J., and Moeller, M.W. 1977. Influence of bambermycins on *Salmonella* infection and antibiotic resistance in calves. J. Anim. Sci. 44: 734-738.
Dellinger, C.A., and Ferry, J.G. 1984. Effect of monensin on growth and methanogenesis of *Methanobacterium formicicum*. Appl. Environ. Microbiol. 48: 680-682.
Dennis, S.M., Nagaraja, T.G., and Bartley, E.E. 1981. Effects of lasalocid or monensin on lactate-producing or -using rumen bacteria. J. Anim. Sci. 52: 418-426.
Dinius, D.A., Simpson, M.E., and Marsh, P.B. 1976. Effect of monensin fed with forage on digestion and the ruminal ecosystem of steers. J. Anim. Sci. 42: 229-234.
Edrington, T.S., Callaway, T.R., Bischoff, K.M., Genovese, K.J., Elder, R.O., Anderson, R.C., and Nisbet, D.J. 2003a. Effect of feeding the ionophores monensin and laidlomycin propionate and the antimicrobial bambermycin to sheep experimentally infected with *E. coli* O157: H7 and *Salmonella* Typhimurium. J. Anim. Sci. 81: 553-560.
Edrington, T.S., Callaway, T.R., Varey, P.D., Jung, Y.S., Bischoff, K.M., Elder, R.O., Anderson, R.C., Kutter, E., Brabban, A.D., and Nisbet, D.J. 2003b. Effects of the antibiotic ionophores monensin, lasalocid, laidlomycin propionate and bambermycin on *Salmonella* and *E. coli* O157: H7 *in vitro*. J. Appl. Microbiol. 94: 207-213.
Federic, F., and Sokol, A. 1973. Effect of peroral application of a colicinogenic strain of *Escherichia coli* on the incidence of different categories of plasmids in *Escherichia coli* isolated from weaned piglets fed on flavomycin. Folia Microbiol. 18: 65.
Fridkin, S.K., Lawton, R., Edwards, J.R., Tenover, F.C., McGowan, J.E., and Gaynes, R.P. 2002. Monitoring antimicrobial use and resistance: comparison with a national benchmark on reducing vancomycin use and vancomycin-resistant Enterococci. Emerg. Infect. Dis. 8: 702-707.
Galyean, M.L., and Owens, F.N. 1988. Effects of monensin on growth, reproduction, and lactation in ruminants. In: ISI Atlas of Science: Anim. Plant Sci. ed. ISI Press, Philadelphia, PA. p. 71-75
Garber, L.P., Wells, S.J., Hancock, D.D., Doyle, M.P., Tuttle, J., Shere, J.A., and Zhao, T. 1995. Risk factors for fecal shedding of *Escherichia coli* O157: H7 in dairy calves. J. Amer. Vet. Med. Assoc. 207: 46-49.
Goodrich, R.D., Garrett, J.E., Gast, D.R., Kirick, M.A., Larson, D.A., and Meiske, J.C. 1984. Influence of monensin on the performance of cattle. J. Anim. Sci. 58: 1484-1498.
Griffin, P.M., and Tauxe, R.V. 1991. The epidemiology of infection caused by *Escherichia coli* O157: H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13: 60-97.
Hegarty, R.S. 1999. Mechanisms for competitively reducing ruminal methanogenesis. Aust. J. Agric. Rev. 50: 1299-1305.
Herriott, D.E., Hancock, D.D., Ebel, E.D., Carpenter, L.V., Rice, D.H., and Besser, T.E. 1998. Association of herd management factors with colonization of dairy cattle by shiga toxin-positive *Escherichia coli* O157. J. Food Prot. 61: 802-807.
Honeyfield, D.C., Carlson, J.R., Nocerini, M.R., and Breeze, R.G. 1985. Duration and inhibition of 3-methylindole production by monensin. J. Anim. Sci. 60: 226-231.
Hooper, D.C. 2002. Fluoroquinolone resistance among Gram-positive cocci. Lancet. Infect. Dis. 2: 530-538.
Hungate, R.E. 1966. The rumen and its microbes. Academic Press, New York, NY.
Johnson, K.A., and Johnson, D.E. 1995. Methane emissions from cattle. J. Anim. Sci. 73: 2483-2494.
Kajikawa, H., and Russell, J.B. 1992. The effect of ionophores on proton flux in the ruminal bacterium, *Streptococcus bovis*. Curr. Microbiol. 25: 327-330.
Katz, M.P., Nagaraja, T.G., and Fina, L.R. 1986. Ruminal changes in monensin and lasalocid fed cattle grazing bloat provocative alfalfa pasture. J. Anim. Sci. 63: 1246-1257.
Krause, D.O., and Russell, J.B. 1996. An rRNA approach for assessing the role of obligate amino acid fermenting bacteria in ruminal amino acid degradation. Appl. Environ. Microbiol. 62: 815-821.
Lana, R.P., Fox, D.G., Russell, J.B., and Perry, T.C. 1997. Influence of monensin on holstein steers fed high-concentrate diets containing soybean meal or urea. J. Anim. Sci. 75: 2571-2579.
Lana, R.P., and Russell, J.B. 1996. Use of potassium depletion to assess adaption of ruminal bacteria to ionophores. Appl. Environ. Microbiol. 62: 4499-4503.
Lewis, K., Naroditskaya, V., Ferrante, A., and Fokina, I. 1994. Bacterial resistance to uncouplers. J. Bioenerg. Biomemb. 20: 639-646.
Losinger, W.C., Garber, L.P., Smith, M.A., Hurd, H.S., Biehl, L.G., Fedorka-Cray, P.J., Thomas, L.A., and Ferris, K. 1997. Management and nutritional factors associated with the detection of *Salmonella* sp. from cattle fecal specimens from feedlot operation in the United States. Prev. Vet. Med. 31: 231-244.
Mitchell, P. 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of

mechanism. Nature. 191: 144-148.
Nagaraja, T.G., Avery, T.B., Bartley, E.E., Roof, S.K., and Dayton, A.D. 1982. Effect of lasalocid, monensin or thiopeptin on lactic acidosis in cattle. J. Anim. Sci. 54: 649-658.
Nagaraja, T.G., and Chengappa, M.M. 1998. Liver abscesses in feedlot cattle: A review. J. Anim. Sci. 76: 287-298.
Newbold, C.J., and Wallace, R.J. 1989. Changes in the rumen bacterium, *Bacteroides ruminicola*, grown in the presence of the ionophore, tetronasin. Asian Austral. J. Anim. Sci. 2: 452-453.
Newbold, C.J., Wallace, R.J., and Walker, N.D. 1993. The effect of tetronasin and monensin on fermentation, microbial numbers and the development of ionophore-resistant bacteria in the rumen. J. Appl. Bacteriol. 75: 129-134.
Owens, F.N. 1988. Protein metabolism of ruminant animals. In: The ruminant animal. D.C. Church, ed. Waveland Press, Prospect Heights, IL. p. 227-249
Paulsen, I.T., Brown, M.H., and Skurray, R.A. 1996. Proton-dependent multidrug efflux systems. Microbiol. Rev. 60: 575-608.
Perry, T.W., Shields, D.R., Dunn, W.J., and Mohler, M.T. 1983. Protein levels and monensin for growing and finishing steers. J. Anim. Sci. 57: 1067-1076.
Poos, M.I., Hanson, T.L., and Klopfenstein, T.J. 1979. Monensin effects on diet digestibility, ruminal protein bypass and microbial protein synthesis. J. Anim. Sci. 48: 1516-1524.
Potter, E.L., Cooley, C.O., Richardson, L.F., Raun, A.P., and Rathmacher, R.P. 1976a. Effect of monensin on performance of cattle fed forage. J. Anim. Sci. 43: 665-669.
Potter, E.L., Raun, A.P., Cooley, C.O., Rathmacher, R.P., and Richardson, L.F. 1976b. Effect of monensin on carcass characteristics, carcass composition and efficiency of converting feed to carcass. J. Anim. Sci. 43: 678-683.
Pressman 1976. Biological applications of ionophores. Ann. Rev. Biochem. 45: 501-530.
Rasmussen, M.A., Wickman, T.L., Cray, W.C., and Casey, T.A. 1999. *Escherichia coli* O157: H7 and the rumen environment. In: *E. coli* O157 in Farm Animals. C.S. Stewart and H.J. Flint, ed. CAB International, p. 39
Raun, A.P., Cooley, C.O., Potter, E.L., Rathmacher, R.P., and Richardson, L.F. 1976. Effect of monensin on feed efficiency of feedlot cattle. J. Anim. Sci. 43: 670-677.
Richardson, L.F., Raun, A.P., Potter, E.L., Cooley, C.O., and Rathmacher, R.P. 1976. Effect of monensin on rumen fermentation *in vitro* and *in vivo*. J. Anim. Sci. 43: 657-664.
Rogers, M., Jouany, J.P., Thivend, P., and Fontenot, J.P. 1997. The effects of short-term and long-term monensin supplementation, and its subsequent withdrawal on digestion in sheep. Anim. Feed Sci. Technol. 65: 113-127.
Rosen, B.P. 1986. Recent advances in bacterial ion transport. Ann. Rev. Microbiol. 40: 263-286.
Rumpler, W.V., Johnson, D.E., and Bates, D.B. 1986. The effect of high dietary cation concentration on methanogenesis by steers fed diets with and without ionophores. J. Anim. Sci. 62: 1737-1741.
Russell, J.B. 1987. A proposed mechanism of monensin action in inhibiting ruminal bacterial growth: effects on ion flux and protonmotive force. J. Anim. Sci. 64: 1519-1525.
Russell, J.B., and Rychlik, J.L. 2001. Factors that alter rumen microbial ecology. Science. 292: 1119-1122.
Russell, J.B., and Strobel, H.J. 1989. Effect of ionophores on ruminal fermentation. Appl. Environ. Microbiol. 55: 1-6.
Rychlik, J.L., and Russell, J.B. 2002. The adaptation and resistance of *Clostridium aminophilum* F to the butyrivibriocin-like substance of *Butyrivibrio fibrisolvens* JL5 and monensin. FEMS Microbiol. Lett. 209: 93-98.
Saier, M.H., Paulsen, I.T., Sliwinski, M.K., Pao, S.S., Skurray, R.A., and Nikaido, H. 1998. Evolutionary origins of multidrug and drug-specific efflux pumps in bacteria. FASEB J. 12: 265-274.
Schnappinger, D., and Hillen, W. 1996. Tetracyclines: antibiotic action, uptake, and resistance mechanisms. Arch. Microbiol. 165: 359-369.
Shoemaker, N.B., Vlamakis, H., Hayes, K., and Salyers, A.A. 2001. Evidence for extensive resistance gene transfer among *Bacteroides* spp. and among *Bacteroides* and other genera in the human colon. Appl. and Environ. Microbiol. 67: 561-568.
Slyter, L.L. 1979. Monensin and dichloroacetamide influences on methane and volatile fatty acid production by rumen bacteria*in vitro*. Appl. Environ. Microbiol. 37: 283-288.
Sokol, A., Kremery, V., Federic, F., Fajtar, V., and Janouskova, J. 1973. The influence of flavomycin on the elimination of R-factors of *Escherichia coli in vitro*. Folia Microbiol. 18: 176.
Stahl, D.A., Flesher, B., Mansfield, H.R., and Montgomery, L. 1988. Use of phylogenetically based hybridization probes for studies of ruminal microbial ecology. Appl. Environ. Microbiol. 54: 1079-1084.
Steen, W., Gay, N., Boling, J.A., Bradley, N.W., McCormick, J.W., and Pendlum, L.C. 1978. Effect of monensin on performance and plasma metabolites in growing-finishing steers. J. Anim. Sci. 46: 350-355.
Stewart, C.S., and Bryant, M.P. 1988. The rumen microbial ecosystem. P.N. Hobson. Elsevier Science Publishers, Ltd, London, UK. p. 21.
Van Kessel, J.S., and Russell, J.B. 1992. Energetics of arginine and lysine transport by whole cells and membrane vesicles of strain SR, a monensin-sensitive ruminal bacterium. Appl. Environ. Microbiol. 58: 969-975.
Van Nevel, C.J., and Demeyer, D.I. 1977. Effect of monensin on rumen metabolism *in vitro*. Appl. Enviro. Microbiol. 34: 251-257.
Vogel, G. 1996. A review of large pen research trials. In: Scientific update on Rumensin/Tylan/Micotil for the professional feedlot consultant. ed. Elanco Animal Health, Indianapolis, IN. p. 16.
Wedegaertner, T.C., and Johnson, D.E. 1983. Monensin effects on digestibility, methanogenesis and heat uncrement of a cracked corn-silage diet fed to steers. J. Ani. Sci. 57: 168-177.

Woodford, N. 1998. Glycopeptide-resistant enterococci: a decade of experience. J. Med. Microbiol. 47: 849-862.

Yang, C.M.J., and Russell, J.B. 1993a. The effect of monensin supplementation on ruminal ammonia accumulation *in vivo* and the numbers of amino acid-fermenting bacteria. J. Anim. Sci. 71: 3470-3476.

Yang, M.J., and Russell, J.B. 1993b. Effect of monensin on the specific activity of ammonia production by ruminal bacteria and disappearance of amino nitrogen from the rumen. Appl. Envir. Microbiol. 59: 3250-3254.