The Science and Mechanisms Behind Ionophores for Pigs and Poultry

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Background

Polyether ionophore antibiotics, colloquially referred to as ionophores, are chemical compounds that are made by *Streptomyces* and other actinomycetes (Kevin, 2009). A number of ionophores have been characterized but only a few are used in veterinary medicine. The antibiotic activity of lasalocid was described in 1951 (Berger, 1951), but monensin was the first ionophore to be chemically characterized and commercialized (Chapman, 2010). As such, monensin is the best-studied ionophore of the group and serves as the representative member of this class of antibiotics. The approval of other ionophore antibiotics soon followed and the introduction of new products continued for some 20 years. The last ionophore antibiotic to be approved in the US was semduramicin in 1995 (Jeffers, 2011).

Ionophores are used in both poultry and swine, but for different purposes. Ionophores are used exclusively in poultry as an aid in the prevention of coccidiosis. Six different ionophores are currently available for use as coccidiostats in broiler chickens (Table 1). Narasin (Skycis® 100) has been recently approved for use in growing-finishing swine for improved feed efficiency and increased weight gain (FOI NADA 141-340). Presently, there are no FDA-approved products for the control of coccidiosis in swine. This paper provides an overview of the salient features of ionophores and their use in monogastric species.

Table 1. Ionophore antibiotics used in US poultry[a]

Ionophore	Approval	Trade Name
Lasalocid	1976	Avatec®
Maduramicin	1989	Cygro®
Monensin	1971	Coban®
Narasin	1988	Monteban®
Salinomycin	1983	Bio-Cox®; Sacox®
Semduramicin	1995	Aviax®

[a]Animal Drugs@FDA

Use of Ionophores in Broilers

Coccidiosis is a parasitic disease that causes significant economic losses to the poultry industry in both the US and worldwide (Anonymous, 2013). Ionophores have been key tools in the fight against this disease for over 40 years (Kevin, 2009; Chapman, 2010). The causative agent, *Eimeria*, has a complex lifestyle which involves both intracellular and extracellular life forms. Monensin is active against sporozoites and merozoites, the extracellular motile forms of the parasite (Chapman, 2010). It has been said that the growth of the poultry industry owes much to the introduction of ionophore antibiotics (Jeffers, 2011).

Ionophores achieve their anti-parasitic effects in a unique way. Cells generate electrical energy to transport various substances across the membrane. This is accomplished by actively maintaining high and low concentrations of various ions in the cell relative to the external environment. For example, the intracellular concentration of potassium ions (K+) is higher than that of sodium ions (Na+) and the opposite is true in the extracellular environment. Ionophores transport additional ions such as calcium (Ca^{+2}), potassium (K^{+}) and sodium (Na^{+}) into the cell thereby disrupting these essential ion gradients (Kevin, 2009; Rutkowski, 2013). The cell expends energy to regain ion homeostasis but to no avail.

Ionophore-treated sporozoites and merozoites exhibit morphological changes in vitro such as swelling of the cell and mitochondria, vacuolation, blebbing and disintegration (Smith, 1981; Mehlhorn, 1983; Chapman, 2010). The accumulation of Na^{+} in the cell and the concomitant influx of water results in osmotic damage and the bursting of the cell (Chapman, 2010). In vitro assays have shown that monensin-treated sporozoites lose their ability to invade host cells (Smith, 1981; Augustine, 1986). This may be due to a disruption of membrane signaling pathways that are essential for entry into the host (del Cacho, 2007). In vivo, the treatment of *Eimeria*-infected broilers with lasalocid resulted in the formation of degenerate intracellular sporozoites (Daszak, 1991). Morphologically aberrant merozoites were also observed in damaged host cells and in the lumen (Daszak, 1991).

Ionophore-resistant coccidia have been described for years (Chapman, 2010). However, such isolates are best described as having reduced susceptibility to these antibiotics (Chapman, 2010). Birds infected with such strains have been shown to have reduced weight gains, decreased feed efficiency, higher lesion scores, and higher oocyte production, but not frank disease (Wang, 2006; Chen, 2008; Jenkins, 2010). Changes in parasite susceptibility are associated with decreased membrane fluidity, decreased antibiotic uptake and a host of changes in gene expression (Augustine, 1987; Wang, 2006; Chen, 2008). With regards to the latter, genes involved in the creation of the extracellular matrix were up-regulated and genes involved in energy generation were down-regulated (Chen, 2008). Reduced ionophore susceptibility in *Eimeria* does not necessarily equate with reduced efficacy in the field (Jeffers, 2013). Explanations for this include the inability of *Eimeria* to acquire full resistance to ionophores and the development of immunity in treated birds (Jeffers, 2011).

Despite sanitation measures, *Eimeria* oocytes persist in the rearing environment and birds are continually at risk for infection. As such, anticoccidial agents are administered to birds throughout their lifetime (Anonymous, 2008; Chapman, 2010). These agents include chemicals, ionophores and vaccines. Maximum year-round protection against coccidiosis in successive flocks usually involves the strategic use of all of these tools. Combination products containing an ionophore and a chemical, e.g. narasin and nicarbazin, act synergistically and stem the development of coccidia isolates with reduced susceptibility (Jeffers, 2011). The rotation of products from flock to flock is also effective at maintaining control. The use of a vaccine after a round of treatment of ionophores can help restore full susceptibility to *Eimeria* populations in the rearing environment (Chapman, 2010).

Use of Narasin in Swine

In addition to *Eimeria*, ionophores also have activity against Gram-positive bacteria (Kevin, 2009; Rutkowski, 2013). Gram-negative bacteria are not susceptible to ionophores because the outer membrane prevents their access to the cell membrane. Ionophores kill susceptible bacteria using a mechanism of action that is similar to that in coccidia. However, the ultimate

lethal event in bacteria is the depletion of energy stores. Treated cells do not burst because the rigid bacterial cell wall prevents osmotic rupture. Rather, after an ionophore-mediated influx of ions, the bacterial cell futilely attempts to regain homeostasis until there is not enough cellular ATP left to sustain growth (Russell, 1987; Newbold, 2013). The activity of ionophores against Gram-positive bacteria is an added benefit in treated broilers because it provides additional protection against necrotic enteritis, which is caused by Clostridium perfringens (Golder, 2011).

Narasin increases weight gain and improves feed efficiency in swine by dual mode of action in selectively altering the microbial population in both the foregut and hindgut. Ionophore activity against Gram-positive bacteria is well documented (Kevin, 2009; Rutkowski, 2013) and act in similar manner as other classes of sub-therapeutic antibiotics in the foregut. Wuethrich (1998) demonstrated that pigs fed low levels of narasin (15 ppm) had improvement ($P<0.03$) in nitrogen digestibility and retention. An internal Elanco metabolism study (T1XCA0202) supported the work of Wuethrich (1998) in finding that the addition of 15 ppm of narasin in the diet of growing pigs increases apparent nitrogen retention (62.02 vs. 56.14%), phosphorous retention (47.64 vs. 40.61%) and ileal energy digestibility (2726 Kcal vs. 2660 Kcal). Thacker (1992) using a different ionophore (salinomycin at 25 ppm) in growing pigs noted a similar increase in protein digestibility ($P<0.05$) when rye based diets were supplemented with salinomycin (25 ppm) alone or in conjunction with a beta-glucanase enzyme. **(NOTE – Salinomycin is NOT approved for use in pigs in the United States).**

Similarly, ionophores also alter the micro flora of the hindgut, which can provide up to 20 to 30% of maintenance energy needs (Yen, 1991). Wuethrich and colleagues (1998) examined the impact of an ionophore (narasin at 15 and 30 ppm) on the VFA production in the cecum, second loop of distal colon and transverse colon. Their findings indicate that the dietary addition of an ionophore increased propionic acid concentration relative to acetic or butyric in a dose dependent manner. This shift in VFA production results in an increase of absorbed gluconeogenic precursors and decreases net energy losses through methane gas production as a by-product of acetic and butyric acid fermentation (Wuethrich 1998). Using the energy conversion efficiency of VFA suggested by Bergman (1989), propionic acid will provide 734 Kcal/mole as compare to 419 and 524 Kcal/mole for acetic and butyric acids and therefore increase whole body energetic efficiency.

Difference in Resistance Development

In bacteria, ionophore resistance occurs as a result of persistence. Although bacterial populations are genetically identical, some heterogeneity in gene expression can occur, which results in physiological differences from one cell to another. These slight differences may allow the bacteria to grow in the presence of an antibiotic without the acquisition of true resistance (Levin, 2004; Shah, 2006; Dhar, 2007; Girgis, 2012). For example, persisters that grow more slowly are not killed by ampicillin because the antibiotic targets actively-growing cells (Balaban, 2004). Likewise, persisters that produce extra polysaccharide survive amoxicillin treatment (Girgis, 2012). Persistence is considered to be a survival mechanism under adverse growth conditions and the "resistant" phenotype is lost after the antibiotic is removed (Girgis, 2012). Monensin-treated bacteria exhibit the hallmarks of persistence: slower growth, mixed cultures containing both susceptible and resistant cells, transient resistance and increased capsule production (Russell, 2003; Simjee, 2012).

There are concerns that the use of antibiotics in food animals will lead to the development of antibiotic-resistant foodborne bacteria. Humans exposed to these bacteria may become sick with infections that cannot be effectively treated with antibiotics. The use of ionophores in

monogastric species does not lead to such problems. Gram-positive foodborne bacteria do not acquire ionophore resistance (Butaye, 2003; Aarestrup, 2011; Simjee, 2012). With no monensin-resistant bacteria or inheritable or transferable monensin resistance genes, cross-resistance with other human use antibiotics and co-selection of other types of resistance genes are not a concern. Ionophores are not currently used in human medicine because of their toxicity profiles (see below) and treatment failure is not of concern (Kevin, 2009).

Toxicity

Individual animal species differ in their tolerance to ionophore antibiotics. For example, the oral LD_{50} of monensin in chickens is 200 mg/kg and that of horses is 2-3 mg/kg (Kart, 2008). Toxicity and death can occur when animals are overdosed or if non-target animals intentionally or unintentionally consume ionophores (Kart, 2008; Kevin, 2009). In animals, the interference of ion flux in cells may lead to, among others, cardiac muscle damage, pulmonary edema, and neuropathy (Kart, 2008). The types of adverse events that are observed is animal species-specific (Kart, 2008; Kevin, 2009). Toxicity may also result from the simultaneous treatment of animals with an ionophore antibiotic and tiamulin (Islam, 2009). Tiamulin interferes with the metabolism of ionophores and toxic levels accumulate in the body. Not all dual dosing conditions lead to toxicity (Islam, 2009; Weber, 2013). Toxicity was not observed in swine given full dietary doses of narasin (30 ppm) and tiamulin (40 ppm) (Weber, 2013).

Summary

Polyether ionophores have successfully been used in food animals for the past 40 years; **first in** ruminants to improve performance by altering VFA production patterns, secondly as effective coccidiostatic agents in broilers and lastly to improve growth and feed utilization in growing-finishing swine. Ionophore's unique mode of action by attaching to the cell wall of coccidia and Gram-positive bacteria allowing influx of extracellular ions has proven effective and resulted in a very low incidence of resistance in all target animal species. However, caution must be observed when using ionophores as toxicity is a concern in non-targeted species and concomital treatment of swine with tiamulin. Ionophores have been deemed as a "non-medically important" class of antibiotic by FDA under GFI document #152; thus offering broiler producers effective coccidiosis control and treatment and swine producers an effective growth performance product that can be an alternative to traditional "medically important" antibiotics.

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