Chapter 9

Antibiotic Use in DDGS Production

Introduction

One of the ongoing challenges in fuel ethanol production facilities is to control bacterial contamination during fermentation. Lactic acid producing bacteria (*Lactobacillus, Pediococcus, Leuconostoc, and Weissella*) are the most common contaminants (Bischoff et al., 2009; Leja and Broda, 2009; Muthaiyan and Ricke, 2010; Skinner and Leathers, 2004). Other bacterial contaminants including *Bacteroides forsythus*, *Fusobacterium nucleatum*, *Propionicbacterium granulosum*, and *Clostridium aerotolerans* also have detrimental effects on ethanol production (Leja and Broda, 2009; Skinner and Leathers, 2004). Lactic acid bacteria are of concern because they compete with yeast (which produce starch) for essential growth factors, and they produce organic acids, including lactic and acetic acid, which inhibit yeast growth (Skinner and Leathers, 2004). In fact, lactic acid concentrations as low as 1 to 4% inhibit yeast growth, while a 0.3% acetic acid concentration stops fermentation (Hynes et al., 1997; Weigel et al., 1996). Lactic acid bacteria are especially problematic because they can tolerate high temperatures, low pH, and high ethanol concentrations encountered in the fuel ethanol production process. Furthermore, lactic acid bacteria grow rapidly and reach high numbers of viable cells prior to the completion of yeast fermentation (Bischoff et al., 2009; Hynes et al., 1997; Leja and Broda, 2009). Failure to identify and control bacterial contamination can lead to a stalled fermentation, in which all of the starch has not been converted to alcohol. Stalled fermentation results in a shutdown of the fermentors and loss of production time while the system is cleaned of contaminants and re-inoculated (Bischoff et al., 2009; Muthaiyan and Ricke, 2010).

Bacterial contamination reduces ethanol yield by 1 to 5% (Narendranath et al., 1997) and results in the production of lower quality DDGS. Bacteria can thrive during fuel ethanol production because the process is not sterile nor in pure culture conditions (Bischoff et al., 2009). Contaminating bacteria may be introduced to the milling process via raw materials used to make ethanol (heat treatment of raw materials does not kill all contaminants), water used for pump and agitator seals, poorly stored backset, active dry yeasts, and yeast slurry used as inocula (Heist, 2009; Leja and Broda, 2009; Makanjuola et al., 1992; Muthaiyan and Ricke, 2010; Skinner-Nemec et al., 2007). Improper cleaning, especially of vessels and transfer lines, allows the bacteria to continue to thrive on equipment used in the dry-grind process. The bacteria form biofilms, which are active bacterial colonies that are possibly resistant to antibiotics and cleaning (Rich et al., 2011; Skinner-Nemec et al., 2007). Impediments to process flow can be a source of bacterial colonization as well (Heist, 2009).

Antibiotic Use in Ethanol and DDGS Production

Antibiotics have been used to control bacterial infections during fermentation in ethanol production for many years (Juranek and Duquette, 2007), and virginiamycin and penicillin have been the most commonly used. When antibiotics are used, they are added to fermenters in very small quantities relative to usage rates in animal feeds. For example, when virginiamycin (Lactrol) is added to fermenters, it is typically added at levels of 0.25 to 2.0 ppm, whereas when

virginiamycin (Stafac) is added to swine feeds it is at levels 5.5 to 110 ppm. No data have been published regarding the extent of antibiotic use in fuel ethanol production.

There are two major concerns with the use of antibiotics in fuel ethanol production. First is the potential for bacteria to develop resistance, rendering antibiotics ineffective to control infections (Muthaiyan and Ricke, 2010). Secondly, there is concern regarding the potential for antibiotic residues to remain in animal feeds (i.e. DDGS), and potentially in animal tissues used for human consumption (Benz, 2007). Antibiotic resistance is thought to develop as a result of misuse of antibiotics. This includes antibiotic overdosing when no effect is observed and underdosing when efficient control is observed. Therefore, concerns that the consumption of DDGS containing antibiotic residues by animals could potentially result in animals, and humans consuming food products from those animals, developing resistance to antibiotics used in fuel ethanol production have been raised.

Regulatory Authority of Antibiotic Use in Ethanol Production

The U.S. Food and Drug Administration (FDA) has regulatory authority for all drugs, additives and ingredients used in animal feeds, as well as establish limits for feed contaminants (Benjamin, 2009; de Alwis and Heller, 2012) related to animal food products eventually consumed by humans. This regulatory authority also includes additives used in the production of DDGS (Benjamin, 2009).

In November, 1993, the FDA's Center for Veterinary Medicine issued a "letter of no objection" for the use of virginiamycin at dosage concentrations between 2 to 6 ppm in the fermentation phase of ethanol and DDGS production, and had no objection to potential virginiamycin residues of 0.2 to 0.5 ppm in DDGS. This statement was based calculating virginiamycin residues resulting from inclusion concentrations in ethanol production, estimated residues in DDGS, and in an animal diet containing no more than 20% DDGS. In addition, it was stated that the CVM was unlikely to take regulatory action against DDGS-containing feed with residual virginiamycin concentrations below 0.5 ppm. Virginiamycin concentrations below 0.5 ppm pose no concern to broiler, turkeys, swine, or cattle consuming the feed, nor to the humans consuming food derived from those animals (Benz, 2007). No other antibiotics were included in this letter of no objection. Currently, there are minimal guidelines and no FDA regulatory enforcement and monitoring of antimicrobial residues in distillers co-products produced by fuel ethanol plants.

Due to the dramatic increase in DDGS production and use in animal feeds during the past few years, the FDA has expressed three primary concerns related to antibiotic residues in distillers grains 1) the potential for transfer of antibiotic residues from distillers grains to animal tissues, 2) the potential harm to humans who eat animal tissues containing antibiotic residues, and 3) the potential harm to animal health if antibiotic residues are present in distillers grains. The prevalence of antibiotic use in the ethanol industry, the level of residue detection, and the presence of biological activity in residues in distiller's grains is unknown.

Because of these concerns and limited data on the extent and levels of antibiotic use in ethanol and distillers grains production, the FDA initiated a nationwide survey in December, 2007, and a multi-analyte method calibrated to only detect residues of virginiamycin, erythromycin, and tylosin was used (National Grain and Feed Association 2010; Olmstead, 2009).. Preliminary

results from this survey were reported in January, 2009. Antibiotic residues were detected in 24 of 45 samples (obtained from ethanol plants in several states) tested thus far, and 15 of the 45 samples contained residues of virginiamycin, 12 contained residues of erythromycin, and 5 samples contained residues of tylosin. The FDA has not published these results, commented on their health and safety implications, or implemented regulatory action to date.

In 2012, the FDA conducted a second survey using an analytical method described by de Alwis and Heller (2010) to check for 13 antibiotic residues. Of the total of 46 samples analyzed, 3 samples had detectable concentrations of erythromycin, virginiamycin, and penicillin. The first sample contained 0.58 ppm erythromycin, the second sample contained 0.24 ppm penicillin and 0.15 ppm virginiamycin, and the third sample contained 0.16 ppm virginiamycin. Erythromycin had a detection limit of 0.5 ppm, penicillin had a detection limit of 1.0 ppm, and virginiamycin had a detection limit of 0.1 ppm (Luther, 2012).

It is important to note that the FDA used a non-FDA approved multi-analyte residue detection method capable of detecting antibiotic residues as low as 0.1 ppm (dry matter basis) in distiller's co-products (Heller and de Alwis, 2008). The accuracy of this method ranged from 88 to 111% over the quantitative range of 0.1 to 1.0 ppm (Heller and Hemakanthi de Alwis, 2008). The only FDA approved method to quantify antibiotic residues in feeds and feed ingredients is for virginiamycin. This approved method is different than the method used by FDA (Heller and de Alwis, 2008) to quantify antibiotic residues in distiller's grains samples in their recent survey. The approved method is a bioassay developed by SmithKline Beecham (now owned by PhibroChem) that has a limit of detection for virginimycin residues of 0.1 ppm, well below the 0.5 ppm level cited in FDA's 1993 "letter of no objection". It is of great importance to use appropriate analytical methodology when attempting to detect antibiotic residues in distiller's co-products. PhibroChem reported in July, 2009, results from testing 42 samples of wet and dry distiller's grains and DDGS, obtained from 11 ethanol plants by an independent laboratory and Phibro's technical service laboratory. No virginiamycin residues were detected in any samples using the FDA approved bioassay procedure.

In January, 2009, at an International Feed Regulators Meeting in Atlanta, GA, a spokesperson from FDA's Center for Veterinary Medicine announced that "the agency is reviewing the appropriateness of its November 1993 "letter-of-no-objection" under which the agency has exercised enforcement discretion allowing residues of up to 0.5 parts per million (ppm) of virginiamycin in distiller's grain products in the current regulatory environment.".

Types of Antibiotics That May Be Used in Ethanol Production

Antibiotics can be bactericidal or bacteriostatic. Antibiotics that kill bacteria in vitro are bactericidal, whereas bacteriostatic antibiotics slow or stop bacterial growth (Merck Sharpe & Dohme, 2004).

Virginiamycin

Virginiamycin is a macro-lide antibiotic and is composed of two components, Factors M and S (Vannuffel and Cocito, 1996). The S and M factors interact synergistically to increase the antimicrobial activity of the product. Virginiamycin is a bacteriostatic when the S and M factors are not associated, and a bactericidal antibiotic when the two components are associated (Merck Sharpe & Dohme, 2004; Hynes et al., 1997; Vannuffel and Cocito, 1996). The two factors are most active in a M to S ratio of 2:1 or 1:1, and the M factor is the first-limiting for antibiotic activity (Cocito, 1979). In combination, the two factors work synergistically to reduce the colony forming capacity of bacteria, but separately, each factor only reduces the viability of most bacteria after an exceedingly long incubation period. In fact, the activity of the two components together is 10 to 100 times greater than the activity of either one individually (Cocito, 1979).

Virginiamycin is a narrow spectrum antibiotic effective at controlling gram positive bacteria including a majority of lactic acid bacteria (Cocito, 1979; Hynes et al., 1997; Islam et al., 1999). The effectiveness of virginiamycin against *Lactobacilli* species is dependent on the strain and growth phase. Currently, some resistance to virginiamycin by several genera of gram positive bacteria, as well as breakdown of virginiamycin by *Lactobacilli* species, has been reported (Hynes et al., 1997). In addition, reports of cross-resistance exist between macrolide antibiotics (e.g. tylosin and erythromycin) and virginiamycin for gram positive bacteria (Cocito, 1979). However, resistance to streptogramins, including virginiamycin, is less common than any other protein synthesis inhibitor (Vannuffel and Cocito, 1996).

In ethanol fermentation, virginiamycin is normally added to fermenters at a level of 0.25 to 2.0 ppm, although the FDA “letter of no objection” allows a maximum use rate of 2 to 6 ppm. Virginiamycin is effective in controlling lactic acid bacteria, preventing ethanol yield reductions as great as 11% in the presence of *Lactobacilli* species (Hynes et al., 1997).The stability of virginiamycin is not greatly affected at temperatures ranging from 25 to 35°C and at pH 3.8 to 4.8 for 72 hours during fermentation (Islam et al., 1999). However, the distillation process (30 minutes at 100°C) has been shown to significantly inactivate virginiamycin where 97.4% of original virginiamycin activity was eliminated under these conditions (Hamdy et al. 1996). PhibroChem, the exclusive producer of virginiamycin for the ethanol industry, indicates that typically drying operations at temperatures as high as 800° F (426.6°C) will result in rapid breakdown of virginiamycin residues in DDGS dryers. From the above references, it can be concluded that virginiamycin residues are easily destroyed if they experience sufficiently high temperatures during the DDGS distillation and drying operations.

Virginiamycin is approved by the FDA for use in animal feed. Consumption of DDGS containing virginiamycin residues by food-producing animals poses little or no harm to animal or human health (Juranek and Duquette, 2007). Several factors prevent virginiamycin in co-products of fuel ethanol production from being harmful to animals and humans. First, virginiamycin is inactivated during the ethanol distillation process (Hynes et al., 1997). Second, virginiamycin is not absorbed in the gut and was not found in the kidneys, liver, or muscle of chickens fed virginiamycin (Butaye et al., 2003; Juranek and Duquette, 2007). Third, virginiamycin has been fed to animals at much higher concentrations than FDA approved with no ill effects on the health of the animals. Finally, virginiamycin concentrations in DDGS (0.2 to 0.5 ppm) are much lower

than those currently approved by the FDA for use in animal feeds (FDA, 2010; Juranek and Duquette, 2007). Results from a SmithKline Beecham study, which were provided to the FDA and foreign regulatory agencies as part of the approval process for virginiamycin to be legally and safely used in animal feeds, are shown in **Table 1**. These results show that even when virginiamycin is fed to animals at levels higher than the legally approved levels, residues were not detected in animal tissues (Juranek and Duquette, 2007).

The FDA also conducted a quantitative risk assessment on virginiamycin and human health in 2004 and concluded that virginiamycin poses no threat to human health. Some of the data to support this conclusion are shown in **Table 2**. Therefore, virginiamycin is a safe and effective antibiotic choice for use in the fuel ethanol industry.

Table 1. Effect of feeding high levels of virginiamycin on tissue residues in various animal species.[1]

Species	Dosage, ppm	Withdrawal period, days*	Muscle ppm	Liver ppm	Kidney ppm	Fat ppm
Swine	170.5 ppm feed (18 wks)	0	< 0.1	< 0.1	< 0.1	< 0.1
Veal calves	50 mg/kg BW oral dose	3	< 0.1	< 0.1	< 0.2	< 0.2
Trout**	40 ppm feed (12 wks)	0	< 0.1	< 0.1	< 0.1	< 0.1
Rabbits**	80 ppm feed (4 wks)	0	< 0.1	< 0.1	< 0.1	< 0.1
Broilers	110 ppm feed (4 wks)	0	< 0.02	< 0.02	< 0.02	< 0.02
			White	Yolk		
Layers –eggs**	80 ppm feed (6 mo)	0	< 0.02	< 0.05		

* Withdrawal period refers to the number of days virginiamycin was removed from the feed before harvest.
**Virginiamycin is not approved for use in trout, rabbits, or layers.
[1] Juranek and Duquette, 2007.

Table 2. Effects of increasing feeding levels of virginiamycin, and exceeding approved usage levels, on health and toxicity of various animal species[1]

Species	Dose	Effects
Cattle	25,75, 125, or 625 g/ton feed, 500 ppm (23 wks)	No adverse health effects or evidence of toxicity
Calves	80 ppm in feed (4 mo)	No adverse effects
Chickens	2,000 ppm feed (24 hrs), 22, 66, or 110 ppm in feed (7 wks)	No evidence of toxicity
Pigs	1,600 mg/kg BW (2 wks), 500 mg/kg BW (3 mo)	No adverse effects

[1]Juranek and Duquette, 2007

Penicillin

Penicillin is not approved by the FDA for use in ethanol and DDGS production. However, penicillin is often added at concentrations above 1.5 mg/L in fuel ethanol production due to the

possibility of induced enzymatic degradation of the antibiotic (Hynes et al., 1997). This concentration is much lower than concentrations approved for use in food animals, and has both bacteriostatic and bactericidal activities on gram positive bacteria and a few gram negative cocci, as well as *actinomycetes* and *spirochaetes*. The stability of penicillin is directly affected by temperature and pH. High temperatures (>35°C) and pH values greater than 8.0 and less than 4.0 cause penicillin to become unstable (Kheirolomoom et al., 1999). Islam et al. (1999) reported that within 48 hours, penicillin G (0.5 unit/mL) was almost inactivated at 35°C and at pH of 3.8, 4.0, 4.2, and 4.5 during sterile malt glucose yeast extract fermentation. They also found that the biological half-life of penicillin decreased dramatically from 24 hours at 25°C to 4 hours at 35°C. Based on these research results, it is expected that the temperature and pH conditions during ethanol and DDGS production will inactivate penicillin. Fermentation occurs over a 48 to 72 hour time period, when pH declines to less than 4, and at a temperature of approximately 32°C. Distillation at temperatures over 78°C for up to 30 minutes will also inactivate any penicillin remaining in the mash, and drying of DDGS at temperatures between 300 and 600 °C in the rotary drum dryers (Bothast and Schlicher, 2005), should completely inactivate the penicillin residues in DDGS.

Erythromycin

Erythromycin is not approved by the FDA for use in ethanol and DDGS production. It is a 14-membered lactone ring macrolide ring antibiotic that is used in fuel ethanol production (Petropoulos et al., 2008) because it is very effective against most gram negative and gram positive bacteria (Chittum and Champney, 1995). Macrolide antibiotics are bacteriostatic and bind to the 50S subunit of the ribosome in a ratio of one molecule per ribosome (Merck Sharpe & Dohme, 2004; Petropoulos et al., 2008; Vannuffel and Cocito, 1996). The stability of erythromycin is pH and temperature dependent, where it is more stable at the pH range from 7.0 to 8.0 and lower temperatures (Brisaert et al. 1996). It is soluble in alcohol and insoluble in water, but it becomes more unstable with higher alcohol concentrations. Like penicillin, erythromycin is likely inactivated by the low pH and high temperatures encountered during the fermentation and distillation of ethanol. Once consumed, erythromycin is diffused well into body fluids, but food consumption decreases its absorption (Merck Sharpe & Dohme, 2004). It is currently approved for use in food animals. However, it is important to note that erythromycin has an antagonistic effect when combined with virginiamycin or penicillin, and it can cause monensin toxicity due to delayed clearance or altered biotransformation of monensin when fed concurrently (Basaraba et al., 1999; Cocito, 1979; Hof et al., 1997).

Tylosin

Tylosin is not approved by the FDA for use in ethanol and DDGS production. It is an effective, 16-membered ring macrolide antibiotic against gram positive and some gram negative bacteria and inhibits bacterial protein synthesis (Omura et al., 1983; Petropoulos et al., 2008). Tylosin consists of tylosin A, tylosin B, tylosin C, and tylosin D, all of which contribute to its antibiotic potency. Tylosin A is, by far, the major component (usually about 90% and not less than 80%). Tylosin solutions are stable at about pH 7 and temperatures of 60 to 90°C. The decomposition rate of tylosin is largely dependent on pH, buffer type and concentration, temperature, as well as the ionic strength (Paesen et al. 1995). Tylosin is most stable at about pH of 3.5 or about pH of 9.0. Outside of those two pH ranges, there is significant inactivation of the antibiotic. In addition, increased temperatures and exposure periods can lead to inactivation (Aksenova et al., 1984).

Therfore, it is likely, that any residue in DDGS would be inactivated due to its low stability at the pH and high temperatures present in the fuel ethanol production process. Currently, tylosin is approved to be fed in livestock.

Tetracycline

Tetracycline is a bacteriostatic antibiotic that is unstable at low pH (pH < 2) and will form anhydrotetracyclines via the loss of water and proton transfer in strongly acidic conditions (Wang et al., 2008). Furthermore, tetracyclines degrade faster at low pH and high temperatures, its absorption is decreases with metal cations (Al, Ca, Mg, Fe), and it is antagonistic when co-administered with penicillin (Merck Sharpe & Dohme, 2004). Tetracycline is currently approved to be fed to livestock. In the body, it penetrates most body tissues and fluids. Previous studies have looked at the effectiveness of heat sterilization of animal feed ingredients in order to reduce the level of active tetracycline residue, and Hassani et al., (2008) reported that low-temperature, long-time treatments with conventional sterilization (121 °C for 20 minutes) are most effective in reducing active residues to less than one percent.

Antibiotic Alternatives

Several ethanol plants are evaluating antibiotic alternatives to control bacterial infections. The two most common alternative products are stabilized chlorine chloride and an enzyme derived from hops. Chlorine dioxide is a buffered sodium chlorite with antimicrobial properties, and is activated from sodium chlorite to chlorine dioxide by acid producing bacteria. Sodium chlorite degrades to nontoxic chloride and sodium ions. Hops extract has antimicrobial properties and contains enzymes that control bacteria but also enhances the ability of yeast to convert starch to ethanol. Limited published scientific data is available on these products but results from a few field studies suggest that they may be cost effective alternatives to antibiotics in controlling bacterial infections in fermenters during ethanol production.

Recent Research Results on the Presence and Biological Activity of Antibiotic Residues in DDGS

Paulus-Compart (2012) recently completed a study at the University of Minnesota to determine if antibiotic residues are present in wet and dried distillers grains with solubles, and if so, whether they have biological activity. The objectives of this study were to: 1) collect and evaluate wet and dried distillers co-products samples from multiple geographical locations and dry-grind ethanol plants in the U.S. for the presence of virginiamycin, penicillin, erythromycin, tetracycline, and tylosin residues, and 2) determine the extent of any antimicrobial activity of samples using sentinel bacteria strains of *Escherichia coli* (ATCC 8739) and *Listeria monocytogenes* (ATCC 19115).

Materials and methods

Approximately 20 wet and 20 dried distillers grains samples were collected by independent nutritional consultants from 43 dry-grind ethanol plants located in 9 Midwestern U.S. states every 3 months during a 12-month period. Samples were sent to the University of Minnesota Animal Science Department and immediately frozen (-21° C). Original samples are subsampled and sent to SGS North America (Brookings, SD, U.S.A.) for proximate nutrient analysis and detection of penicillin, erythromycin, tetracycline, and tylosin residues using procedures (de Alwis and Heller, 2010). An additional extraction using phosphate buffer solution (PBS) is utilized to minimize antibiotic residue damage during the extraction process. Residues recovered using PBS extraction were tested against sentinel bacteria strains for *Escherichia coli* (ATCC 8739) and *Listeria monocytogenes* (ATCC 19115) to determine biological activity. Bacterial thresholds were determined for antibiotic residues by adding sentinel bacteria at concentrations of 10^4, 10^5, 10^6, and 10^7 to the antibiotic extract in broth for 18 to 24 hours at 37°C, and then samples were examined for bacterial growth. Bacterial inhibition was also determined by plating 10 mL from each broth on blood agar plates. After 18 to 24 hours of incubation at 37°C, bacterial colonies were counted and recorded as colony forming units (CFU) per mL. Another set of quarterly subsamples were sent to Phibro EPG Laboratory (St. Paul, MN) for detection of virginiamycin residues using a proprietary, FDA-approved bioassay procedure.

Data were analyzed using the Mixed procedure of SAS with sampling period and ethanol plant of origin as random effects, and fixed effects included type of distillers (wet or dry), and the interactions of distillers type × ethanol plant and distillers type × sampling period. Effects were considered significant when P values were ≤ 0.05 and trends were considered when $0.05 > P$ values ≤ 0.10.

Results

One-hundred and fifty-nine samples (79 wet and 80 dried) were been analyzed for tetracycline, tylosin, erythromycin, and penicillin residues. As shown in **Figure 1**, one sample contained detectable concentrations of tetracycline and one sample contained detectable levels of penicillin, but none of the samples contained tylosin residues. Erythromycin was found in 16 of the samples (10.1%). Only two samples had detectable concentrations of virginiamycin (> 0.3 ppm) using Phibro's FDA-approved bioassay (**Figure 2**). One sample contained 0.6 µg/g and the other contained 0.5 µg/g virginiamycin.

Residue concentrations for all other antibiotics tested were extremely low with mean concentrations (dry matter basis) for dried samples were less than 0.8 µg/g for erythromycin, less that 1.2 µg/g for tetracycline, and less than 0.12 µg/g for penicillin. Residue concentration distribution among samples is shown in **Figures 2, 3, 4, and 5** for virginiamycin, tetracycline, erythromycin, and penicillin, respectively.

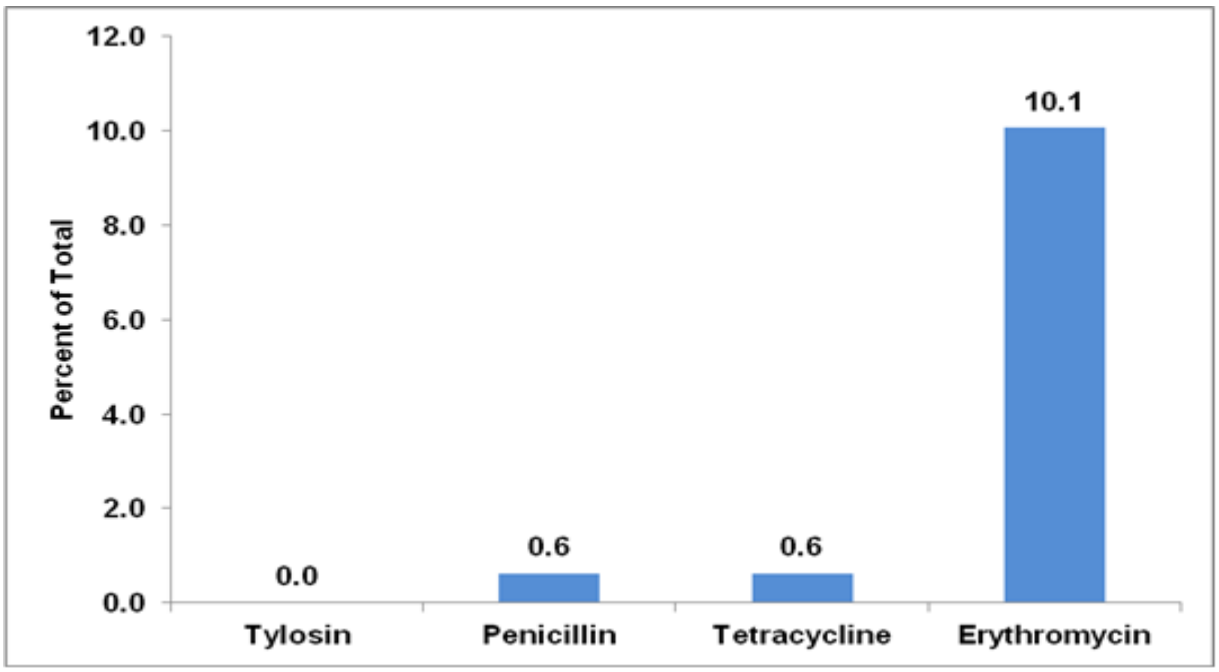

Figure 1. Percentage of samples containing antibiotic residues

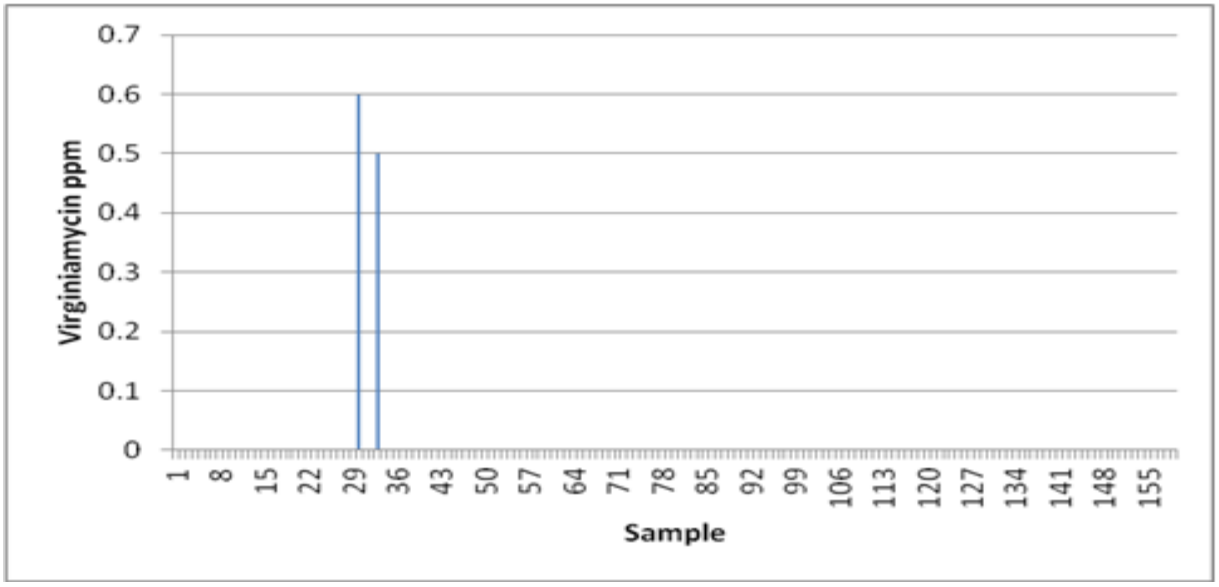

Figure 2. Virginiamycin residue concentrations of wet and dried distillers grains with solubles (DM basis

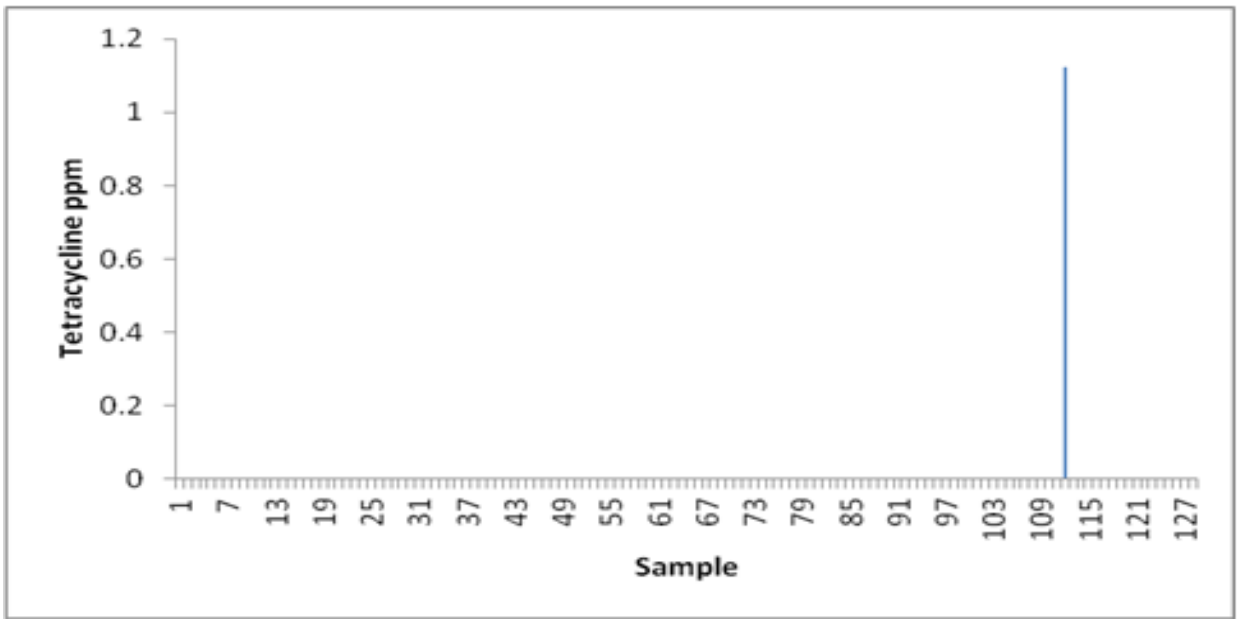

Figure 3. Tetracycline residue concentrations of wet and dried distillers grains with solubles (DM basis).

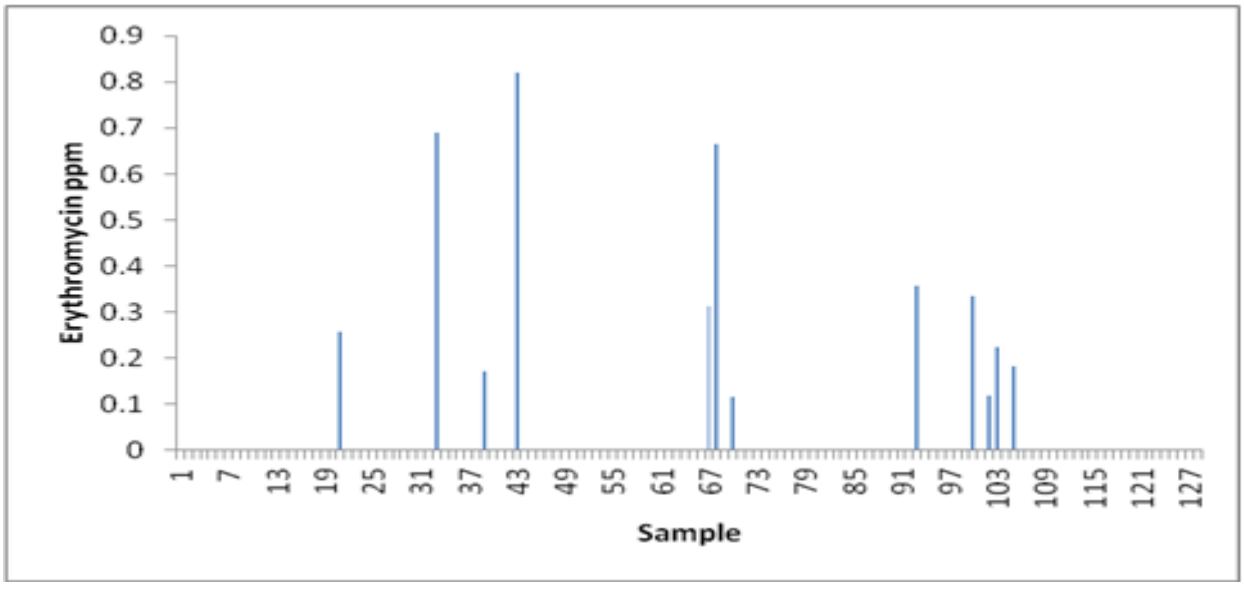

Figure 4. Erythromycin residue concentrations of wet and dried distillers grains with solubles (DM basis).

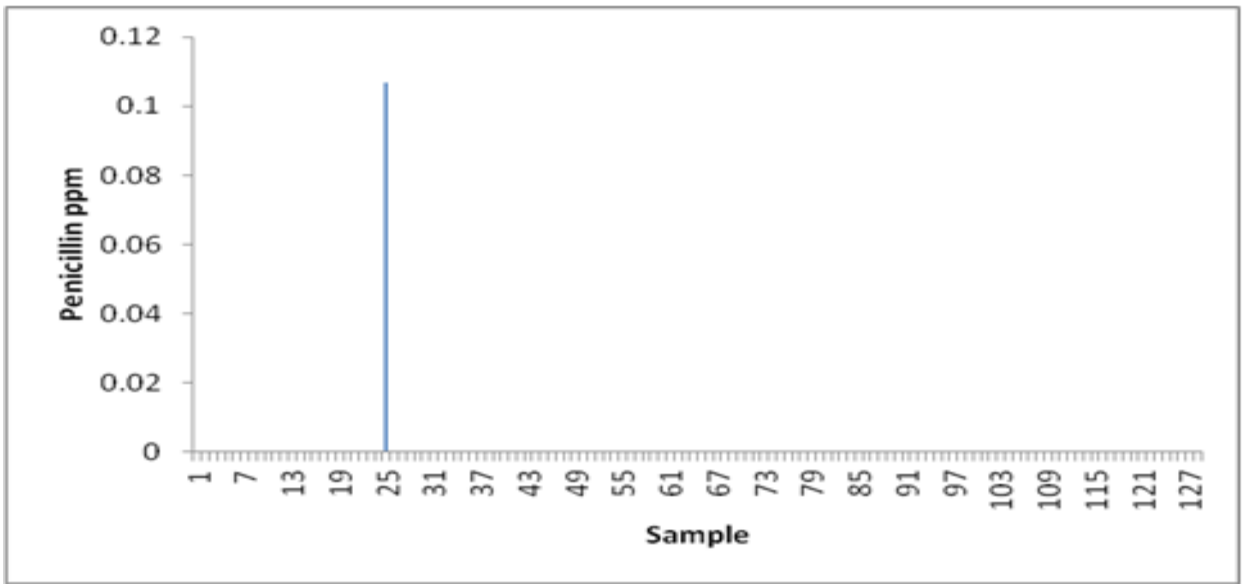

Figure 5. Penicillin residue concentrations of wet and dried distillers grains with solubles (DM basis).

The extract from only one sample was found to have any inhibitory properties for *E. coli*, but not *L. monocytogenes* growth. The extract inhibited *E. coli* (ATCC 8739) at concentrations between 10^4 and 10^5. However, there were no detectable concentrations of residues from the 5 antibiotics tested in this sample. Therefore, the cause of bacterial inhibition produced by this sample is unclear. All of the other samples tested for antibiotic residue activity showed no bacterial inhibition, and produced plates with too many colonies to count for both *E. coli* and *L. monocytogenes* (ATCC 19115). The results of this study indicate that 20 of the 159 samples (12.6%) tested in this survey contained detectable levels of antibiotic residues. Furthermore, the concentrations of the residues detected in wet and dried distillers co-products were extremely low, and no tylosin residues were detected. Less than 1.3% of the samples tested contained low (0.5 to 0.6 µg/g), but detectable concentrations of virginiamycin residues using the FDA-approved bioassay. However, it appears that there is no concern of residues having inhibitory properties when using sensitive strains of *E. coli* and *L. monocytogenes* as sentinel bacteria. These results indicate that antibiotic residues in distillers grains are inactivated during the distillers grains production process, and detectable antibiotic residues had no effect on sentinel bacteria chosen to test their antimicrobial activity.

Conclusions

Antibiotics are often used to control bacterial infections in the dry-grind fuel ethanol production process to enhance ethanol yield and nutritional quality of distillers co-products. Virginiamycin is the most widely used antibiotic in ethanol production, is the best researched and understood. All scientific evidence to date suggests that using virginiamycin in ethanol production poses no concerns for residues or risks for animal and human health. Less than 1.3% of the samples tested contained low (0.5 to 0.6 µg/g), but detectable concentrations of virginiamycin residues. Only 1 sample contained penicillin residues, 1 sample contained tetracycline residues, and no

samples contained tylosin residues. Extremely low concentrations of penicillin, erythromycin, and tetracycline residues were detected in wet and dried distillers co-products. However, it appears that there is no concern of residues having inhibitory properties when using *E. coli* (ATCC 8739) and *L. monocytogenes* (ATCC 19115) strains as sentinel bacteria.

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