J. Dairy Sci. 98:1–6
http://dx.doi.org/10.3168/jds.2015-9932

Short communication: No antimicrobial effects from one source of commercial dried distillers grains with solubles

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ABSTRACT

Because residual antibiotics in dried distillers grains with solubles (DDGS) could lead to inadvertent feeding of antibiotics to animals, the objective of our study was to determine if a commercial DDGS contained antibiotics. The DDGS used in a feeding study, and milk from cows fed the DDGS, were below the detection limits for at least 17 antibiotics. Additionally, we evaluated if DDGS had any antimicrobial effect against *Salmonella* Typhimurium, *Listeria innocua*, *Escherichia coli* ATCC 25922, *Staphylococcus aureus*, *Pediococcus acidilactici*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bacillus licheniformis*, *Paenibacillus odorifer*, *Pseudomonas fluorescens*, and *Paenibacillus amylolyticus* using the disk diffusion seeded agar overlay method. Neither the buffered nor nonbuffered water-soluble fractions of DDGS yielded clear zones around disks, indicating that the water-soluble DDGS fraction had no antimicrobial properties against any of the microorganisms tested. The absence of antibiotic residues in DDGS and milk samples in this study confirmed that this source of DDGS can be used as livestock feed without fear of inadvertent feeding of antibiotics.

Key words: antibiotics, clostridia, milk, total mixed ration

Short Communication

Ethanol production from corn involves fermentation by yeast (*Saccharomyces cerevisiae*; US Grains Council, 2012). After fermentation and distillation of ethanol, whole stillage (water and solids) is produced as a coproduct. Through centrifugation, whole stillage is separated into thin stillage and coarse solids or wet distillers grains (**WDG**, ~35% DM). These WDG are further heat-dried with air temperatures averaging 430°C (Saunders and Rosentrater, 2009) in a rotary drier to form dried distillers grains (**DDG**). The mixture of syrup (30% DM), obtained after evaporation of thin stillage, and DDG results in the production of dried distillers grains with solubles (**DDGS**, ~90% DM). As an often economical energy, fiber, and protein source, DDGS have been used as feed for lactating dairy herds for over a decade (Schingoethe, 2007; Giuntoli et al., 2009; Hoffman and Baker, 2011; Testroet et al., 2015).

Virginamycin, a streptogramin antibiotic, is used in industrial ethanol fermentations to control the growth of contaminating lactic acid bacteria that could interfere with desirable yeast fermentation (Hynes et al., 1997). Currently, virginiamycin is the only antibiotic that has been provided a "no objection letter" from the Food and Drug Administration (**FDA**; Nov. 16, 1993) for use in the ethanol fermentation process. Virginiamycin is effective against 7 strains of lactobacilli during alcoholic fermentation, but not against *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, and *Lactobacillus plantarum* (Hynes et al., 1997). Virginiamycin also has been used in the livestock industry, particularly in ruminant diets, as a growth inhibitor of gram-positive bacteria. Although virginiamycin is not currently used in the US dairy or beef industry, previous research has shown that virginiamycin improves gain to feed ratios in beef cattle (Salinas-Chavira et al., 2009) and improves milk yield in lactating dairy cattle (Clayton et al., 1999). Current FDA recommendations discourage the use of antibiotics for improving production parameters in livestock, and instead, the FDA recommends judicious use of antibiotics in livestock to help prevent the development of antibiotic-resistant microorganisms (FDA, 2014).

Because antibiotics are used routinely in the production of ethanol, and consequently, the production of DDGS, feeding DDGS could potentially result in inadvertent feeding of antibiotics to ruminant animals. A study conducted by the FDA in 2012 (Fairfield, 2012) reported that 3 out of 28 (10.7%) samples of distillers grains (**DG**) had detectable antibiotic residues. Out of those positive samples, one contained 0.16 mg/kg of virginiamycin M1 residue; a second sample contained

Received June 9, 2015.
Accepted August 14, 2015.
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0.15 mg/kg of erythromycin; a third sample contained approximately 0.15 mg/kg of virginiamycin M1 residue and 0.24 mg/kg of penicillin G. Determination of the presence or lack of antibiotics in DG is important, but it is additionally important to determine the biological activity of these residual antibiotics. In a more recent study by Paulus Compart et al. (2013), antibiotic residue concentrations in DG (wet: 79 samples, dry: 80 samples) were found to be very low and less than the maximal concentrations approved by FDA in feed for food-producing animals.

Any residual antibiotics remaining in DDGS could be detrimental in animal feeding programs because misuse of antibiotics could lead to development of antibiotic resistance in bacteria. Consumption of DDGS that contains antibiotic residues by food-producing animals could lead to development of resistance to those antibiotics (US Grains Council, 2012). Inadvertent feeding of antibiotics to dairy cows through DDGS could have important implications for the dairy industry at large, particularly if any antibiotics in DDGS are transferred into milk. The objective of this study was to determine if one source of commercially available DDGS contained antibiotic residues and if these DDGS possessed any antimicrobial properties against select pathogens and milk spoilage microorganisms.

The DDGS used in this study were acquired from Heartland Cooperative (Prairie City, IA). Dietary treatments were formulated for a dairy cow feeding study reported separately (E. D. Testroet, S. Clark, and D. C. Beitz; unpublished data). Diets included (1) TMR with no DDGS, (2) TMR with 10% DDGS by dietary DM, and (3) TMR with 20% DDGS. To obtain a random sample representative of how feed is presented to the cows, samples of feed from all 3 treatments were collected by holding Ziploc bags (S. C. Johnson, Racine, WI) directly at the conveyer of the feed wagon. Sample bags from each of 3 mixed rations from 3 consecutive days of feeding were collected and pooled into a large rubber basin, minimally mixed by hand to avoid particle stratification, and subsampled randomly by hand and sent immediately to Dairyland Laboratories (Arcadia, WI) for proximate analyses by wet chemistry methods. Duplicate samples from all feed collected were frozen at −20°C until antibiotic/antimicrobial analyses were performed.

One sample for each dietary treatment was sent to the University of Nebraska–Lincoln Food Processing Center for microbial analysis. Aerobic mesophilic spore counts were determined on the feed samples as based on AOAC International, 1998; method 990.12. Feed samples were diluted (1:10 wt:wt) in Butterfield's phosphate buffer, heated to 80°C, and held at that temperature for 12 min. Serial dilutions were plated on Standard Methods agar and incubated at 32°C for 48 h (Wehr and Frank, 2004). Plates were checked for growth, and visually distinct bacterial isolates were streaked for purity on Standard Methods agar.

Two representative samples of DDGS and 6 samples of milk (2 samples per treatment) collected from cows fed DDGS treatments underwent full drug screening tests at the Cyclone Custom Analyte Detection Service (CyCads) at the Iowa State University Veterinary Diagnostic Laboratory (Ames). Samples (approximately 1 kg) of DDGS were collected from the feed bin from both shipments of DDGS that were used in this study. Dried distillers grains with solubles samples were collected and stored in Ziploc bags (S. C. Johnson) at −20°C until antibiotic screening. Neither the dairy management nor the personnel at the DDGS supplier were aware of the collection dates or of the antibiotic testing. Milk samples (about 50 mL) were taken directly from freshly collected pooled milk of 3 sets of 10 cows (3 different diet treatments) in a feeding trial (Sankarlal et al., 2015), placed on ice, and delivered to CyCads for antibiotic screening where they were frozen at 20°C until antibiotic screening. Screening included quantification of β-lactams and cephalosporins, tetracyclines, sulfonamides, phenicols, macrolides/lincosamides/streptogramins, quinolones, aminoglycosides, and anthelmintic drugs by using liquid chromatography and mass spectrometry.

The antimicrobial properties of the water-soluble fraction of DDGS was tested by determining the zone of inhibition by using the disk diffusion agar overlay method as described in Xia et al. (2012). For this method, overnight pure cultures (10^8 cfu/mL) were used to create lawns on solid agar. Pour plates were made with 1.5% agar (Fischer Scientific, Fair Lawn, NJ) and allowed to cool/harden. Specifically, tryptic soy broth (BD Chemicals, Sparks, MD) was used for *Salmonella* Typhimurium ATCC 14028, *Listeria innocua* (ATCC 30090, Microbiologics (St. Cloud, MN), *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* (ATCC 25923, Microbiologics). MRS Lactobacillus Broth (BD Chemicals) was used for *Pediococcus acidilactici* (USDA NRRL, Beltsville, MD), *Lactobacillus casei* (Microbiologics), *Lactobacillus acidophilus* (USDA NRRL). Brain heart infusion broth (BHI Teknova, Hollister, CA) was used for *Bacillus licheniformis* (FSL J3–0143; Cornell University, Ithaca, NY), *Paenibacillus odorifer* (FSL H8–0237; Cornell University), *Pseudomonas fluorescens* FSL W5–0203; Cornell University), and *Paenibacillus amylolyticus* (FSL H7–0689; Cornell University). A 0.5-mL overnight culture suspension of each culture was added to 4.5 mL of tempered (≤50°C) 0.75% overlay