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SPOILAGE OF WET DISTILLERS GRAINS PLUS SOLUBLES WHEN STORED IN A BUNKER

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by

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Five studies evaluated the impact of spoilage of wet distillers grains plus solubles (WDGS) on nutrient composition, nutrient losses, and cattle performance. Exp. 1 and Exp. 2 utilized barrels to evaluate the ability of various cover treatments to prevent nutrient changes and losses due to spoilage. In Exp. 3, a 140-d barrel study was conducted to mimic bunker storage under ambient temperature but with no precipitation. Barrels were weighed and sampled on 28 day intervals. In Exp. 4, a 130-d finishing study utilized 60 individually-fed steers fed 3 treatments: a dry-rolled corn based diet (control) and 2 diets containing 40% WDGS replacing DRC. The WDGS was stored in either an uncovered bunker or a silo bag and stored anaerobically. An 84 day growing study utilized 60 individually fed steers in a 2x2 factorial design in Exp. 5. Treatments were bunkered vs. bagged WDGS fed at 15 or 40% of diet DM. Exp. 1 and 2 found that covering wet by-products with plastic resulted in the least nutritional losses and changes. Exp. 3 found that spoilage increased the pH and the amount of OM lost. Calculations suggest 12% of DM was lost during storage in the bunker in Exp. 4. Feeding control, non-spoiled WDGS, or spoiled WDGS did not affect DMI. No differences in ADG, final

BW, or G:F were observed between non-spoiled and spoiled WDGS treatments. In Exp. 5, calculations suggest that 6.0% of DM was lost in the bunker. Feeding bunkered WDGS decreased DMI across both levels of dietary WDGS compared to bagged WDGS. The diets containing bunkered WDGS had statistically similar ADG and G:F compared to diets with bagged WDGS.

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Introduction

The fermentation of cereal grains to produce beverage alcohol has been around for centuries. Ethanol was first used to power an engine in 1826, but wasn't used as an octane booster until the 1920s and 1930s. It became very popular during World War II due to shortages in fuel. The predominant cereal grain used in the ethanol industry is corn due to its availability and ability to easily convert to alcohol (Bevil et al. 2008). The ethanol industry has grown substantially since the 1900s. In 2011 biorefineries used 5 billion gross bushels of corn, which produced 13.9 billion gallons of ethanol and more than 39 million metric tons of livestock feed (RFA, 2012). An assortment of livestock feeds are produced as byproducts of the ethanol industry, one of the most commonly used byproducts is distillers grains plus solubles.

In 2011, ethanol producers provided 35.7 million metric tons of distillers grains for animal feed (RFA, 2012). Distillers grains are a popular feed source for cattle today, since the nutrients are concentrated three-fold compared to that of the original feed source from which they were derived. Distillers grains offer many feeding options in either a pasture or feedlot situation (Erickson et al., 2007). They have proven to be an excellent source of protein and energy for growing and finishing cattle. Distillers grains have been found to have a greater energy value relative to corn, especially wet distillers grains plus solubles (WDGS), which is ~130% the energy value of corn (Bremer et al. 2008). However, WDGS has a high moisture content (~30% DM) which causes some

storage and shelf life issues. Research has shown that once WDGS is stored and exposed to oxygen, it has a short shelf-life. Spoilage can occur within a few days depending on the amount of oxygen exposure and ambient air temperature (Christensen et al. 2010). These shelf life issues can be avoided if producers keep a fresh supply, and utilize all the WDGS within a few days of receiving a load. However, this limits the ability of smaller cattle operations to use WDGS, because most milling plants prefer to deliver semi-load quantities (25-30 tons), making it difficult for smaller cattle operations to utilize all of the WDGS before spoilage starts to occur. Similarly, cow-calf producers may want to use WDGS, but only on a seasonal basis. It is difficult for milling plants to accommodate seasonal usage due to the constant production of WDGS. Not only do milling plants have to deal with seasonal users of WDGS, but they also have to deal with seasonal changes in the number of cattle in feedlots (Erickson, 2008; NASS, 2012; RFA, 2012).

Past trends indicate that fewer cattle are fed during the summer months (i.e. July, August, and September) and more cattle on feed from November-June (NASS, 2012). Due to the seasonality of cattle numbers in feedlots, the price of distillers grains tends to be lower during the summer months due to a decrease in demand. This makes it practical and economical for producers to stockpile large quantities of distillers grains during the summer months for use during the winter feeding period. However, producers will again be faced with the issues of storage and shelf-life of WDGS (Erickson, 2008). This review will focus on the production, feeding value, storage, and spoilage of WDGS.

Dry Milling

Introduction. The fermentation of cereal grains (i.e. corn) has been utilized for centuries. There are two processes that are currently used to ferment cereal grains to

produce ethanol; with each process producing very different by-products. The dry milling process (Figure 1) produces distillers grains plus solubles, while the wet milling process produces corn gluten feed (Stock et al., 2000). The dry milling industry represents roughly 60% of the fuel grade ethanol produced from grain fermentation, and in 2011 was responsible for the production of roughly 35.7 million metric tons of distillers grains (RFA, 2012; Shurson, 2005). For the purpose of this review we will focus on describing the dry milling process and its by-products.

The process. The first step of the dry milling process is screening the grain to separate any debris (i.e. stalks) that might be mixed in (ICM, 2012). The grain is then sent through the hammer mill to be ground down to a flour consistency. Common grains used in this process are corn, grain sorghum, wheat, barley, or a mixture of two or more grains (Stock et al. 2000). Once the grain is ground, water is added to make a mixture called slurry (ICM, 2012).

After the water is added to make slurry, pH is adjusted to 5.8 (ICM, 2012). Alpha amylase and ammonia are then added to the slurry. The enzyme converts starch to dextrose, while the ammonia controls the pH and is a nutrient for the yeast (Wikipedia, 2012). The slurry is then heated to 82-88°C for 30-45 minutes. This whole process takes place at high temperatures to minimize bacteria. The slurry is then sent through a pressurized jet cooker and held for five minutes at 105°C. This is referred to as the primary liquefaction phase. The secondary liquefaction phase consists of holding the mixture for 1 to 2 hours at 82-87°C. The purpose of this phase is to allow time for the alpha amylase to convert starch to short chain dextrin's. Once both liquefaction phases are completed the mixture is adjusted to a specific pH and temperature. Then a second

enzyme, glucoamylase, is added as the mixture is moved into tanks to ferment (ICM, 2012).

After all the previous processing steps, the slurry mixture is now converted to a product referred to as mash. The mash is held in the fermentation tanks for 50 to 60 hours, which gives the glucoamylase enough time to convert dextrin's to their simple sugars. Yeast is also added during this phase, which converts the sugars to ethanol and CO_2 (ICM, 2012). After the mash has finished fermenting, the mash (whole stillage) contains 15% ethanol and solids. These solids are from the grain and added yeast (ICM, 2012). The mash must be processed so that the alcohol and water are removed. The step to remove the alcohol is referred to as the distillation step.

The distillation step utilizes the different boiling points of water and ethanol to separate the two liquids (ICM, 2012). This allows the fermentation system to boil off the ethanol and separate if from the water. This process results in two different products; a product that is 95% ethanol and a product that is referred to as whole stillage. The whole stillage consists of non-fermentable solids and water. The stillage is removed from the distillation tanks and transferred to the centrifuge (ICM, 2012). The centrifuges remove a majority of the solids from the liquid portion of the whole stillage (Stock et al., 2000). The two products that come out of the centrifuge process are wet cake and thin stillage (ICM, 2012). The wet cake product can either be sold as wet distillers grains (WDG) or dried and sold as dry distillers grains (DDG). The thin stillage is further processed by sending it through a series of evaporators to remove moisture. This process produces a product called condensed distillers solubles (CDS). The CDS can be added back to either

the DDG or WDG to produce dry distillers grains plus solubles (DDGS) and wet distillers grains plus solubles (WDGS) or sold as an individual product (Stock et al., 2000).

Nutrient Composition

Introduction. The dry milling process consists of fermenting grain, primarily corn, to produce ethanol. Roughly two-thirds of corn is starch, which is the component that is fermented in the dry milling process. Protein, fat, fiber, and phosphorus are the remaining nutrients recovered in the stillage. Once the starch is fermented and removed, the concentrations of these remaining nutrients are all increased three fold compared to the corn they came from (Stock et al. 2000). The protein content increases from 10 to 30%, fat from 4 to 12%, NDF from 12 to 36%, and P from 0.3 to 0.9% (Erickson et al. 2007). However, these increases can be variable between loads delivered and between various ethanol plants (Shurson, 2005). This variability makes it difficult to specifically pin point the exact nutrient composition of distiller grains.

Variability. Spiehs et al. in 2002 conducted a study to evaluate the nutrient content and variability of dry distillers grains plus solubles (DDGS). Ten ethanol plants, 8 from Minnesota and 2 from South Dakota, contributed to this study. There were a total of one hundred and eighteen samples collected once all the ethanol plants were sampled. The DDGS samples ranged from 87.2-90.2% DM, 28.1-31.6% CP, 8.2-11.7% fat, and 7.1-9.7% fiber. Therefore, the average DDGS nutrient composition was 89.9% DM, 30.2% CP, 10.9% fat, and 8.8% crude fiber. Since there is variation in DDGS there is going to be similar variation in WDGS on a DM basis. Some might think that this variation is attributed to the corn brought into the plant to be fermented. However, according to research conducted by Beylea et al. in 2004, DDGS variation is not due to

the nutrient composition of the corn initially brought in. This variation in nutrient content may be due to the amount of condensed distillers solubles (CDS) added back to the distiller's grains during the dry milling process.

Buckner et al. in 2011 conducted a study to evaluate nutrient content of dry milling by-products. Nutrient composition was determined for WDGS and modified distillers grains plus solubles (MDGS). There were six ethanol plants that participated in this study. Ten samples were collected per day over a 5 day collection period. Sampling was repeated every four months. Each sample was analyzed for fat, CP, P, S, and DM. They determined that WDGS averaged 31% CP, 11.9% fat, 0.84% P, 0.77% S, and was roughly 33% DM. They reported that the greatest fat variation was observed between ethanol plants. Sulfur content varied greatly among days; as did DM. This indicates that it is difficult to successfully identify the nutrient composition of WDGS without monitoring the composition on a regular basis. These results were similar to those found in a previous study conducted by Holt et al. (2004). In this study four regional ethanol plants were sampled. They determined that WDGS ranged from 29.5- 36.48% DM, 34.39-36.58 %CP, 36.10-48.18 % NDF, 9.81-16.93% ADF, 2.75-4.23% ash, and 11.04-13.12% fat. Even though there is variability in the nutrient composition of distillers grains; DDGS, MDGS, and WDGS have a similar nutrient profile. On average, distillers grains contain 10-15% fat, 40-45% neutral detergent fiber, 30-35% crude protein, and 4% ash (NRC, 1996).

Feeding distillers grains

Introduction. There are two rationales as to why producers replace corn with distillers grains in feedlot diets. These consist of including distillers in the diet to meet

protein requirements, or including it in the diet to meet the protein and energy needs of the cattle. Diets containing 15 to 20% of the diet DM or less of distillers grains are typically utilizing the distillers grains as a protein source. If producers want to utilize distillers grains as a protein and energy source they need to add above the 15 to 20% inclusion level in the diet DM (Erickson et al. 2007).

Many trials have been conducted to compare the feeding value of WDGS and dry distillers grains plus solubles (DDGS) to corn. These research studies have indicated that both WDGS and DDGS have greater energy values than corn. However, when comparing WDGS and DDGS, WDGS has the higher energy value relative to corn (Bremer, 2008).

Feeding Value. A number of experiments conducted by Larson et al. (1993) evaluated WDGS fed as either a protein or an energy source. The WDGS was fed at 5.2 and 12.6% of the diet DM to supply only protein to the ruminant animals. When WDGS was fed at 40% of the diet DM it was providing protein and energy to the animals. The authors determined that when WDGS was fed at 40% of the diet DM, feed efficiency was improved 14% compared to corn based control diets. It was then calculated that WDGS had 135% the feeding value of corn.

Similarly, Ham et al. (1994) conducted five studies to evaluate the feeding value of both wet and dry distillers grains as either a protein or energy source for cattle. Cattle being fed WDGS or DDGS gained faster and more efficiently than cattle being fed corn based diets without any distillers grains. Even though the gains were similar for cattle fed WDGS vs. DDGS, cattle being fed WDGS consumed less feed and in turn were more efficient than the cattle being fed DDGS. It was calculated that WDGS contained 47% greater feeding value than corn, and DDGS contained 24% greater value.

Vander Pol et al. (2006) conducted a 126 d finishing study utilizing 288 crossbred yearlings. Similar to the previous studies discussed, this study evaluated the feeding value of WDGS relative to a DRC:HMC blend. Six dietary treatments were fed to the steers on study consisting of a control with no WDGS, 10% WDGS, 20% WDGS, 30% WDGS, 40% WDGS, or 50% WDGS inclusion in the ration on a DM basis. As inclusion increased, WDGS replaced a 1:1 ratio of DRC and HMC. The authors observed a quadratic increase for ADG, DMI, and G:F, with optimum inclusion being 30 to 40% of diet DM. The calculated energy value for WDGS relative to the HMC:DRC mixture was greater than 100% at any of the inclusion levels. Another study conducted by Buckner et al. (2007) evaluated the effect of increasing levels of DDGS in corn-based diets on steer performance. Treatments consisted of diets that included 0, 10, 20, 30, 40, and 50% (DM basis) DDGS. Similar to the studies conducted previously using WDGS, quadratic trends were observed for final BW and ADG with increasing levels of DDGS. All DDGS treatments resulted in improved G:F compared to the 0% treatment, however, 20% inclusion showed the most improvement. The energy value of DDGS at 10 to 40 % stayed above 100%. Average relative energy value of DDGS was determined to be 125% the value of corn when DDGS was fed at 10 to 20% of the diet DM.

Bremer et al. (2008) conducted a meta-analysis of studies replacing DRC or HMC with WDGS. There were 34 studies pooled for this meta-analysis. Only studies that replaced DRC, HMC, or a combination of the two types of corn with corn WDGS (0% to 50% of diet DM) were included in this analysis. This analysis indicated that WDGS fed between 15 to 40% of the diet DM was 130% the feeding value of corn. In most of the studies, performance and carcass characteristics improved up to 30 to 40% inclusion.

Most of these studies have looked at the energy value of WDGS in corn based diet. However there have been studies that have looked at the energy of WDGS in high forage diets. A study by Nuttelman et al. (2009) was conducted to compare the energy value of WDGS to DRC in a forage based diet using 160 crossbred steers. Diets consisted of DRC or WDGS, sorghum silage, grass hay, and a supplement. These diets were formulated to meet degradable intake protein and metabolizable protein requirements. This study calculated the energy value of WDGS to be 130% the value of DRC when fed in forage based diets. Another study by Nuttelman et al. (2010) was conducted to evaluate the energy value of WDGS to DRC in high forage diets. Similar to the previous study, cattle consuming WDGS gained more than DRC cattle. The energy value of WDGS was calculated to be 146, 149, and 142% the energy value of DRC when included at 15, 25, and 35% of the diet DM.

Similar to the studies conducted by Nuttelman et al (2009; 2010), Ahern et al. (2011) conducted a study to compare the energy value of DDGS and WDGS, at differing levels, to DRC in a forage-based diet. One hundred and twenty crossbred steers were utilized in this study. Diets included DDGS, WDGS, or DRC, with sorghum silage, grass hay, and a supplement. Diets were formulated to meet energy and metabolizable protein requirements. Diets were also calculated to contain the same amount of energy assuming the distillers grains contains 108% TDN compared to 90% TDN for DRC. In this study WDGS had an energy value of 120% the value of corn, while the DDGS has an energy value of 114% the value of corn.

As previously discussed in the dry milling process, WDGS and DDGS are not the only distillers grains produced in the dry milling process. A study conducted by

Nuttelman et al. (2011) compared WDGS, DDGS, and MDGS effects on feedlot cattle performance. The treatments were arranged in a 3 x 3 plus 1 factorial, with three types of distillers grains (WDGS, MDGS, or DDGS), three inclusions (20, 30, or 40% diet DM), and a corn-based control diet. There was no effect of type of distillers grains on ADG, however, DMI increased for MDGS and DDGS compared to WDGS. Therefore, G:F was improved for cattle being fed the WDGS treatment compared to the corn-based diet, and cattle fed MDGS fell intermediate. All distillers grains treatments had a greater gain and a more efficient G:F compared to the corn control treatment. The feeding value of WDGS was 35.4% and 17.8% greater than DDGS and MDGS, respectively. The feeding value was 45.7%, 26.5%, and 9.3% more than corn for WDGS, MDGS, and DDGS.

Based on these studies we can conclude that distillers grains, whether it is DDGS, MDGS, or WDGS, have a higher energy value when compared to the corn it originated from; making them excellent feed sources for producers. However, throughout the studies, it is apparent that the feeding value of WDGS (~130% the value of corn) is greater compared to DDGS or MDGS (Bremer, 2008). Not only does WDGS have a higher feeding value than DDGS and MDGS, it also reduces the biorefineries energy usage because it doesn't have to go through the drying process (Bremer, 2010). That being said, WDGS has higher moisture content than both DDGS and MDGS, however MDGS is still wet (40-45% DM). The high moisture content of WDGS brings up some concerns with storage.

Storage of WDGS

Introduction. Distillers grains are an excellent feed source for cattle. However, research has indicated that WDGS has the greater feeding value out of all three types of

distillers grains. On the other hand, WDGS has a very high moisture content which makes it difficult for producers to store it. Spoilage will usually start occurring within 3 to 14 days when exposed to air. Another issue with utilizing WDGS is that most milling operations prefer to deliver semi-truck load quantities (~30 ton loads), which makes it difficult for smaller operations to utilize the large quantity fast enough to avoid spoilage. Cattle operations may also want to purchase large quantities of WDGS during the summer months when the prices are lower due to lower numbers of cattle on feed, and store the WDGS until the winter feeding period (Erickson, 2008).

Storing WDGS is similar to the process of storing silage or high moisture corn. The goal is to minimize exposure to oxygen. The two most commonly used storage methods for WDGS is either a silo bag or bunker. Eliminating the presence of oxygen in silo bags is fairly easy. WDGS can be successfully stored in silo bags without any pressure; however, the weight of WDGS causes the bag to spread out and take up more storage space. When storing WDGS in the bunker the surface is left exposed to oxygen, therefore it is common for producers to put some form of a cover on top of the WDGS stored in a bunker (Erickson, 2008). When storing the WDGS in a bunker its high moisture content doesn't allow it to be compacted into the bunker, which increases the chances that oxygen will be present in the bunker. To minimize the presence of oxygen within the bunker, most producers will blend a roughage source with WDGS before storing it in a bunker, this roughage will act as a bulking agent and allow the WDGS to be compacted in the bunker (Erickson, 2008).

There were six studies conducted by Adams et al. (2008) evaluating storage methods for WDGS with added forages. In the first study conducted, WDGS were mixed with one of five different feedstuffs including grass hay, alfalfa hay, wheat straw, DDGS, and wet corn gluten feed (WCGF). Grass hay was tested at levels of 17.5%, 15%, 12.5% 10%, and 7.5% with the remaining amount being WDGS in the mixture (DM basis). Alfalfa was tested at 25%, 22.5%, 20%, 17.5%, and 15% inclusion on a DM basis. Wheat straw was mixed with WDGS at 15 and 12.5% DM basis. Ratios of DDGS:WDGS were also evaluated. These ratios were 50:50 and 60:40 (DDGS:WDGS DM basis). Another ratio was tested with WCGF. These ratios were 40:60 and 50:50 (WCGF:WDGS). These mixtures were then evaluated on their ability to be placed efficiently into the silo bag under pressure.

The second study conducted by Adams was similar to the first, however two semi-loads of WDGS were mixed with 30% grass hay and another two loads of WDGS were mixed with 40% grass hay for storage in a silo bunker. This study also evaluated mixing WDGS with 29% corn stalks (DM basis) and storing the mixture in a bunker. In the fourth experiment WDGS was mixed with 67% or 33% wheat straw (DM basis) and stored in a silo bag. In the fifth experiment WDGS and cornstalks were mixed in a 50:50 ratio and stored in a silo bag. Lastly, in the sixth experiment, WDGS and grass hay were mixed in a ratio of 56:44 grass hay to WDGS (DM basis). The silo bag split open during the bagging process in the first experiment when bagging the 7.5% and 10% grass hay levels. The bag also split open when bagging the 40% and 50% WCGF/WDGS mixture. Based on results from the second experiment the minimal level of grass hay mixed with WDGS was 40%, because the 30% mixture was not able to hold the weight of the skid

loader packing it into the silo bunker. Based on all these experiments, 30 to 40% grass hay with WDGS should be included when storing WDGS in a bunker, and lower levels of rough inclusion when using wheat straw or cornstalks. Once the WDGS is packed into the bunker the focus needs to shift to the surface of the bunker, which is still going to be exposed to oxygen.

Cover Treatments. The bunker storage method has the most issues with spoilage due to the fact that the surface of the WDGS is exposed to oxygen. However, this could be avoided by applying various cover treatments to prevent the surface from being exposed to oxygen. A study conducted by Christensen et al. (2010) evaluated various covers for WDGS mixed with forages and stored in a bunker. A combination of 70% WDGS and 30% cornstalks (DM basis) were mixed together and packed in 55 gallon steel barrels. This was done to simulate bunker storage at a smaller level. All barrels were filled to the same weight and height. Five cover treatments were applied to the barrels; control (no cover), plastic cover (6 mil thickness) weighted with sand, salt (2.2 kg per 0.09 m^2), solubles (7.62 centimeters thick), and solubles plus salt (7.62 centimeters thick). Environmental conditions were also evaluated on open barrels (no cover) by placing some barrels inside in a temperature controlled room and other barrels outside. Certain barrels also had water applied to them one time per week, which is equivalent to 1.52 cm of rain. This was conducted on open barrels and barrels that had a solubles plus salt cover. Barrel losses were extrapolated out to a bunker, which gave producers an idea of the amount of loss that could be expected when placing this mixture in a bunker. Based on barrel storage, leaving barrels uncovered resulted in 3.5 to 5.0% DM losses in a 10-ft height bunker situation. If spoilage was considered a loss then the percentage

ranged from 7.5 to 9.3% of DM. Plastic was the most effective cover for reducing DM loss and spoilage, followed by solubles, salt, or a combination of the two. It is also noted that if solubles is used as a cover, solubles will lose 25 to 50% of their DM during storage.

Previous research has indicated that there are issues with spoilage and losses when it comes to storing WDGS in a bunker. However, when WDGS is treated with a cover like plastic or solubles plus salt spoilage is decreased, thus decreasing nutritional losses. These studies indicate that applying a cover to a bunker reduces spoilage and losses, but does not eliminate it completely.

Spoilage

Spoilage is defined as a metabolic process that causes food to be undesirable or unacceptable for human or animal consumption. Spoilage usually causes a change in texture, smell, taste, or appearance. These changes cause animals or humans to reject the food (Doyle, 2007). Some ecologists believe that the smells coming from spoiled foods are actually produced by microbes to repulse animals or humans from consuming the food supply, thus keeping the food supply for themselves (Burkepile, 2006; Sherratt, 2006). There are many factors that can cause food to spoil and become undesirable. These factors consist of 1) endogenous enzymes in plants that oxidize phenolic compounds or degrade pectin; 2) insects or rodents consuming the feed source; 3) parasites on/in the food; 4) bacteria, molds, or yeasts growing on the food and metabolizing it for their own energy; 5) light degrading pigments and proteins which cause changes to odor and flavor; 6) high and low temperatures causing changes in food texture; 7) oxygen oxidizing lipids; and 8) too much or too little moisture. Not just one of

these factors alone contributes to food spoilage. If certain temperatures, moisture, and oxygen levels are at their optimum levels, activities of enzymes and microbes will increase, which sequentially increases the amount of spoilage occurring in the food (Doyle, 2007).

Most people don't realize spoilage is occurring until they visually see it, however most spoilage starts to occur prior to the visual changes. Typically spoilage micro flora exceed 10^7 organisms/g of food by the time these visual observations are made (Sperber, 2009). Microbes will initially start to utilize sugars and any easily digested carbohydrates first. Once all of these sugars and carbohydrates are consumed, the microbes will begin degrading proteins. As the microbes degrade nutrients volatile compounds are produced, which could contribute to the strong odor from spoiled food (Ellis, 2006). A variety of microbes contribute to the spoilage of a food source. The main three types of spoilage organisms consist of bacteria, yeasts and molds (Gram, 2002). These three organisms are at a constant struggle to survive in their ecological environment. It has been determined that the faster the organisms grow, the more likely they are to survive compared to the slower growing organisms. In most cases, bacteria grow faster than yeasts, and yeasts grow faster than molds (Frazier, 1958). However, even though bacteria tend to grow faster, yeasts and molds have the ability to withstand harsher environments (Sperber, 2009).

Spoilage organisms come from a wide variety of places which include soil, water, air, and even some insects (Doyle, 2007). There is also the possibility that some microbes are present in the food when it is produced. A study conducted by Lehman et al. (2007) evaluated microbial development in distillers wet grains produced during fuel

ethanol production from corn. This study found that fungi averaged 3.9 x 10^5 cells/g dry mass in fresh WDGS which were predominately yeasts and some molds. Yeasts and molds were roughly 3.8 x 10^2 CFU/g dry mass initially, and after 4 days of storage they increased to 1.0 x 10^3 CFU/g dry mass. Five different yeasts and five different molds were identified in the WDGS. Three of the molds were Alter aria sp, Fusarium sp., and a Penicililium sp. These three molds are common to cereal grains and consist of species that produce mycotoxins.

Organisms. Yeasts are a division of organisms called fungi. Yeasts are single-celled organisms that are usually present in high moisture environments. However, yeasts do not produce any toxic metabolites. Yeasts are a facultative organism, which means they can survive with or without oxygen (Doyle, 2007). As previously discussed, yeasts are used in the fermentation process of the production of WDGS (Stock, 2000). The four main groups of spoilage yeasts consist of zygosaccharomyces, saccharomyces spp., candida, and dekkera/brettanomyces. However, dekkera/brettanomyces are the yeasts most common to fermented foods (Doyle, 2007).

Molds are another division of fungi. Molds are most commonly associated as a recycling agent for dead plants and animals. However, they can also utilize other food sources. They generally produce airborne pathogens and unlike yeasts require oxygen to survive. Molds have the ability to grow in a pH range of three to eight. Different molds have the ability to grow at different temperatures, so as the temperature changes the types of molds you will see on the feed can change as well. Mold secondary metabolism is responsible for the production of mycotoxins. Common molds are zygomycetes, penicillium, and aspergillus (Doyle, 2007). Molds are the most common form of spoilage

when it comes to baked goods or bread. Mold contamination usually occurs by mold spores traveling through the air and landing on the food supply (Pateras, 2007).

Bacteria are also organisms associated with food spoilage (Doyle, 2007). In order for bacteria to become a problem, warmer temperatures must be present (Pateras, 2007). Spore-forming bacteria can withstand very high temperatures, which is why they are most prevalent in heat treated foods. There are two types of bacteria that can be present on a food source that is spoiling, these bacteria are either anaerobes or facultative. The most common bacteria associated with spoilage are lactic acid bacteria, pseudomonas, and enterobacteriaceae (Doyle, 2007).

As food spoils, the microbial profiles will change along with the nutrient profile of the feedstuff. This is primarily due to the fact that different organisms require different nutrients to survive. However, some organisms can't survive in the presence of other organisms. A good example of this occurring would be with lactic acid bacteria and molds. Both of these organisms secrete certain compounds to inhibit any competitor microbes that would utilize their energy source (Gram, 2002). Most spoilage microorganisms interact with their surrounding organisms in an antagonistic way; constantly trying to maintain dominance in their environment. However, some microorganisms tend to grow together in a synergistic association. This relationship between microorganisms is quite rare. Lastly, microorganisms can interact in a metabiotic relationship. This means that as one organism grows it produces an environment suitable for another organism, and so on (Frazier, 1985). As these microorganisms are struggling for survival on a food source, the food is going to start spoiling, making it undesirable for animals.

Mechanisms. The whole reason food spoilage occurs is because of the biochemical activities of microorganisms in the food. Spoilage of food is not described by a step by step process that occurs, it is caused by many microorganisms digesting the nutrients a particular food has to offer and utilizing it for their own growth and development (Sperber, 2009). As previously described, spoilage is defined as a food source becoming undesirable whether it be a change in odor, taste, or appearance (Doyle, 2007). Microorganism's digestion of sugars, proteins, complex carbohydrates, and fats are all possible causes for the undesirable changes in spoiled food. The metabolic processes that cause spoilage of food include sugar fermentation, protein hydrolysis, carbohydrate digestion, lipolysis, organic acid and alcohol oxidation, and surface growth (Sperber, 2009).

When microorganisms metabolize sugars they can produce gas or acid. There are a variety of catabolic pathways that bacteria utilize to digest pentoses and hexoses. Lactic acid is a primary product produced by these pathways, which causes a sour taste when present in food. As one would expect, lactic acid bacteria is the primary producer of lactic acid. If a food supply is experiencing this kind of spoilage, the pH of the food would decrease from what is was originally. Bacteria can metabolize sugars with or without producing gas (carbon dioxide). If fermentative yeasts are present, sugars can be metabolized, thus producing ethanol and carbon dioxide. The food supply would have to have a low pH and contain large amounts of sugars for this type of spoilage to occur (Sperber, 2009).

Many bacteria that are responsible for spoilage produce proteolytic enzymes. These enzymes have the ability to hydrolyze proteins in a variety of food sources (i.e.

milk, meat, and seafood). This process can cause the spoiled food to give off a very rotten smell. In some instances, the enzymes not only hydrolyze the proteins, they can actually metabolize amino acids. This also produces very rancid smells (Sperber, 2009).

Bacteria and molds can also produce pectinase. Pectinase is the enzyme responsible for the degradation of pectin. Degradation of the pectin causes the feed to become very soft and mushy. Amylolytic enzymes are also produced by molds and bacteria. These enzymes are responsible for the degradation of starches to simple sugars. This type of spoilage will cause the food to lose its thickness (Sperber, 2009).

Spoilage microorganisms not only produce enzymes to metabolize complex carbohydrates, they can produce lipolytic enzymes, which are enzymes that are responsible for the hydrolysis of lipids. This process also leaves the feed with a very rancid odor. These spoilage microorganisms also have the ability to metabolize the organic acids that might be present in the feed. If there is a large of amount of organic acid being metabolized the pH of the feed will start to increase. If the feed had a low pH to begin with, the digestion of the organic acids could cause the pH to increase enough that other spoilage organisms will start to grow (Sperber, 2009).

Lastly, the other type of spoilage mechanism described is surface growth. This is perhaps one of the most common types of spoilage, due to the fact that most microorganisms can spoil food by simply growing on the surface. This type of spoilage is caused by a large number of microorganism growing on the surface of food, but it is important to note that it is not caused by any microbial nutrient metabolism. Surface growth typically causes color and texture changes to the feed (i.e. red spots on bread) (Sperber, 2009).

Mycotoxins. Five different yeasts and five different molds have been identified in WDGS (Lehman et al. 2007). Three of the molds were Alternaria sp, Fusarium sp., and a Penicililium sp. These three molds found are common to cereal grains and consist of species that produce mycotoxins. Mycotoxins are secondary metabolites produced by molds. These secondary metabolites are produced by fungus but are not essential for fungi to grow (Whitlow, 2004). It is unknown why fungi produce mycotoxins; however there are some speculations that those mycotoxins are produced as a means of protection for the fungi, which in turn enhances the ability of the fungi to survive in its environment (CAST, 2003). Mycotoxins cause some side effects when animals are exposed to them, this is known as mycotoxicosis (Nelson, 1993). Most animals are exposed to mycotoxins by consumption, dermal contact, or inhalation. Effects of mycotoxin exposure are quite variable, but it is common to see a reduction in feed intake, decline in weight gain, decline in performance, reproductive issues, vomiting, diarrhea, degrading of tissues, tumors, and possibly death. The major classes of mycotoxins are aflatoxins, zearalenone, trichothecenes, fumonisins, ochratoxin A, and the ergot alkaloids (Whitlow, 2004). For the purpose of this review we will focus on zearalenone and fumonisins.

Zearalenone is an estrogenic metabolite that is derived from many species of Fusarium. Zearalenone is most commonly associated with hyperestrogenism in swine (Whitlow, 2004). The main effects of zearalenone are reproductively related, and seem to be predominant in swine. However, when heifers were fed 15 ppm zearalenone (diet DM), it was observed that they had reduced ovulation. This indicates that the biggest concern of zearalenone contamination would be if the contaminated feed was being fed to

breeding stock. It has been reported that maximum levels of zearalenone in feeder cattle should not exceed 15 ppm (DM basis; Vincelli, 2002).

Fumonisins are mycotoxins that are predominant in corn. There are Fumonisins B_1, B_2, and B_3. Roughly 75% of the total fumonisins content is made up of Fumonisin B_1 in a contaminated feed (Vincelli, 2002). These fumonisins are the most predominant mycotoxin in contaminated feed. Fumonisins are responsible for equine leucoencephalomalacia (ELEM) in horses (Wilson, 1985; Marasas, 1988), pulmonary edema in swine (Harrison, 1990), and hepatoxicity in rats (Gelderblom, 1991). Conversely, diets containing 150 ppm fumonisins have been fed to steers and other than some liver lesions, the cattle were not affected (Vincelli, 2002; Osweiler, 1993). The U.S. Food and Drug Administration determined that the maximum levels of fumonisins in feedlot cattle diets was 60 ppm and it be included in no more than 50% of the diet DM.

Objectives

Distillers grains are an excellent feed source, no matter which of the three types a producers chooses to feed. However, WDGS has proven to be the better of the three in multiple studies. The downside of feeding WDGS is storage and spoilage issues. Research previously discussed has indicated that WDGS that is stored in a bunker is going to have some spoilage occur even if a cover treatment is applied. It has been well documented that this spoilage has caused a decrease in fat while increasing NDF, CP, pH, and inorganic matter. These nutrient losses are a concern, because the WDGS could be losing feeding value as it sits in storage. Since most producers do not separate the spoiled WDGS from the non-spoiled material these losses could be impacting cattle performance. Therefore, the objectives of this research was to 1) evaluate the effects of spoilage of

WDGS on the nutrient profile over time, and 2) to determine the impact of feeding spoiled WDGS to growing and finishing cattle.

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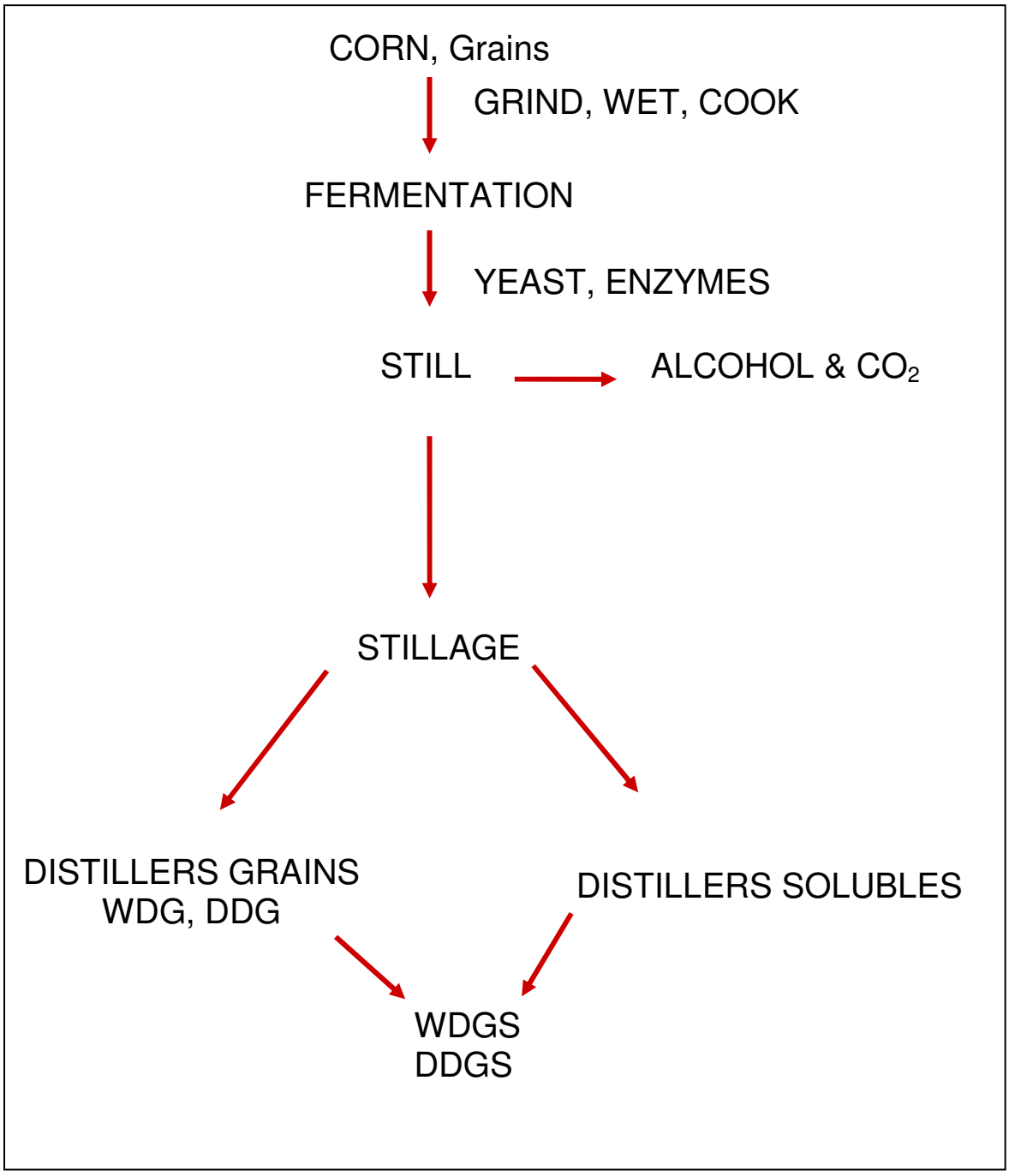
Figure 1. Dry Milling Process



CHAPTER II

Nutrient composition and losses of spoiled and non-spoiled wet by-products mixed and stored with straw.[1]

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ABSTRACT

Two experiments were conducted to evaluate different cover treatments that can be applied to the surface of wet distillers grains plus solubles (WDGS) stored in a bunker to minimize spoilage. Experiment 1 evaluated nutrient composition of spoiled (S) and non-spoiled (NS) fractions of 70% wet distillers grains with solubles (WDGS) mixed with 30% straw (7x2 factorial) or 60% solubles with 40% straw (2x2 factorial) that were stored in 200 L barrels for 56 d. The 7 WDGS treatments were kept in a building and included no cover with or without water added to the surface to simulate rain; covered with solubles, salt, or a mixture of solubles and salt; mixed solubles and salt with water added to the surface; and covered with plastic. The 2 solubles treatments included no cover stored inside or outside to be exposed to rainfall. Barrels (2 replications/treatment) were separated into S and NS portions after storage. Interactions ($P < 0.01$) resulted between cover treatment and S layer for pH, CP, fat, NDF, and ash with the WDGS:straw. Nutrient composition changes for the S layer led to increased pH, NDF, and ash and decreased fat ($P < 0.01$) compared to the NS layer for both mixtures. Using solubles as a cover preserved the nutrient content of the S fraction. Experiment 2 evaluated nutrient losses of WDGS mixed with 30% straw or straight MDGS stored in 200 L barrels for 60 d, similar cover treatments described in Exp. 1 were applied. There was an effect ($P < 0.01$) of cover treatment and the amount of spoilage, DM loss, OM loss, fat loss, and pH for the WDGS: Straw mixture and straight MDGS. Barrels using plastic and distillers solubles + salt as covers had the least amount of DM, OM, and fat lost, as well as spoilage. The spoilage process also caused the pH of the original mixtures

to increase from an initial pH of 4.42 to 6.77 with a plastic cover and 6.11 with a solubles + salt cover.

Keywords: bunker, spoilage, storage, WDGS

INTRODUCTION

The past trends have shown that there are fewer cattle fed during the summer months (i.e. July, August, and September) and more cattle on feed from November-June (NASS, 2012). Due to the seasonality of cattle numbers in the feedlot, the price of distillers grains plus solubles tends to be lower during the summer months due to a decrease in demand. This makes it practical and economical for producers to stockpile large quantities of distillers grains plus solubles during the summer months for use later during the winter feeding period (Erickson, 2008). However, the high moisture content of and particle size of wet distillers grains plus solubles (WDGS; 30% DM), modified distillers grains plus solubles (MDGS; 44% DM), and distillers solubles (DS; 35% DM) makes storage difficult (Buckner, 2011; Erickson, 2008).

By mixing WDGS or DS with straw, the product can be packed to remove oxygen, which leads to better storage in bunkers. Research conducted by Adams et al. (2008) reported that 30 to 40% grass hay mixed with WDGS (DM basis) allows for storage of WDGS in a bunker. Lower roughage inclusion is possible with lower quality forages such as wheat straw or cornstalks. Once the WDGS is packed into the bunker appropriately, the spoilage will likely only occur on the surface of the bunker, which may be exposed to oxygen. When the surface of WDGS is exposed to the air, spoilage can occur fairly quickly. Christensen et al. (2010) reported that the spoilage process will result in loss of DM at the surface of the bunker. The spoilage can be minimized with the

application of a cover treatment. However, no research has been conducted to evaluate the changes in nutrient composition and nutritional losses that might be occurring during this spoilage process.

Two experiments were conducted to evaluate various cover treatments and their effects on spoilage losses and nutrient composition changes due to spoilage. The objective of Exp. 1 was to determine the nutritional composition of the spoiled feed fractions and how different covers affect the nutrient concentrations within the spoiled and non-spoiled layers. The objective of Exp. 2 was to evaluate six different cover treatments applied to a WDGS: straw mix and how each cover affects losses due to spoilage. Another objective was to study the effects of storing MDGS (with no added forage) with or without a cover on spoilage losses.

MATERIALS AND METHODS

Exp. 1.

To replicate bunker storage, 200 L barrels were packed with one of two mixes: 70% WDGS and 30% straw mixture or a 60% DS and 40% straw mix (both on a DM basis). The mixtures were made at the Agricultural Research and Development Center research feedlot near Mead, NE. Barrels were filled to approximately the same weight (136 kg) and packed to similar heights (0.29 m^2 surface was exposed). All barrels (except DS:straw open-outside) were stored inside the Animal Science building at the University of Nebraska – Lincoln in a temperature (18.3°C) controlled room. The barrel covers (Table 1) were assigned randomly with two replications (barrels) per treatment. Cover treatments for the WDGS: straw mixture included no cover, no cover with water added, plastic, salt (NaCl), DS, DS plus salt, and DS plus salt with water added.

Treatments for the DS:straw mixture included barrels uncovered stored outside and barrels left uncovered stored inside.

After 56 days of storage, each barrel was opened by carefully removing the solubles layer (if applied), the spoiled portion, and then the non-spoiled portion. When salt was used as a cover it was collected and analyzed as part of the spoiled layer. As in previous research, it was assumed that all of the spoilage occurred from the top down due to oxygen exposure. The spoilage was determined by appearance and texture. Representative samples were collected by removing the spoiled layer, mixing it in a small mixer (#2-100DA, Leland Detroit Mfg Co., Detroit, MI), and sub-sampling that mixture. A sub-sample was dried in a 60°C forced air oven for 48 hours to obtain DM percentages. Likewise, a subsample was freeze dried (Virtis Freezemobile 25ES) and ground through a Wiley Mill (1mm screen) and analyzed for pH (Mettler Toledo,OH), fat, neutral detergent fiber (NDF), ash, and CP and reported on a % DM basis. Ash and OM were determined by placing samples in a muffle furnace for 6 h at 600°C. Ether extract was determined by performing a biphasic lipid extraction procedure described by Bremer (2010). The NDF analysis was conducted using the procedure described by Van Soest et al. (1991) with modifications described by Buckner et al. (2010). The CP was determined by using a combustion chamber (TruSpec N Determinator, Leco Corporation, St. Joseph, MI) (AOAC, 1999). The non-spoiled material was assumed to be unchanged and, therefore, equivalent to the starting mix.

Data were analyzed using the mixed procedures of SAS (Version 9.2, SAS Inc., Cary, NC). The model for the WDGS:straw mixture included type (spoiled or non-spoiled), cover treatment (Table 1), and type x treatment comparisons. The model for the

DS:straw mixture included type (spoiled or non-spoiled), cover treatment (Table 1), and type x treatment comparisons. Barrel was used as the experimental unit. Probabilities less than or equal to alpha ($P \leq 0.05$) were considered significant.

Exp. 2

Similar to Exp. 1, 200 L barrels were packed with one of two treatments: 70% WDGS and 30% straw mixture (DM) or 100% MDGS (46% DM). Barrels were filled to approximately the same weight (136 kg) and packed to similar heights. All barrels were stored in a barn, open to the south, at the Agricultural Research and Development Center research feedlot near Mead, NE, subject to ambient temperature but not precipitation, for approximately 60 days. Storage was initiated on June 2, 2010. Table 2 describes the cover treatments assigned randomly to each barrel (3 barrels/treatment).

After 60 d of storage, each barrel was opened as described in Exp. 1. When salt was used as a cover, it was collected and analyzed as part of the spoiled layer. As in Exp. 1, spoilage was determined by appearance and texture. As each layer (solubles layer if applied, spoiled layer, and non-spoiled portion) was removed, representative samples were collected (as described in Exp.1), freeze dried, and analyzed for DM, pH, fat, NDF, CP, ash and OM. These analyses were conducted using the same methods as described in Exp. 1. Nutrient analyses for both the spoiled and non-spoiled layers, along with nutrient analyses of the original WDGS sample, were used to determine nutrient losses. When calculating losses, the spoiled layer was included in the recovered DM, OM, fat, NDF, and CP, assuming that it would be fed. Therefore, if the spoiled layer were discarded, the loss would be the total of DM loss plus spoilage amount.

Data were analyzed using the mixed procedures of SAS (Version 9.2, SAS Inc., Cary, NC). The model included effect of cover treatment applied to the mixtures (Table 2). Barrel was used as the experimental unit. Probabilities less than or equal to alpha ($P \leq 0.05$) were considered significant.

RESULTS AND DISCUSSION

Exp. 1

Interactions ($P < 0.01$) resulted between cover treatment applied and spoilage layer for pH, % fat, % NDF, % ash, and % CP with the WDGS:straw mixture. Overall there was a decrease in % fat and increases in pH, % NDF, % ash, and % CP. However, there was only an interaction ($P < 0.01$) between cover treatment and the spoiled layers for CP concentration in the DS:straw mixture (Table 3). The spoilage process caused the pH of the WDGS:straw mixture to increase from its initial pH of 4.0 to a final pH of 8.5 in the spoiled layer when salt was used as a cover. When DS+salt+H_2O were used as a cover the pH changed from 4.0 to 6.0 in the spoiled layer. The other five cover treatments fell intermediate in terms of pH change. Similar changes in pH were reported with spoilage in silage placed in bunker, where pH was increased from 3.90 in normal silage to 4.89 in the spoiled material (Bolsen, 2011).

The DM content of the spoiled material also changed between cover treatments. The greatest increase in DM (7.7 % units) was observed when barrels were left uncovered. Suggesting that the WDSG:straw mixture was drying out as it was stored. The barrels left open and treated with H_2O resulted in the greatest decrease in DM content (8.4 %). This was to be expected, due to the added water. Barrels treated with a plastic cover had a 2.2 percentage unit decrease in DM content in the spoiled layer, while barrels

treated with salt had a 4.2 percentage unit increase in DM content within the spoiled layer. When DS, by itself, was used as a cover treatment there was a 1.9 percentage unit decrease in DM content. Conversely, when DS+salt were used as a cover treatment there was a 0.3 percentage unit increase in DM content, and when water was added to this cover treatment it resulted in a 1.9 percentage unit decrease in DM content.

The most important of these changes to consider is the decrease in fat content within the spoiled layer. The greatest decrease in fat resulted when salt was used as a cover or when barrels were left uncovered. Fat decreased from 10.2 to 4.1% of DM in barrels covered with salt and 10.6 to 4.9% DM when barrels were left uncovered. This could be due to microorganisms causing the spoilage. Spoilage microorganisms can produce lipolytic enzymes, which are enzymes that are responsible for fat hydrolysis (Sperber, 2009). Fat hydrolysis is a chemical reaction that affects food that is being stored. The hydrolysis reaction consists of degrading the acyl groups of triglycerides to produce free fatty acids. This reaction causes off flavors in food (Adawiyah, 2012). Using DS as a cover resulted in no change in fat content for the spoiled fraction. It was difficult to separate the spoiled layer from the DS cover, so there could be DS remaining with the spoiled layer. The other treatments were intermediate in terms of changes in fat concentration during the storage process.

The NDF content (% of DM) generally increased as spoilage occurred. The greatest change occurred with the open barrels with or without water added with a 12.3 and 10.6 percentage unit increase in NDF content. A 2.2 percentage unit increase was the smallest change recorded with the salt covering, but it must be noted that the salt covering was not separated from the spoiled material. When separating the DS layer

from the spoiled layer, not all of the DS could be removed, therefore, some was collected in the spoiled layer. This may explain why the spoiled portions of the barrels covered with DS resulted in a decreased NDF content. The fat from the DS cover could be diluting the NDF concentration in the spoiled layer. Similar results were reported when analyzing silage that had spoiled in a bunker. The silage increased from 42.6% to 48.9% NDF when it spoiled (Bolsen, 2011).

The results for OM content of the WDGS:straw mixture showed the greatest decrease with the salt covering (10.8 percentage units decrease), but presumably all related to salt being included in the spoiled material (salt is inorganic matter). Barrels that were left open (3.9 percentage units) and covered with plastic (3.8 percentage units) had the smallest decrease in OM content. The other five cover treatments fell intermediate to these two cover treatments. Again, these results are comparable with results reported on OM changes in silage that contained spoilage. When silage spoils decreases from 94.7% to 90.9% OM were observed (Bolsen, 2011).

The CP % generally increased in the spoiled layer, with any cover treatment, of both the WDGS:straw and DS:straw mixtures. This is likely due to the microbes utilizing different types of OM. If microorganisms utilize carbohydrates instead of protein, the proportion of CP in the feed will become more concentrated. Another explanation would be microorganism producing microbial protein while degrading the nutrients, thus increasing the CP concentration. This is also seen when silage spoils; % CP increases from 6.9% in the original silage to 9.4% in spoiled silage (Bolsen, 2011).

Exp. 2

There was a significant ($P < 0.01$) effect of cover treatment on pH of the spoiled and non-spoiled layer of the WDGS:straw mixture (Table 4). The spoilage process caused the pH of the WDGS:straw mixture to increase from its initial pH of 4.42 to a final pH of 7.11 in the spoiled layer when salt was used as a cover. When DS was used as a cover the pH changed from 4.42 to 6.88 in the spoiled layer. The other four cover treatments fell intermediate in terms of pH change. Barrels treated with soluble+straw, solubles, salt, plastic, and left open had statistically similar pH's within the spoiled layer. Barrels treated with solubles+salt showed the least change (4.42 to 6.11) of pH within the spoiled layer. There was no effect of cover treatment on pH in the spoiled layer of the MDGS ($P > 0.05$).

The DM content of the spoiled material also changed between cover treatments. Initial DM content of the WDGS:straw mixture prior to storage was 42.6%. The greatest change, numerically, in DM content was in the WDGS:straw barrels covered with plastic (7.7 percentage unit decrease). The smallest change in the WDGS:straw mixture, numerically, was in barrels covered with salt or solubles (0.2 percentage unit increase). While the other three treatments fell intermediate. However, these changes in DM content are not the same as DM losses within the spoiled layer. There was a significant ($P < 0.01$) effect of cover treatment on the amount of DM lost due to spoilage within the WDGS:straw mixture. The greatest DM loss was found in the barrels with solubles+straw as covers (11.05% DM loss), and the smallest DM loss was in barrels covered in solubles+salt (1.6% DM increase), plastic (3.5% DM loss), or solubles (5.2% DM loss). The other two cover treatments fell intermediate in terms of DM loss. Barrels of straight MDGS left open had the greatest increase (9.43 percentage units) in DM content, while

the barrels with the barrels covered with plastic had a decrease (8.27 percentage units) in % DM. There was a significant effect of cover treatment on the amount of DM actually lost due to spoilage. Barrels of MDGS left open (12.2%) had greater DM loss than barrels covered in plastic (2.8%).

Similar to Exp. 1, fat content (% of DM) changed within the spoiled layer. The initial fat content of the WDGS:straw mixture was 7.8%. The greatest decrease, numerically, in fat content for the WDGS:straw mixture was observed in barrels covered in salt (4.3 percentage units), solubles+salt (4 percentage units), and left open (4.02 percentage units). There was a significant ($P < 0.01$) effect of cover treatment on the amount of fat lost due to spoilage in the WDGS:straw mixture. The greatest loss was observed in the barrels covered in solubles plus straw (28.93%), while the least amount of fat loss was observed in barrels covered in either plastic (4.80%) or solubles plus straw (4.88%). Similarly, barrels of straight MDGS showed decreases, 4.56 percentage units (open) and 3.55 percentage units (plastic), in % fat. Barrels covered in plastic showed the least ($P < 0.01$) amount of fat lost (3.89%) compared to barrels left open (24.03%).

The NDF content (% of DM) generally increased in the WDGS:straw mixture as spoilage occurred. However, barrels that were treated with the solubles plus salt cover showed a 13.7 percentage unit increase in NDF content. When separating the solubles plus salt from the spoiled layer, some of the cover could have remained with the spoiled layer. This may explain why the spoiled portions of the barrels covered with solubles plus salt resulted in a decreased NDF content. This was observed with barrels treated with DS in Exp. 1. Even though there were increases in % NDF in the spoiled layer of WDGS:straw mixture, there were still NDF losses due to spoilage. The greatest NDF

losses ($P < 0.01$) were observed in the barrels covered with solubles+straw (15.55% lost). The other 5 treatments applied to the WDGS:straw mixture reported similar NDF losses ($P < 0.01$). Similar observations were made in the MDGS barrels. There was 7.49 percentage unit increase in % NDF for barrels left open, and a 8.54 percentage unit increase in barrels covered with plastic. Losses in NDF due to spoilage were not significantly different ($P = 0.17$).

The results for OM content of the WDGS:straw mixture showed the greatest decrease with the salt cover (10.3 percentage units) and the solubles plus salt cover (13.3 percentage units), but this is likely because the cover was included in the spoiled material (salt is inorganic matter). Barrels that were left open (5.3 percentage units) and covered with plastic (5.6 percentage units) had the smallest decrease in OM content. The other three cover treatments fell intermediate to these two cover treatments. Again, these results are comparable with results reported in Exp. 1. Losses in OM due to spoilage were greatest ($P < 0.01$) in barrels covered with solubles plus straw (19.54%), while barrels covered with salt, solubles, or left open showed the least amount of OM lost. Barrels containing MDGS that were left open had a 1.62 percentage unit decrease in %OM, while the barrels covered in plastic reported a 2.94 percentage unit decrease in %OM. However, barrels left open had greater ($P < 0.01$) amounts of OM loss due to spoilage when compared to the barrels covered in plastic.

Plastic resulted in resulted in the least ($P < 0.01$) amount of spoilage, 7.8% spoilage in the WDGS:straw mixture and 4.6% spoilage in the MDGS. Decreasing the amount of spoilage thus decreases the amount of nutritional losses. The barrels left uncovered resulted in the greatest amount of spoilage, 19% in the WDGS:straw mixture

and 38.7% in the MDGS. The plastic reduced the amount of oxygen that reached the surface of the by-products, which helped retain feeding value. The barrels left uncovered also resulted in the greatest losses in DM, OM, fat, and NDF in both the WDGS:straw mixture and MDGS barrels. Barrels covered with plastic or solubles plus salt resulted in the least amount of DM, fat, and NDF loss.

IMPLICATIONS

The results of these two studies illustrate that the nutritional changes and overall losses due to spoilage are inevitable. However, these changes can be minimized by applying a cover treatment to reduce the amount of oxygen exposure on the surface of the bunker. Based on the results from these two studies, plastic and solubles plus salt seem to have the greatest impact on reducing nutritional changes and losses due to spoilage.

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Table 1. Cover treatments (Exp. 1).

WDGS : Straw	
Open	Barrels were left uncovered.
Open + H_2O	Uncovered with water added at a rate of 1.52 cm. weekly to mimic average Nebraska precipitation.
Plastic	6 mil plastic covering the surface of the mixture weighed down with sand and the edges were sealed with tape. This treatment would be comparable to plastic and tires in a bunker setting.
Salt	Salt was sprinkled over the surface of the mixture at a rate of 0.45 kg/ 929.03 cm^2.
DS[1]	DS were poured over the surface of the mixture to make a 7.62 cm layer (20.4 kgs as-is).
DS + Salt	DS and salt added at rates previously discussed and mixed together before application.
DS + Salt + H_2O	DS and salt added at rates previously discussed and water added at 1.524 cm weekly.
WDGS:straw	
Open – Inside	Barrels left uncovered and stored inside.
Open – Outside	Barrels left uncovered and stored outside at the University of Nebraska Feedlot near Mead, NE and exposed to any rainfall.

[1]Distillers Solubles- thin stillage taken off during the milling process.

Table 2. Cover treatments (Exp. 2).

WDGS : Straw	
Open	Barrels were left uncovered.
Plastic	6 mil plastic covering the surface of the mixture weighted down with sand and the edges were sealed with tape. This treatment would be comparable to plastic and tires in a bunker setting.
Salt	Salt was sprinkled over the surface of the mixture at a rate of 0.45 kg/ 929.03 cm^2 (1.25 kg total).
DS[1]	DS were poured over the surface of the mixture to make a 7.62 cm layer (20.4 kg as-is).
DS + Salt	DS and salt added at rates previously discussed and mixed together before application.
DS + Straw	DS and straw (60:40 blend) added over the surface to make a 7.62 cm layer (11.3 kg as-is).
MDGS	
Open	Barrels left uncovered and stored.
Plastic	6 mil plastic covering the surface of the mixture weighted down with sand and the edges sealed with tape. This treatment would be comparable to plastic and tires in a bunker setting.

[1]Distillers Solubles- thin stillage taken off during the milling process.

Table 3. Effects of different cover treatments on nutrient composition of WDGS:straw and DS:straw mixtures (Exp 1).

	Oven DM%		pH		Fat%		NDF%		OM%		CP%	
WDGS[3]: Straw	**SP**	**N**	**SP**	**N**	**SP**	**N**	**SP**	**N**	**SP**	**N**	**SP**	**N**
Open	44.0	36.3	8.1	4.1	4.9	10.6	52.9	42.2	88.0	91.9	28.7	27.6
Open + H_2O	25.3	33.7	7.6	4.5	6.0	10.5	55.3	43.0	85.8	91.3	25.9	27.9
Plastic	39.0	41.2	7.2	3.9	7.2	10.1	49.3	45.4	88.0	91.8	29.3	25.7
Salt	43.6	39.4	8.5	4.0	4.1	10.2	50.5	48.3	80.9	91.7	24.0	25.5
DS	37.4	39.3	6.5	3.9	10.0	10.1	38.1	44.3	86.1	91.2	29.9	23.7
DS + Salt	39.3	38.0	5.4	4.1	7.4	10.5	35.2	40.9	80.0	89.0	25.6	25.5
DS + Salt + H_2O	32.3	34.2	6.0	4.0	9.5	9.4	41.7	43.7	82.3	88.6	26.1	24.7
D[4]: Straw												
Open – Inside	41.3	44.5	7.5	4.0	5.9	13.2	46.2	35.1	81.0	87.9	23.2	18.2
Open – Outside	43.2	41.5	7.0	4.1	7.1	13.0	43.8	36.5	81.7	88.2	22.3	19.4

[1]S-Spoiled material.
[2]NS-Non-spoiled material.
[3]Wet distillers grains plus solubles (30% DM)
[4]Distillers solubles

Table 4. Effects of different cover treatments on nutrient composition, losses, and pH of WDGS plus straw (Exp. 2).

	WDGS+ Straw (Open)	WDGS + Straw (Plastic)	WDGS + Straw (Salt)	WDGS + Straw (Solubles)	WDGS + Straw (Solubles + Salt)	WDGS + Straw (Solubles + Straw)	P-Value
pH							
Initial pH	4.42	4.42	4.42	4.42	4.42	4.42	-
Non-spoiled pH after[1]	4.33[a]	4.03[b]	4.33[a]	4.03[b,d]	4.03[b]	4.31[a]	<0.01
Spoiled pH after[2]	6.72[a]	6.77[a]	7.11[a]	6.88[a]	6.11[b]	6.82[a]	<0.01
DM							
Initial DM, %	42.6	42.6	42.6	42.6	42.6	42.6	-
Spoiled DM, %	41.6	34.9	42.8	42.8	43.8	37.3	-
Non-spoiled DM, %	37.5	39.1	37.8	38.3	38.7	37.1	-
DM Loss, %	8.1[a,d]	3.5[b]	7.3[a,b,d]	5.2[a,b]	-1.6[c]	11.05[d]	<0.01
OM							
Initial OM, %	92.6	92.6	92.6	92.6	92.6	92.6	-
Spoiled OM, %	87.3	87.9	82.3	86.2	79.3	86.7	-
Non-spoiled OM, %	91.8	92.3	91.8	91.7	91.7	92.2	-
OM Loss, %	9.08[a]	3.89[b]	9.47[a]	13.59[c]	7.82[a]	19.54[d]	<0.01
Fat							
Initial Fat, %	7.8	7.8	7.8	7.8	7.8	7.8	-

Spoiled Fat, %	3.78	6.5	3.5	4.5	8.0	3.8	-
Non-spoiled Fat, %	10.1	9.7	10.1	9.9	9.2	9.8	-
Fat Loss, %	17.33[a]	4.80[b]	21.75[c]	24.70[d]	4.88[b]	28.93[e]	<0.01
NDF							
Initial NDF, %	41.9	41.9	41.9	41.9	41.9	41.9	-
Spoiled NDF, %	49.8	45.5	46.5	46.3	28.2	51.8	-
Non-spoiled NDF, %	35.1	35.6	35.7	35.7	37.0	37.2	-
NDF Loss, %	4.85[a]	2.47[a]	5.20[a]	7.63[a]	6.05[a]	15.55[b]	<0.01
Spoilage							
Spoil, %	19.0[a]	7.8[b]	23.4[c]	17.8[a,d]	15.0[d]	17.2[a,d]	<0.01
Non-Spoil, %	81.0[a]	92.2[b]	76.6[c]	82.2[a,d]	85.0[d]	82.8[a,d]	<0.01
Nutrient recovery for covers							
OM recovered, %	-	-	-	43.15	59.51	32.41	0.44
Fat recovered, %	-	-	-	12.10[a]	96.13[b]	7.11[a]	<0.01

[a,b,c] means with different superscripts are different ($P < 0.05$)

Table 5. Nutrient composition, losses, and pH of modified distillers grains plus solubles alone stored with no cover (Open) or with plastic covering (Plastic) in Exp. 2.

	MDGS (Open)	MDGS (Plastic)	P-Value
pH			
Initial pH	4.63	4.63	-
Non-spoiled pH	4.27	4.31	0.60
Spoiled pH after	6.70	6.82	0.77
DM			
Initial DM, %	46.01	46.01	-
Spoiled DM, %	55.44	37.74	-
Non-spoiled DM, %	41.44	44.72	-
DM Loss, %	12.2	2.8	<0.01
OM			
Initial OM, %	95.52	95.52	-
Spoiled OM, %	93.90	92.58	-
Non-spoiled OM, %	94.76	95.48	-
OM Loss, %	12.49	2.92	<0.01
Fat			
Initial Fat, %	13.40	13.40	-
Spoiled Fat, %	8.84	9.85	-

Non-spoiled Fat, %	13.53	14.26	-
Fat Loss, %	24.03	3.89	<0.01
NDF			
Initial NDF, %	23.09	23.09	-
Spoiled NDF, %	30.58	31.63	-
Non-spoiled NDF, %	19.79	22.24	-
NDF Loss, %	5.77	2.25	0.17
Spoilage			
Spoil, %	38.7	4.6	<0.01
Non-Spoil, %	61.3	95.4	<0.01

[a,b,c] means with different superscripts are different (P<0.05)

CHAPTER III

Effects of spoilage of wet distillers grains plus solubles when stored in a bunker on nutrient composition and performance of growing and finishing cattle.[1]

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ABSTRACT

Three studies evaluated the impact of spoilage of wet distillers grains plus solubles (WDGS) on nutrient composition and cattle performance. In Exp. 1, a 140-d barrel study was conducted to simulate bunker storage. Two barrels were weighed and sampled on d 7, 14, 28, 56, 84, 112, and 140 of storage. An interaction between days of storage and DM, OM, and NDF recovered was observed at the surface in spoiled material. Spoilage led to increased pH of the WDGS from 3.95 to 6.72 on d 140 ($P <$ 0.01). Spoilage over time increased (P =0.10) from d 7 to 140 (6.35-11.70%). The amount of OM lost increased from 4.9% on d 14 to 22.6% on d 140 ($P <$ 0.01). In Exp. 2, a 130-d finishing study utilized 60 individually-fed steers (399 ± 30 kg) fed 3 treatments: a dry-rolled corn (DRC) based diet (control) and 2 diets containing 40% WDGS replacing DRC. The WDGS was stored in either an uncovered bunker (spoiled WDGS) or anaerobically in a silo bag (non-spoiled WDGS). Calculations suggest 12% of DM was lost during storage in the bunker. Feeding control, non-spoiled WDGS, or spoiled WDGS did not affect DMI (P = 0.50). No differences ($P \geq 0.26$) in ADG (1.39± 0.30 kg/d), final BW (571 ± 46 kg), or G:F were observed between non-spoiled and spoiled WDGS treatments. However, both WDGS treatments were greater ($P \leq 0.04$) in ADG, final BW, and G:F than the control. In Exp. 3, an 84 day growing study utilized 60 individually fed steers (332 ± 15 kg) in a 2x2 factorial. Treatments were bunkered vs. bagged WDGS fed at 15 or 40% with 81% or 57% grass hay (DM basis). Calculations suggest that 6.0% of DM was lost during storage in the bunker. Feeding spoiled WDGS decreased DMI ($P <$ 0.01) across both levels of dietary WDGS compared to non-spoiled WDGS (7.41± 1.2 vs.

8.08± 0.92 kg/d). The diets containing spoiled WDGS had statistically similar ADG (0.42 ±0.17 vs. 0.46± 0.13 kg/d) and G:F compared to diets with non-spoiled WDGS ($P \geq$ 0.16). Feeding WDGS containing spoilage did not affect performance of finishing steers. However, when fed to growing steers spoilage did decrease DMI, but had little impact on ADG and no effect on G:F.

Key Words: cattle, spoilage, storage, wet distillers grains plus solubles

INTRODUCTION

Distillers grains have been found to have a greater energy value relative to corn, especially wet distillers grains plus solubles (WDGS), which is roughly 130% the energy value of corn (Klopfenstein, 2008). However, WDGS has a high moisture content, 30-35% DM (Buckner, 2011), which causes storage and shelf life issues. Research has shown that once WDGS is stored and exposed to oxygen, spoilage can occur. Spoilage can occur within a few days depending on the amount of oxygen exposure and ambient air temperature (Christensen, 2010). These shelf life issues can be avoided if producers keep a fresh supply, and utilize all the WDGS within a few days of delivery. However, this limits the ability of smaller cattle operations, because milling plants prefer to deliver semi-load quantities (25-30 tons), which makes it difficult for smaller cattle operations to utilize all of the WDGS before spoilage starts to occur. Similarly, cow-calf producers may want to use WDGS, but only on a seasonal basis. Milling plants have a difficult time accommodating seasonal usage, because they produce WDGS consistently throughout the year. Not only do milling plants have to deal with seasonal users of WDGS, but they also have to deal with seasonal changes in the number of cattle in feedlots (NASS, 2012).

Past trends indicate that fewer cattle are fed during the summer months (i.e. July, August, and September) and more cattle on feed from November-June (NASS, 2012). Due to the seasonality of cattle numbers in the feedlot, the price of distillers grains tends to be lower during the summer months due to a decrease in demand. This makes it practical and economical for producers to store large quantities of distillers grains during the summer months for use later during the winter months. Again, producers will be faced with the issues of storage and shelf-life of WDGS (Erickson, 2008).

Christensen et al. (2010) and Yelden et al. (2011) determined that storing WDGS decreased fat and increased pH, NDF, ash, and CP. Fat decreased from 10.2% to 4.1% DM and 10.6 to 4.9% DM, respectively. These changes in the nutrient composition of WDGS during storage could be impacting cattle performance, especially since most producers do not separate the spoiled material from the non-spoiled material. Therefore, the objectives of this research were to evaluate the effects of spoilage of WDGS on the nutrient profile over time, and to determine the impact of feeding spoiled WDGS to growing and finishing cattle.

MATERIALS AND METHODS

All animal care and management procedures were approved by the University of Nebraska-Lincoln Institution of Animal Care and Use Committee.

Exp. 1-Barrel Study

A barrel study was conducted over a 140 d period. Fourteen 200 L barrels packed with wet distillers grains plus solubles (WDGS) were used to mimic bunker storage on a smaller scale. The barrels were filled to approximately the same weight (136 kg) and

height. All barrels were stored in a building, where they were subjected to ambient temperatures, but not precipitation. The barrels were filled and placed in storage on June 2, 2010. Barrels were stored for 7, 14, 28, 56, 84, 112, or 140 d. On each of these days, two barrels were weighed, and sampled. This process consisted of separating the spoiled and non-spoiled material. The spoilage was determined by appearance and texture, with the spoiled material having a dark brown appearance and the non-spoiled material having the typical golden appearance. Once the spoiled layer had been separated, the spoiled layer and the remaining unspoiled WDGS were then measured for height, weight, and sub-sampled for analysis.

The spoiled and non-spoiled samples were analyzed for DM, ash, OM, fat, NDF, CP, and pH. Samples were placed in a forced-air oven at 60°C for 48 h to determine DM (Buckner, 2011). Ash and OM were determined by placing samples in a muffle furnace for 6 h at 600°C. Ether extract was determined by performing a biphasic lipid extraction procedure described by Bremer (2010). Neutral detergent fiber analysis was conducted using the procedure described by Van Soest et al. (1991) with modifications described by Buckner et al. (2010). Crude protein was determined by using a combustion chamber (TruSpec N Determinator, Leco Corporation, St. Joseph, MI) (AOAC, 1999; method 990.03). Nutrient analyses for both the spoiled and non-spoiled layers, along with nutrient analysis of the original WDGS sample, were used to determine the nutrient losses. Losses were calculated using the weights and nutrient composition of both the spoiled and non-spoiled layer. In the calculations, the spoiled layer is included in the recovered DM, fat, NDF, CP, and OM; assuming that the spoiled and non-spoiled WDGS

would be fed. Therefore, if the spoiled layer were discarded, the loss would be the total of DM loss plus spoilage amount.

Data were analyzed using the mixed procedures of SAS (Version 9.2, SAS Inc., Cary, NC). The model included effect of days (7, 14, 28, 56, 84, 112, and 140 d) in storage. Barrel was used as the experimental unit. Contrasts were used to test the linear and quadratic effects of the number of days in storage on nutrient losses. Probabilities less than or equal to alpha ($P \leq 0.05$) were considered significant.

Exp. 2-Finishing Trial

A 130-d finishing trial was conducted utilizing 60 individually fed steers (398 ± 30 kg). Five days prior to the start of the experiment, steers were limit fed to minimize variation in initial BW due to gut fill, weighed for three consecutive days (Stock et al. 1983) (d -1, 0, and 1) to obtain initial BW. Steers were stratified by BW based on d -1 and 0 BW and then assigned randomly to treatments. During the initial weighing process, all steers were implanted with Revalor-S (120 mg of trenbolone acetate and 24 mg of estradiol, Merck Animal Health, De Soto, KS). Animals were individually fed one of three treatments using the electronic Calan gate system (American Calan, Northwood, NH). There were twenty steers per treatment, with steer as the experimental unit.

The 3 treatments included a dry-rolled corn based control diet (CON) and two diets containing 40% WDGS that was stored in either a silo bag or bunker replacing DRC. All three treatments also contained alfalfa at 7.5% of the diet DM, and supplement at 5.0% of the diet DM (Table 6).

The WDGS was purchased from one ethanol plant (Abengoa Bioenergy, York, NE) and split equally within semi-load into either an uncovered bunker (spoiled WDGS)

at a depth of 37 cm or into a silo bag and stored anaerobically (non-spoiled WDGS). The WDGS was bagged (Kelly Ryan 2W08, Blair, NE) under no pressure. Storage was initiated on June 2, 2010; 38 d prior to the start of the experiment (July 10, 2010) to allow for spoilage to occur.

Samples of WDGS (from both storage methods) were collected daily after allowing the WDGS to mix alone in the truck prior to diet mixing to ensure accurate sampling occurred throughout. Daily samples of WDGS were composited by week for nutrient analysis. Weekly composites were analyzed for DM, ash, fat, NDF, CP, and pH. Analyses for nutrient composition were conducted with the same procedures as described in Exp. 1. A composite of the bagged and bunkered WDGS were analyzed for mycotoxins (Romer Labs; Union, MO). Feed refusals were weighed, sampled, and placed in a 60°C forced air-oven to determine DM twice per week to calculate accurate DMI for each steer.

All steers were harvested on d 130 at Greater Omaha (Omaha, NE). Hot carcass weight (HCW) and liver abscesses were recorded on the day of harvest; USDA marbling score, 12th rib fat thickness, and LM area were collected after a 48 hr chill. For USDA calculated YG, KPH fat was assumed to be 2.5%. Hot carcass weights were used to calculate adjusted final BW by dividing HCW by a common dressing percentage (63%). Yield grade was calculated using the equation: USDA YG = 2.5 + 2.5(12th rib fat thickness, cm) – 0.32(LM area, cm^2) + 0.2(KPH fat, %) + 0.0038 (HCW, kg) (Boggs and Merkel, 1993).

Data were analyzed using the Proc Mixed procedure of SAS (Version 9.2, SAS Inc., Cary, NC) as a CRD. The model included effect of treatment (control, WDGS stored

in silo bag, WDGS stored in a bunker). Steer was the experimental unit. Treatment differences with alpha=0.05 were considered significant.

Exp. 3-Growing Study

An 84-d growing trial was conducted utilizing 60 individually fed crossbred steers (332 ± 30 kg). Initial processing when calves were received in the feedlot included vaccination with Bovi-Shield Gold 5 (a modified live virus vaccine for protection against: IBR, BVD Types I & II, PI3, and BRSV) and Somubac (for prevention of *Haemophilus somnus;*28 Pfizer Animal Health, New York, NY), and injection with Dectomax (paraciticide; PfizerAnimal Health) and Micotil (antimicrobial; Elanco Animal Health, Greenfield, IN). Approximately 14 d later, cattle were revaccinated with Piliguard Pinkeye + 7 (for prevention of pinkeye and clostridial infections; Merck Animal Health, Summit, NJ) and Ultrabac-7 Somubac (*Haemophilus somnus* booster; Pfizer Animal Health).

Steers were limit fed a common diet at 2.0% of BW for 5 d, weighed 3 consecutive d (d -1, 0, and 1) to obtain initial BW (Stock, 1983). Steers were stratified by BW based on d -1 and 0 BW and then assigned randomly to treatments. Steers were implanted with Revalor-G (40 mg trenbolone acetate and 8 mg estradiol, Merck Animal Health, Summit, NJ) on d 0 of BW collection. Animals were individually fed one of four treatments using the electronic Calan gate system (American Calan) with 15 steers per treatment. The 4 treatments were designed as a 2x2 factorial. The two factors included: WDGS that was stored in a bunker (spoiled) or stored in a silo bag (non-spoiled), and dietary inclusion of 15% or 40% WDGS (DM basis).

The treatments with 15% WDGS were formulated to meet the protein needs of the steers. The 40% inclusion treatments were formulated to meet the protein needs of steers and provide additional energy. The WDGS was purchased from an ethanol plant (Abengoa Bioenergy, York, NE) and split equally within semi-load into either an uncovered bunker (spoiled WDGS) or into a silo bag under no pressure (Kelly Ryan 2W08) and stored anaerobically (non-spoiled WDGS). Storage was initiated 5 months (October, 2010) prior to starting the experiment (March 24, 2011) to allow for spoilage to occur throughout the winter months. Samples of WDGS (from both storage methods) were collected daily after allowing the WDGS to mix alone in the truck prior to diet mixing to ensure accurate sampling occurred throughout. Daily samples of WDGS were composited every 35 d for nutrient analysis. Composites were analyzed for DM, ash, fat, NDF, CP, and pH. The analyses were conducted using the same procedures as described in Exp. 1. A composite of the bagged and bunkered WDGS were analyzed for mycotoxins (Romer Labs, Union, MO). Feed refusals were weighed, sampled, and placed in a 60°C forced air-oven to determine DM twice per week to calculate accurate DMI for each steer.

Growth performance data were analyzed using the mixed procedures of SAS (Version 9.2, SAS Inc., Cary, NC) as a CRD. The model included source of WDGS (silo bag or bunker), WDGS inclusion (15 or 40% diet DM), and source x inclusion comparisons. Steer was the experimental unit. Treatment differences with alpha=0.05 were considered significant.

RESULTS AND DISCUSSION

Exp. 1

There was a linear increase ($P < 0.01$) in the amount of DM (8.6% on d 7 and 21.1% on d 140), OM (8.8% on d 7 and 22.6% on d 140), and NDF (1.20% on d 7 and 27.10% on d 140) lost as storage time of WDGS increased (Table 8). Spoilage caused a loss of DM, OM, and NDF. These losses were comparable to those reported by Harding et al. (2012). Spoilage also increased the pH of the WDGS from 3.95 (initial pH) to 6.72 on d 140 ($P < 0.01$) in the spoiled layer. There was a linear increase in the pH of the spoiled and non-spoiled layer ($P < 0.01$) as storage progressed. The non-spoiled layer pH increased from 3.95 to 4.12 on d 140 ($P < 0.01$). The increased pH in the spoiled material was similar to previous barrel studies conducted by Yelden et al. (2011) and Harding et al. (2012). Harding et al. (2012) reported that as spoilage occurred in a 70:30 blend of WDGS and straw, the pH increased from 4.42 in the original WDGS:straw mixture to a pH of 6.72 after 60 days of storage. There was no statistical effect on CP losses; however CP concentrations increased numerically from d 7 to 140. Days 7, 14, and 28 showed the least amount of DM loss, averaging 6.73% DM lost ($P <0.01$). Numerically days 112 and 140 showed the greatest loss of DM (22.4% and 21.1%), while days 56 and 84 fell intermediate ($P< 0.01$). Again, there was a linear increase in the amount of DM lost ($P < 0.01$). Similar losses (8.1%) were reported when storing a WDGS:straw mixture uncovered for 60 days by Harding et al. (2012). There was also a linear increase in the amount of spoiled material over time ($P=0.10$) d 7 to 140 (6.35-11.70%).

The amount of OM lost due to spoilage increased from 4.85% on day 14 to 22.60% on d 140 ($P < 0.01$). These losses increased linearly over time ($P < 0.01$).

However, there was no statistical effect of time on the amount of fat lost ($P = 0.67$), indicating that the amount of fat lost due to spoilage didn't depend on the length of time the WDGS was stored.

Based on previous research reported by Yelden et al. 2011, there are two things that happen during the spoilage process; organic matter loss and nutrient composition change within the spoiled layer. We hypothesized that the change within the spoiled layer is related to the organic matter loss. Since the barrels contained the same WDGS as the WDGS fed in Exp. 2 we were able to use the relationship found between percent spoiled and the percent ash (combining both spoiled and non-spoiled ash content) in the barrels to estimate the amount of spoilage in the bunker (Figure 2) for both Exp. 2 and 3.

Exp. 2

The WDGS used in Exp. 1 was from the same semi-load as the WDGS used in this experiment. The regression equation (Figure 2) estimated that steers fed the spoiled treatment (WDGS stored in the bunker) consumed WDGS that contained 7% spoilage on average. Trace amounts of mycotoxins were present in the WDGS (Table 9). However, it is important to note that the WDGS stored in either the bunker or bag contained trace amounts, which indicates that the mycotoxins were present prior to storing the WDGS in the bag or bunker. However, the U.S. Food and Drug Administration limits the level of fumonisin in feedlot cattle diets to 60 ppm, and it be included in no more than 50% of the diet DM (Vincelli,2002). Based on these limits the small amounts present in the WDGS stored in either the bag or bunker were not a concern.

Nutrient analysis of the spoiled WDGS (WDGS stored in the bunker) and non-spoiled (stored in a bag) WDGS (Table 10) indicated WDGS containing spoilage was 0.7

percentage units lower in fat content throughout the feeding period compared to the non-spoiled WDGS. The WDGS containing spoilage (bunker) has greater % DM (dried out), ash, NDF, pH, and no change in CP compared to the non-spoiled (bagged) WDGS. These changes are consistent with the previous barrel study conducted by Yelden et al. (2011). They reported that spoilage caused a decrease in fat, which in turn increased the concentrations of ash and NDF as a percent of the spoiled material. However, these changes in nutrient composition reported by Yelden are within the spoiled layer of WDGS, not an overall nutrient profile of the mixture of both the unspoiled and spoiled layers of the WDGS that are placed in the bunker. The nutrient composition from the bunkered WDGS in this study is derived from analyzing samples that are a mixture of both the spoiled and non-spoiled layers (i.e. what the cattle are actually eating). Therefore, when comparing the nutrient profiles of the bagged and bunkered WDGS in this study, the differences are much less pronounced.

The ash content was used as a marker to calculate the overall loss of DM of the spoiled WDGS from the day (June 2, 2010) it was stored in the bunker (Table 10). The calculated loss indicated that WDGS stored uncovered in a bunker lost 12.3% DM. Also, storing WDGS this way resulted in 16% fat, 8% NDF, and 12.3% CP to be lost (as % of initial amounts). These data suggest that the WDGS containing spoilage changed in composition compared to the initial WDGS purchased on June 2 because 16% fat was lost compared to 12.3% DM. These losses in DM are similar to the barrel study described in Exp. 1, and those reported by Harding et al. (2012). It is important to note that these losses are not the same as the nutrient composition reported previously. These are amounts lost that occur within the bunker during the storage process.

Despite nutrient losses, feeding the control, non-spoiled WDGS, or spoiled WDGS treatments did not affect DMI (Table 11). No differences in ADG, final BW, or G:F were observed between non-spoiled and spoiled WDGS treatments. This disproves the initial hypothesis that cattle consuming WDGS that contained spoilage would have a decrease in performance due to the losses, nutrient composition changes, or palatability of the bunkered WDGS.

However, cattle fed either WDGS treatments had greater ($P \leq 0.04$) ADG, final BW, and G:F compared to the DRC based control. These data are in agreement with previous studies comparing diets containing WDGS vs. diets only containing DRC (Klopfenstein, 2008). Vander Pol et al. (2005) reported a quadratic increase in ADG, DMI, and G:F for animals being fed WDGS, with optimum inclusion of WDGS being 30 to 40% of diet DM. Bremer et al. (2008) reported that performance and carcass characteristics improved up to 30 to 40% inclusion of the diet DM. Bremer also reported that when WDGS is fed between 15 to 40% of the diet DM, it is 130% the feeding value of corn. Even though the spoiled WDGS changed in composition from the initiation of the trial to the end; these data suggest the spoilage occurring when WDGS is stored in a bunker has no effect on the performance of finishing steers. However, storing WDGS in the bunker did result in a 12% DM loss over 140 d. This suggests that there will be minimal loss in normal situations where WDGS are stored for less than a week in commercial feedlots.

Exp. 3

The regression equation (Figure 2) was used to estimate the amount of spoilage in the bunkered WDGS. Steers receiving the spoiled treatments consumed WDGS that

contained 7% spoilage on average, which was similar to the amount being fed in Exp. 1. Trace amounts of mycotoxins were observed in both the WDGS placed in the bunker and the WDGS placed in bag. However, mycotoxin limits did not exceed FDA limits as described in Exp. 2.

Nutrient analysis of the WDGS containing spoilage and the non-spoiled WDGS indicated WDGS with spoilage were 1.6 percentage units higher in fat content throughout the feeding period compared to the non-spoiled WDGS. The WDGS with spoilage were higher in DM, ash, NDF, pH, and CP throughout the 84 d feeding period (Table 12) as well.

Ash was used as a marker to calculate the overall loss of DM from the spoiled WDGS from the day (October 26, 2010) it was stored in the bunker (Table 12). There was a 6.0% DM loss for the WDGS stored in a bunker, which was less than the WDGS stored in the bunker during Exp. 2. However, seasonal storage could have caused a slight difference, as this experiment was stored throughout the winter months, whereas WDGS was stored throughout the summer months in Exp 2. The ash content was also used to calculate NDF, CP, and fat losses and suggest that WDGS stored in a bunker lost 10.3% NDF and 4.9% CP, and increased 2.6% fat, indicating that the fat was becoming more concentrated in the spoiled layer due to the other nutrient losses. These data contradict previous trials (Harding, 2012), which showed losses in fat as WDGS spoiled. It also differs from the 16% fat loss for WDGS stored in a bunker reported in Exp. 2. It is unclear if season or temperature could impact these variable responses.

There was no interaction (Table 13) between inclusion of WDGS (15% or 40%) and source of WDGS (bag or bunker). Therefore, only main effects are presented. Steers

consumed more DM (P <0.01) when they were fed 40% WDGS (8.31 kg) compared to 15% WDGS (7.49 kg). This intake effect is similar to Ahern et al. (2011) who observed increased DMI when WDGS was increased in grass hay diets (P <0.01). Ahern also reported a linear increase in ending BW, ADG, and G:F as the level of energy (i.e. distillers grains) increased in the diet. Similar results were observed in this study. The diets containing 40% WDGS performed better in ending BW, ADG, and G:F (P <0.01) compared with steers fed 15% WDGS. Nuttelman et al. (2010) reported WDGS having 142% to 149% the feeding value of DRC in high forage diets.

Cattle consuming diets that contained spoiled WDGS had decreased DMI (P < 0.01) compared to non-spoiled WDGS. Feeding WDGS that was stored in the bunker (contained spoilage) had statistically similar ending BW, ADG, and G:F compared to diets containing WDGS stored in the bag (with no spoilage; P > 0.05). Therefore, there was no overall effect of source (WDGS with or without spoilage) on ending BW, ADG, or G:F. This suggests that the minimal nutritional differences between the two sources of WDGS (bag or bunker) had no effect on cattle performance. However, feeding WDGS that contained spoilage did affect intakes of growing steers. The effects of spoilage of WDGS on performance were also different in this study compared to what was observed in Exp. 2.

IMPLICATIONS

The results from these three studies indicate that the spoilage process that occurs when WDGS is stored in a bunker causes a loss of DM and nutrients, with decreases in % fat and small increases in ash content (i.e., lower OM). However, feeding WDGS that contains some spoilage does not affect finishing cattle performance. Feeding WDGS that

contained spoilage to growing steers did decrease DMI, but had little impact on ADG and no effect on G:F.

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Table 6. Dietary treatments (% of diet DM) fed to finishing steers evaluating spoilage of stored wet distillers grains plus solubles for Experiment 2.

Ingredient	Control	Spoiled	Non-spoiled
Dry-rolled corn	82.5	47.5	47.5
WDGS, Bag[1]	--	--	40.0
WDGS, Bunker[2]	--	40.0	--
Molasses	5.0	--	--
Alfalfa Hay	7.5	7.5	7.5
Supplement[3]			
Fine ground corn	1.798	2.965	2.965
Limestone	1.420	1.500	1.500
Salt	0.300	0.300	0.300
Urea	1.247	--	--
Tallow	0.125	0.125	0.125
Thiamine[4]	0.015	0.0159	0.0159
Beef Trace Minerals[5]	0.050	0.050	0.050
Vitamin A-D-E[6]	0.015	0.015	0.015

Rumensin-80[7]	0.0187	0.0187	0.0187
Tylan-40[8]	0.009	0.009	0.009

[1] Bagged wet distillers grains plus solubles stored anaerobically to minimize spoilage (non-spoiled)

[2]Bunker wet distillers grains plus solubles that was allowed to have more spoilage occurring during storage prior to and during feeding (Spoiled).

[3]Supplement formulated to be fed at 5% of diet DM.

[4]Premix contained 88 g of thiamine·kg-1.

[5]Premix contained 10% Mg, 6% Zn, 4.5% Fe, 2% Mn, 0.5% Cu, 0.3% I, 0.05% Co.

[6] Premix contained 1,500 IU of vitamin A, 3,000 IU of vitamin D, 3.7 IU of vitamin E·g-1.

[7]Premix contained 198 g of monensin·kg-1 (Elanco Animal Health, Greenfield, IN).

[8]Premix contained 88 g of tylosin·kg-1 (Elanco Animal Health).

Table 7. Dietary treatments fed to growing steers where 15 or 40% wet distillers grains were fed that had spoiled (Bunker) or not (Bag) for Experiment 3.

Ingredient[1]	15% Bunker[3]	40% Bunker[4]	15% Bag[3]	40% Bag[4]
WDGS, Bag	-	-	15.0	40.0
WDGS, Bunker	15.0	40.0	-	-
CRP Hay[2]	81.0	57.0	81.0	57.0
Supplement				
Fine Ground Corn	1.14	1.34	1.14	1.34
Limestone	0.920	1.22	0.920	1.22
Urea	1.50	-	1.50	-
Salt	0.300	0.300	0.300	0.300
Tallow	0.075	0.075	0.075	0.075
Beef Trace Minerals[3]	0.05	0.05	0.05	0.05
Vitamin A-D-E[4]	0.015	0.015	0.015	0.015

[1]Inclusion on a DM basis

[2]Low quality grass hay with a 48% TDN, 72.7% NDF, and 5.3% CP

[3]Premix contained 10% Mg, 6% Zn, 4.5% Fe, 2% Mn, 0.5% Cu, 0.3% I, 0.05% Co.

[4] Premix contained 1,500 IU of vitamin A, 3,000 IU of vitamin D, 3.7 IU of vitamin E·g-1.

Table 8. Nutrient losses (expressed as a % of the original amount of nutrient) of wet distillers grains plus solubles stored uncovered over time (140 days) in Exp. 1.

									P-Values		
	Day 7	Day 14	Day28	Day 56	Day 84	Day 112	Day 140	SEM	F-test	Linear[4]	Quad[5]
DM Loss, %	8.6[a,b]	5.0[a]	6.6[a]	17.3[b,c]	17.6[b,c]	22.4[c]	21.1[c]	2.05	<0.01	<0.01	0.04
Spoil, %	6.4	6.0	5.8	5.8	9.6	12.5	11.7	1.76	0.10	<0.01	0.99
Non-Spoil, %	93.7	94.1	94.2	94.2	90.4	87.6	88.3	1.76	0.10	<0.01	0.99
OM Loss, %	8.80[a,b]	4.85[a]	6.35[a]	18.15[b,c]	18.75[b,c]	23.90[c]	22.60[c]	2.25	<0.01	<0.01	0.04
Fat Loss[3], %	3.15	-0.75	-2.70	5.75	3.35	5.10	2.70	3.67	0.67	0.06	0.37
NDF Loss[3], %	1.20[a,b]	-12.60[b]	0.50[a,b]	17.60[b,c]	16.75[b,c]	21.45[b,c]	27.10[c]	4.82	<0.01	<0.01	0.03
CP Loss[3], %	3.95	-2.60	-5.80	0.80	1.15	8.20	-7.05	3.06	0.08	0.08	0.08
Non-spoiled pH after[1]	3.67[a]	3.87[a,b]	3.93[a,b,c]	4.26[c]	4.22[c,b]	4.09[c,b]	4.12[c,b]	0.07	<0.01	<0.01	<0.01
Spoiled pH after[1]	4.78[a]	6.18[b]	6.50[c]	6.60[c,d]	6.43[c]	6.55[c,d]	6.72[d]	0.05	<0.01	<0.01	<0.01

[a,b,c] means with different superscripts are different (P<0.05)
[1]Non-spoiled layer of WDGS pH after storage, original pH was 3.7
[2]Spoiled layer of WDGS pH after storage, original pH was 3.7
[3]Negative numbers indicate an increase in that nutrient
[4]Linear contrasts for simple effect of number of days of storage
[5]Quadratic contrasts for simple effect of number of days of storage

Table 9. Mycotoxin amounts present in the WDGS fed in Exp. 2.

Mycotoxin	Bunkered (ppm)	Bagged (ppm)
Aflatoxin B1	ND	ND
Aflatoxin B2	ND	ND
Aflatoxin G1	ND	ND
Aflatoxin G2	ND	ND
Ochratoxin A	ND	ND
T-2 Toxin	ND	ND
HT-2 Toxin	ND	ND
Diacetoxyscirpenol	ND	ND
Neosolaniol	ND	ND
Zearalenone	0.4	0.5
Fumonisin B1	0.9	0.9
Fumonisin B2	0.2	0.2
Fumonisin B3	0.2	0.2
Citrinin	ND	ND

ND- non-detectable amounts

Table 10. Weekly nutrient composition of spoiled and non-spoiled WDGS in Experiment 2.

Nutrient	Spoiled[1]	Non-spoiled[2]	Calculated Loss[3]
DM, %	35.2	33.4	12.3
Ash, %	6.4	5.6	-
Fat, %	14.1	14.8	16.0
NDF, %	33.3	31.7	8.0
CP,%	30.8	30.8	12.2
pH	4.8	4.2	-

[1]WDGS stored in a bunk.

[2]WDGS stored in a bag.

[3]Calculated using $(1-((\text{ash}_{\text{initial}}/\text{ash}_{\text{final}})*(\text{nutrient}_{\text{final}}/\text{nutrient}_{\text{initial}})$

Figure 2. Relationship between the percent of total spoilage in the barrel relative to the percent of total ash in the barrel of WDGS.

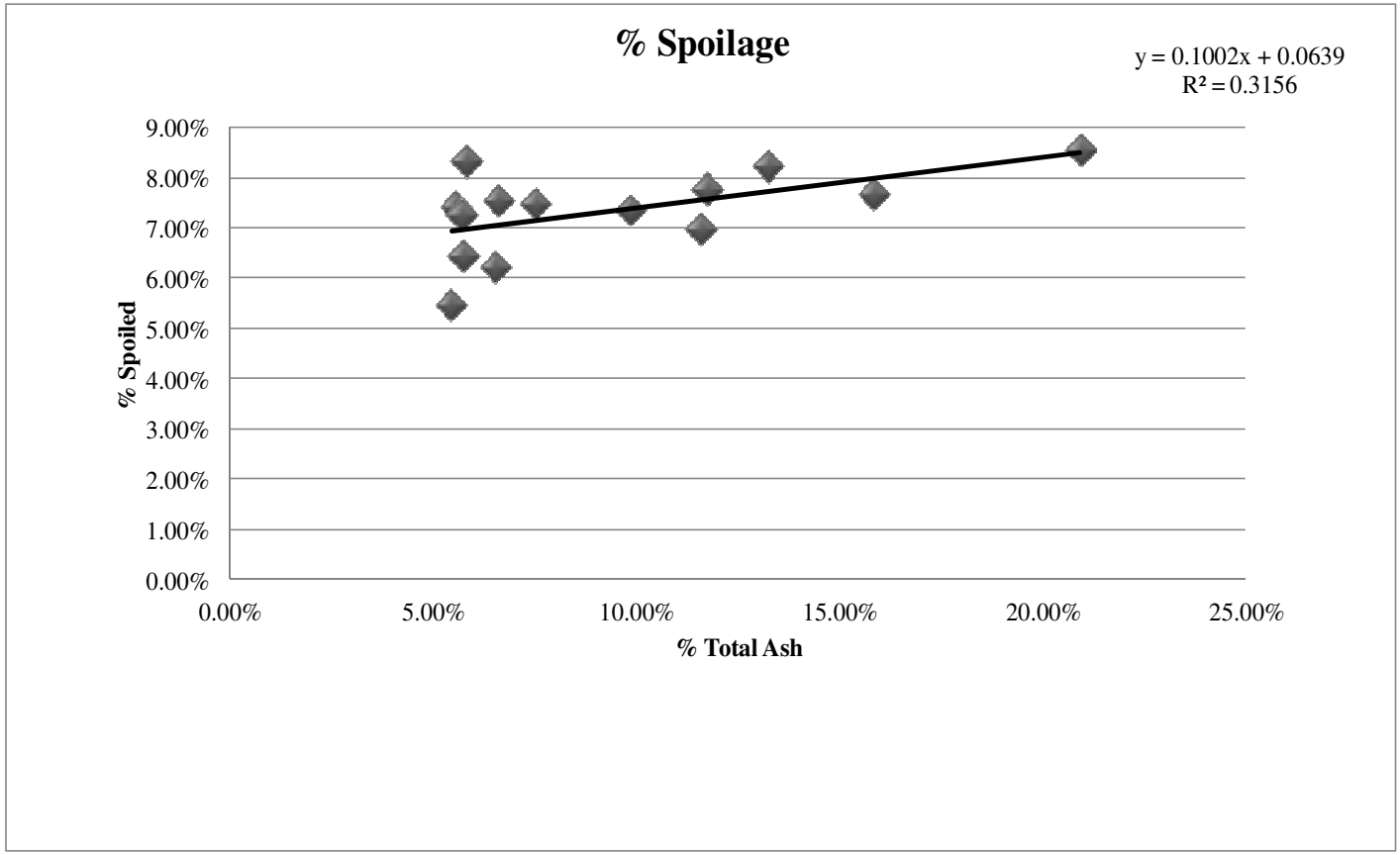

Table 11. Performance and carcass characteristics for steers fed wet distillers grains that had spoilage or not compared to a corn control diet in Experiment 2.

Variable	Control	Non-Spoiled[3]	Spoiled[4]	SEM	F-test
Initial BW, kg	395	401	399	15.3	0.81
Final BW, kg[1]	549[a]	576[b]	586[b]	22.5	0.04
DMI, kg/d	10.29	10.01	10.35	0.48	0.54
ADG, kg	1.18[a]	1.34[b]	1.44[b]	0.14	0.02
G:F	0.115[a]	0.134[b]	0.139[b]	0.34	0.01
HCW, kg	346[a]	363[b]	369[b]	14.2	0.04
LM Area, cm^2	80.6	84.5	82.6	0.3	0.35
Fat, cm	1.17	1.19	1.23	0.03	0.86
Marbling[2]	522.5	526.5	505.7	14.6	0.57
YG	3.03	3.01	3.16	0.13	0.67

[1] Final BW was calculated by taking HCW*0.63 dressing percentage.
[2] Marbling score 400=slight (Select); 500=small (Choice-); 600=modest marbling (Choice).
[3]WDGS stored in a silo bag
[4] WDGS stored in a bunker
[a, b, c] Means with different superscripts within a row are different (P<0.05).

Table 12. Weekly nutrient composition of spoiled and non-spoiled WDGS in Experiment 3.

Nutrient	Spoiled[2]	Non-spoiled[3]	Calculated Loss[1]
DM, %	37.0	35.1	6.0
Ash, %	5.8	5.2	-
Fat, %	12.8	11.2	-2.6
NDF, %	35.1	34.9	10.3
CP,%	35.2	33.1	4.9
pH	4.8	4.0	-

[1] Calculated using $(1-((\text{ash}_{\text{initial}}/\text{ash}_{\text{final}})*(\text{nutrient}_{\text{final}}/\text{nutrient}_{\text{initial}})$

[2]WDGS stored in the bunker

[3]WDGS stored in the silo bag

Negative losses indicate an increase in that nutrient

Table 13. Performance characteristics of growing steers Experiment 3

	15%		40%			P-value		
Variable	S[1]	NS[2]	S	NS	SEM	Interaction	Level	Source
Initial BW, kg	332	332	332	331	7.99	0.94	1.0	1.0
Ending BW, kg	357	360	378	380	9.27	0.83	<0.01	0.56
DMI, kg/d	6.8	7.5	8.0	8.7	0.049	0.94	<0.01	<0.01
ADG, kg	0.30	0.34	0.54	0.54	0.474	0.71	<0.01	0.13
G:F	0.041	0.043	0.067	0.067	1.11	0.42	<0.01	0.67

[1] WDGS stored in the bunker (spoiled)

[2] WDGS stored in the silo bag (non-spoiled)

Table 14. Mycotoxin amounts present in the WDGS fed in Exp. 3.

Mycotoxin	Bunkered (ppm)	Bagged (ppm)
Aflatoxin B1	ND	ND
Aflatoxin B2	ND	ND
Aflatoxin G1	ND	ND
Aflatoxin G2	ND	ND
Ochratoxin A	ND	ND
T-2 Toxin	ND	ND
HT-2 Toxin	ND	ND
Diacetoxyscirpenol	ND	ND
Neosolaniol	ND	ND
Zearalenone	ND	ND
Fumonisin B1	2.3	3.5
Fumonisin B2	0.6	0.8
Fumonisin B3	0.4	0.4
Citrinin	ND	ND

ND- non-detectable amounts