Microbial and nutritional changes of wet brewers grains inoculated with a commercial preservative

Susan P. Marston

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MICROBIAL AND NUTRITIONAL CHANGES OF WET BREWERS GRAINS
INOCULATED WITH A COMMERCIAL PRESERVATIVE

BY

SUSAN P. MARSTON
B.S. University of New Hampshire, 2004

THESIS

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Master of Science
in
Animal Science

December, 2007
This thesis has been examined and approved.

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ACKNOWLEDGMENTS

First and foremost, I would like to thank my advisor, Dr. Peter Erickson, for his support throughout both my undergraduate and graduate education and for giving me the opportunity to further my education in dairy science.

I am also grateful to the members of my committee, Dr. Sheila Andrew, Dr. Charles Schwab and the late Dr. Thomas Fairchild for their much appreciated suggestions on my project and this thesis.

Thank you to the Department of Animal and Nutritional Sciences for providing me with financial support, teaching experience and the opportunity to work with an outstanding group of researchers.

I appreciate the financial support of Agri-King, Inc. for this experiment and I would like to thank everyone at ANALAB who helped analyze my many samples. I am especially grateful to Dave Spangler for all his assistance with this project and for giving me the opportunity to spend a week at the lab. I am also grateful to Kristi Thompson and Lola Manning for spending their evenings with me and making me feel right at home in the Midwest.

In addition to the financial support of Agri-King, I would like to thank Blue Sky Ag Commodities for donating the WBG for this project.

I would also like to thank Nancy Whitehouse for all her advice and the much needed help she gave me while cannulating my cows, running my statistics and downloading data.

To the staff at the Fairchild Dairy Teaching and Research Center, thank you for feeding and taking care of my cows during my in situ trial.
To Aaron Palmer, thank you for constructing the wooden storage bins for this project.

Thank you Tom Oxford for driving the tractor and mixer wagon over from Burley-Demeritt and helping us get the experiments started!

I need to send a giant thank you to my fellow graduate students Erin Shea, Sarah Boucher, Carly Crawford and Kim Morrill for their help and support.

Erin: Your assistance with this project meant a lot to me, but your friendship meant more than you’ll ever know. Best of luck in New York!

Sarah: Thank you for always being there with a good explanation when I’m confused. Your experience and expertise are invaluable to all of us!

Carly: Thank you for helping take samples so I could get away for a weekend even though my brewers grain totally grossed you out!

Kim: Thanks for being there this past summer while I was trying to finish this thesis, your friendship and humor made many frustrating days much better. Good luck when it’s your turn!

To Fred Lundy, thank you for taking me under your wing when was first starting out. You were always willing to help me when I needed and were a great friend when I needed one. I can’t thank you enough!

Last, but not least, thank you to my family. Thank you for all the love and encouragement over the years and for supporting my decision to continue my education. I could not have done this without your support.
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LIST OF ABBREVIATIONS

WBG = wet brewers grains
DBG = dried brewers grains
DM = dry matter
CP = crude protein
d = day
h = hour
g = gram
kg = kilogram

Log CFU = logarithmically transformed number of colony forming units
ABSTRACT

MICROBIAL AND NUTRITIONAL CHANGES OF WET BREWERS GRAINS
INOCULATED WITH A COMMERCIAL PRESERVATIVE

by

Susan P. Marston

University of New Hampshire, December 2007

Two experiments were conducted to compare the deterioration of wet brewers grains (WBG) treated with an enzyme and bacterial inoculant (Silo-King GPX, Agri-King Inc., Fulton, IL). Another experiment investigated ruminal DM and CP degradability of samples taken from experiment 1. Wet brewers grains were divided into separate piles upon delivery. Silo-King GPX was added to WBG at 0 kg/kg (control), 0.45 kg/900 kg WBG, and 0.9 kg/900 kg WBG, mixed for 5 min, and then stored in bins. Piles were left for 28 d to simulate farm storage practices. Samples (300 g) were taken every 2 d, 20.3 cm below the surface and analyzed for nutrients, VFA, molds, yeasts, mycotoxins and clostridia. Treated piles had quadratic increases in fat, acetic and butyric acids and quadratic decreases in starch, Ca, Mg, K, and Mn concentrations. In experiment 1, piles treated with preservative had linear decreases in pH and increases in lactic acid. The log CFU of yeast, mold and clostridia were reduced linearly and quadratically in the treated piles. In experiment 3, treatment resulted in linear and
quadratic increases for DM, starch and K concentrations. The log CFU counts for yeast and mold decreased linearly and quadratically with treatment. Crude protein decreased linearly, while lactic acid concentration increased with treatment. A quadratic increase in ADF concentration was observed, while fat, NDIP, Na and acetic acid concentrations decreased quadratically. Covering increased NDF, ADF, NDIP, K and tended to increase log CFU mold. Three non-lactating cannulated cows were used in a 3 x 3 Latin square to determine the DM and CP degradability of samples taken from experiment 1. Cows were fed a diet supplemented with WBG at a rate of 1 kg/d. Dacron bags containing 5.3 g of dried sample were incubated in the rumen for 0, 2, 4, 8, 12, 16, 24 and 48 h (2 bags/sample d). Treatment had no effect on ruminal DM or CP degradability of WBG. This study indicates that using Silo-King GPX may reduce spoilage of WBG, without altering the degradability of the feed.
CHAPTER I

REVIEW OF LITERATURE

Feeding By-products

Incorporating by-product feeds into dairy cattle rations benefits both the agricultural and food-processing industries by providing an outlet for waste products, and benefits dairy producers through a reduction in feed costs. From a nutritional standpoint, by-product feeds can be used to increase the nutrient density of the diet, to raise the level of rumen undegradable protein (RUP) in dairy cattle diets, or can be substituted for forages to increase the fiber content of the ration (Mowrey and Spain, 1999). Due to the high moisture content of some by-products, storage and handling of these feeds can be challenging. The moist nature of wet feeds, combined with aerobic conditions, provides hospitable conditions for mold, bacteria, yeast and mycotoxin growth within the feed.

Description of Brewers Grains

Brewers grains (BG), often referred to as spent grains, are the solid residue left after the extraction of malt in the production of beer, malt extracts and malt vinegar. These by-product grains make a palatable feed that has been used for centuries as an ingredient in rations for cattle and sheep. Brewers grains are composed primarily of two nutrient constituents, crude protein (CP) (~25 % of grain dry matter (DM)) and neutral detergent fiber (NDF) (~50 % of grain DM) (Westendorf and Wohlt, 2002). Table 1 shows a comparison of the nutrient analyses of wet and dry brewers grains (WBG, DBG) as reported by the NRC (2001) and DePeters et al. (2000).
Table 1. A comparison of wet and dry brewers grains analyses. All values expressed on the basis of DM.

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<tbody>
<tr>
<td>DM, %</td>
<td>90.7 (3.5)</td>
<td>21.8 (5.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CP, %</td>
<td>29.2 (4.0)</td>
<td>28.4 (4.0)</td>
<td>23.6 (0.65)</td>
<td>27.0 (2.2)</td>
</tr>
<tr>
<td>NDF, %</td>
<td>47.4 (6.6)</td>
<td>47.1 (6.8)</td>
<td>51.4 (1.2)</td>
<td>37.3 (3.4)</td>
</tr>
<tr>
<td>ADF, %</td>
<td>22.2 (3.9)</td>
<td>23.1 (3.8)</td>
<td>25.7 (2.3)</td>
<td>18.0 (1.9)</td>
</tr>
<tr>
<td>TDN</td>
<td>71.3 (0.0)</td>
<td>71.6 (0.0)</td>
<td>71.2 (2.9)</td>
<td>75.9 (1.3)</td>
</tr>
<tr>
<td>NE_L, Mcal/kg</td>
<td>1.71 (0.0)</td>
<td>1.71 (0.0)</td>
<td>1.71 (0.03)</td>
<td>1.71 (0.02)</td>
</tr>
<tr>
<td>NE_M, Mcal/kg</td>
<td>1.84 (0.0)</td>
<td>1.84 (0.0)</td>
<td>1.80 (0.04)</td>
<td>1.91 (0.02)</td>
</tr>
<tr>
<td>NE_G, Mcal/kg</td>
<td>1.21 (0.0)</td>
<td>1.21 (0.0)</td>
<td>1.17 (0.04)</td>
<td>1.28 (0.02)</td>
</tr>
<tr>
<td>Fat, %</td>
<td>5.2 (1.6)</td>
<td>5.2 (0.0)</td>
<td>9.6 (0.3)</td>
<td>6.3 (0.4)</td>
</tr>
<tr>
<td>Lignin, %</td>
<td>5.0 (2.7)</td>
<td>4.7 (0.9)</td>
<td>8.7 (1.9)</td>
<td>5.8 (1.1)</td>
</tr>
<tr>
<td>Ash, %</td>
<td>4.3 (0.9)</td>
<td>4.9 (0.9)</td>
<td>4.5 (0.25)</td>
<td>4.28 (0.34)</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.3 (0.11)</td>
<td>0.35 (0.22)</td>
<td>0.23 (0.03)</td>
<td>0.24 (0.03)</td>
</tr>
<tr>
<td>P, %</td>
<td>0.67 (0.06)</td>
<td>0.59 (0.1)</td>
<td>0.63 (0.02)</td>
<td>0.65 (0.06)</td>
</tr>
<tr>
<td>Mg, %</td>
<td>0.26 (0.35)</td>
<td>0.21 (0.26)</td>
<td>0.25 (0.01)</td>
<td>0.27 (0.02)</td>
</tr>
<tr>
<td>K, %</td>
<td>0.50 (0.26)</td>
<td>0.47 (0.26)</td>
<td>0.36 (0.04)</td>
<td>0.26 (0.05)</td>
</tr>
<tr>
<td>Na, %</td>
<td>0.04 (0.06)</td>
<td>0.01 (0.01)</td>
<td>0.02 (0.0)</td>
<td>0.05 (0.02)</td>
</tr>
<tr>
<td>Fe, mg/kg</td>
<td>224.0 (119.0)</td>
<td>247.0 (270.0)</td>
<td>123.4 (16.5)</td>
<td>138.4 (17.6)</td>
</tr>
<tr>
<td>Cu, mg/kg</td>
<td>11.0 (6.0)</td>
<td>9.0 (7.0)</td>
<td>17.4 (2.1)</td>
<td>10.6 (3.1)</td>
</tr>
<tr>
<td>Mn, mg/kg</td>
<td>45.0 (12.0)</td>
<td>49.0 (13.0)</td>
<td>48.7 (8.8)</td>
<td>49.4 (3.7)</td>
</tr>
<tr>
<td>Zn, mg/kg</td>
<td>85.0 (15.0)</td>
<td>91.0 (17.0)</td>
<td>93.9 (23.4)</td>
<td>88.4 (8.9)</td>
</tr>
</tbody>
</table>

Standard deviation (SD) appears in parentheses.

Adapted from Westendorf and Wohlt (2002).

Production of Brewers Grains

The first step in beer production, as described by Crawshaw (2001) and Westendorf and Wohlt (2002), called malting or germinating, involves soaking barley for 2 to 3 days in water (13-18°C). This process is carried out in drums to allow regular turning and to control the aeration of the malting barley (Crawshaw, 2001). During germination, starch and protein within the grain are solubilized, while the enzymes that are produced break down the β-glucans within the cell wall.
Once the seeds have sprouted, the grain is dried. The malting by-products are removed at this point and the second phase of the process, called mashing, begins. During mashing, the malted grains are crushed, re-hydrated and heated for approximately 1 h at 65°C. Heating promotes enzymatic action and the further conversion of starch to sugar (Crawshaw, 2001). Additional grains, including corn grits and rice, are added to the mash and are heated until the majority of the starch has been converted to sugar (Westendorf and Wohlt, 2002). By the end of the mashing phase, 35 to 40% of the initial proteins within the grains have been converted into polypeptides and free amino acids, which form part of the liquid portion, known as wort. However, the solubilization and removal of the carbohydrate fraction of these grains is much greater than that of protein, resulting in an overall net increase in the CP content of the brewers spent grains (Crawshaw, 2001). The mash is then pressed and centrifuged to separate the wort from the spent grains, which are transferred to a silo for use as an animal by-product feed.

The final stages of beer production involve only the liquid wort. Once the spent grains have been removed, hops are added to the wort. The mixture is boiled and then filtered to remove residual hops. Once cooled, the wort is inoculated with a specific culture or a mixed culture of yeasts, containing two or more strains of *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*, at a rate of 10-15 cells/ml. Cell multiplication begins after a 12 - 20 h lag phase, and continues for two to three days, at which point the rate of multiplication declines. Yeast is then separated from the fermenting wort either by floatation or sedimentation, depending on the strain of yeast and the type of beer being produced (Crawshaw, 2001). The yeast is removed by pressing and dried to remove excess beer and can then be marketed as brewers yeast.
another by-product feed used by the animal industry (Crawshaw, 2001; Westendorf and Wohlt, 2002).

**Historical Use of Brewers Grains**

The earliest use of brewers grains as an animal feed is not on record. The production of fermented drinks has been traced back five thousand years to the Sumerians and there is thought to be a connection between the production of these drinks and the feeding of their by-products (Crawshaw, 2001). Prior to the establishment of large scale brewing factories in the 1880’s, the majority of the beer consumed was produced on farms or in monasteries. These brewers grew most of the barley used in the brewing process and residues of the process were then used to feed their own livestock (Crawshaw, 2001). Following the change to factory breweries beer production increased, but has made very little overall change in the last hundred years, falling slightly during prohibition and then peaking in the 1970’s (Crawshaw, 2001). Despite the changes in production patterns, the demand for brewing by-products as animal feeds has remained constant.

**Animal Feeding Trials**

By-product feeds have been fed to dairy cattle and other livestock for many years. This practice will likely continue to increase in the future as it prevents waste disposal problems in the food and beverage industries and reduces the amount of expensive concentrate that must be included in the diet to ensure maximum animal performance. Rakes and Davenport (1975) determined the apparent ruminal digestibility coefficients for various components of wet brewers grain (34 % DM) in dairy cows. The digestion
coefficients were determined to be as follows: gross energy 69.3 %, CP 77.4 %, crude fiber 39.7 %, nitrogen–free-extract 74.8 %, ether extract 83.4 % and TDN 72.1 %.

Poultry

Due to their high fiber content, utilization of DBG in poultry diets is limited (Westendorf and Wohlt, 2002). However, a few experiments have been conducted to compare DBG to other more costly concentrates, such as soybean meal (SBM) and corn meal (CM). Dried brewers grains have been shown to improve growth and egg production in laying hens (Thornton, 1962) as well as improve interior egg quality (Eldred et al., 1975; Jensen et al., 1976; Damron et al; 1976; and Lopez et al., 1981). Increases in fertility and hatchability in chickens and turkeys were reported by Kienholz and Jones (1967) when DBG was included in breeder diets. Pfaff et al. (1988) observed increased hatchability when DBG was included at 30 and 40 % of the diet of pheasant-breeder hens. However, Ademosun (1973) reported lower rates of gain in growing chickens between 8 and 18 weeks of age and concluded that should not be fed DBG in excess of 30 % of diet DM in order to optimize efficiency.

Swine

Dried brewers grains should be limited in the diets of lactating or growing pigs because of its bulkiness (13 - 16 % crude fiber) and low energy content (Holden and Zimmerman, 1991). However, BG may make up a substantial portion of sow diets during gestation (Westendorf and Wohlt, 2002). The reproductive performance of pregnant sows was not affected when DBG was fed at 20 or 40 % of the diet. In this study, litter size and weight of individual piglets were not affected by treatment, nor was
litter weight at birth. Therefore, DBG may be an acceptable alternative to more costly protein sources in the diets of gestating sows (Wahlstrom and Libal, 1976).

Altizio et al, (2000) compared the use of WBG to a CM and SBM diet for market hogs. The diets varied in DM, but were balanced to be isonitrogenous at 16 % CP. Feed intake and conversion efficiency were similar among the three diets, as was carcass quality. Dry matter intake (DMI) and average daily gain (ADG) were not effected by the differences in fiber and moisture content.

**Horses**

Research investigating the use of WBG in horse diets is limited. However, Westendorf and Wohlt (2002) suggest that when used in pelleted or complete diets the high fiber and protein levels of DBG are ideal for horse diets. Ten to twenty percent of DMI for young, growing horses may be supplied by DBG, while DBG may be included in the diets of mature horses between 20 – 40 % DMI (Henry and Morrison, 1920; Cunha, 1991). When greater than 20 % of diet DM consists of BG, attention should be paid to the lysine requirement of the animal, as this amino acid (AA) may be limited in diets that are high in BG (Westendorf and Wohlt, 2002).

**Ruminants**

A diet consisting of DBG up to 30 % of diet DM may be fed to sheep and goats without adverse effects; however, higher levels may result in decreased growth rates and efficiency of feed utilization (Adebowale and Ademosun,1981). Similar results were reported by Ademosun (1973) and Adeyanju and Ilori (1976). In contrast, Preston et al. (1973) observed greater ADG in steers fed rations containing either 25 or 50 % DBG in place of corn. However, this improvement in performance may have been a result of the
decrease in parakeratosis and liver abscesses in the cattle fed DBG (Preston et al., 1973). Rate of gain and feed efficiency were improved in steers fed DBG compared to SBM or urea (Klopfenstein et al., 1977). Stanton et al. (1996) reported greater feed efficiency and higher carcass quality in steers fed 16% WBG, compared to 8% corn silage, 8% WBG, or 24% WBG.

Rogers et al. (1986) observed a decrease in protein solubility, reduced ruminal microbial population and less protein availability postruminally in steers fed DBG compared to WBG. Armentano et al. (1984) reported that feeding DBG increased the postruminal protein supply relative to SBM.

Crickenberger and Johnson (1982) studied the effects of WBG on the wintering and reproductive performance of beef heifers, as WBG is a good source of natural Se. However, no apparent beneficial or detrimental effects of WBG on the reproductive performance of heifers were observed. Intake of calves was reported to be less for WBG than DBG, while nitrogen (N) retention and utilization were reported to be greater in calves fed WBG than those fed DBG (Conrad and Porter, 1976).

Conrad and Porter (1976) compared the nutritive value of WBG and DBG in lactating dairy cows and found no differences in milk production between the two forms; however, cows fed WBG consumed less feed, indicating that WBG is utilized more efficiently for milk production. Conrad and Rogers (1977) observed greater milk yields (MY) per unit of DM when WBG were included in a ration, compared to DBG. Feeding WBG or DBG at 15% of dietary DM had no influence on feed intake by dairy cows (Dhiman et al., 2003). Davis et al. (1983) observed a decrease in DMI when the diet of lactating cows consisted of 30% or 40% WBG compared to controls, but 4% fat
corrected milk (FCM) was similar for all diets, data indicates that efficiency of milk production favored WBG diets over controls. Early lactation dairy cows fed isonitrogenous diets containing 21.5 % DBG or 23.5 % WBG had similar feed intakes, MY and milk composition, despite the differences in DM content of the diet (69.9 % and 47.3 %, respectively; Hoffman and Armentano, 1988). Feeding WBG at 0, 15 or 30 % of diet DM during hot, humid weather did not affect DMI or MY in Jerseys, but an increase in MY was observed when liquid brewers’ yeast was added to the 30 % WBG diet (West et al., 1994). No effects were observed for milk fat (MF) percentage in this experiment, but milk protein was lower for the WBG fed cows and this group had higher serum N concentrations. Belibasakis and Tsirgogianni (1996) observed an increase in MY (actual and 4% FCM) and MF % during hot weather in Holsteins when WBG was fed to replace portions of corn silage, SBM and wheat bran in the diet.

Lactating dairy cattle require diets that have a high energy density. To meet the high energy demands of lactation, the concentrate portion of the diet is often increased at the expense of the effective fiber. Various by-product feeds (such as BG) have been used to balance both the energy and fiber needs. However, the NDF from these by-products only provides half as much ruminally effective fiber as NDF from alfalfa silage (Swain and Armentano, 1994). These researchers replaced a portion of the forage in the diet of lactating cows with either DBG or WBG and observed no evidence of milk fat depression or change in feed intake. Dry matter intake was not affected by decreasing forage neutral detergent fiber (NDF) or total non-fibrous carbohydrate (NFC) with increasing levels of WBG, but when WBG replaced the concentrate portion of the diet, DMI decreased linearly (Firkins et al., 2002). Younker et al. (1998) observed a DMI depression when
BG replaced both forage and concentrate and reported that BG can be substituted for forage on a short-term basis, as BG may provide enough effective fiber to help counteract the acidogenic effects of starch, but long-term use of BG as a fiber source is questionable. The combined filling effects of both forage and BG may be responsible for the reduction in DMI when BG replaces concentrate in a ration. The replacement of forage NDF with NDF from WBG may have decreased the digestion rate, while simultaneously increasing the rate of passage, thus counteracting the effects of rumen fill and DMI (Firkins et al., 2002).

Several authors have compared the use of BG (wet and dried) to SBM as a protein supplement for lactating dairy cows. Johnson et al. (1987) compared different forms of WBG and concluded that all are equal as replacements for SBM in lactating cows and that greater income over feed costs can be realized when feeding WBG in place of SBM. Polan et al. (1985) evaluated various protein sources at 14.5 - 15.0 % of dietary CP and observed a milk response above the 11.7 % CP basal diet. With greater protein supplementation, milk production did not respond to SBM but did respond to WBG and DBG. Murdock et al. (1981) reported that WBG may be included in lactating cow rations up to 30 % and was utilized on an equivalent CP basis with SBM for milk production. Danielson et al. (1981) observed increased milk production in fat cows (but not thin cows) when half of the dietary SBM was replaced with DBG, suggesting that tissue mobilization was increased by this protein supply in fat cows. No differences in milk production, feed intake, and body weight change were observed during subsequent lactation when SBM and DBG diets were fed during gestation (Seymour and Polan, 1986). However, reduced intestinal digestibility of RUP was observed in DBG fed cows.
as a result of the high amount of bound N, and may explain the lack of production response in these cows. Armentano et al. (1984, 1986) reported that feeding DBG increased postruminal protein supply relative to SBM. Dried brewers grains provided more methionine and a similar quantity of lysine to the intestine compared to SBM and cows fed DBG produced more milk and milk protein than those fed SBM (Cozzi and Polan, 1994). Hoffman and Armentano (1988) fed isonitrogenous diets containing WBG, DBG or SBM during the first third of lactation and observed no differences in MY, 3.5% FCM, milk fat % or milk protein % among treatments, however, diets containing WBG or DBG tended to be lower in digestibility than the SBM diet.

**Storage and Preservation of Wet Brewers Grains**

Due to their high moisture content, the storage life of many wet by-product feeds (including WBG) is short (Nofsinger et al., 1983). As moisture drains away, the palatability of WBG decreases. Intake of WBG by lactating cows is often reduced due to spoilage (Dixon and Combellas, 1983) and intake of a total mixed ration (TMR) containing brewers grain will also decrease. Small dairies or feedlots often find it difficult to keep feeding ahead of spoilage, especially during the summer months (Westendorf and Wohlt, 2002). Unless stored under anaerobic conditions, these feeds rapidly deteriorate and promote mold and mycotoxin growth. Several authors have compared inoculants and different storage methods in order to improve the preservation of ensiled WBG (Allen and Stevenson, 1975; Allen et al., 1975; Lilly et al., 1980; Schneider et al., 1995). However, due to the seasonal production patterns of breweries, delivery of WBG can be sporadic, making long-term anaerobic storage challenging (Schneider et al., 1995).
Mycotoxins are chemicals produced by fungi that are toxic to humans and animals. Over 400 different mycotoxins have been identified; however, only small percentages appear to be involved with agricultural feedstuffs (Kuldau and Woloshuk, 2002). In domestic animals, mycotoxin contamination reduces growth efficiency, lowers feed conversion and reproductive rates, impairs resistance to infectious diseases, reduces vaccination efficacy and induces pathologic damage to the liver and other organs (Coulombe, 1993; Diekman and Green, 1992).

Approximately 25% of the world’s food supply is contaminated annually by mycotoxins (Akande et al., 2006), which could be the result of either pre- or post harvest contamination. The severity of mycotoxin contamination of agricultural commodities varies yearly (Coulombe, 1993). Major environmental factors that determine the level of contamination include excessive moisture in the field and storage, temperature extremes, humidity, drought, variations in harvesting practices, and insect infestation (Coulombe, 1993).

Deoxynivalenol (DON), also known as vomitoxin, is the most common mycotoxin found in silages (Charmley et al., 1993; Whitlow and Hagler, 1997). It is most often produced by Fusarium graminearum. Several other species of Fusarium are capable of producing mycotoxins, including F. sporotrichoides, F. poae, F. graminearum, F. verticillioides and F. proliferatum, which produce T-2 toxin, diacetoxyscirpenol, zearalenone, and fumonisins, respectively (Kuldau and Woloshuk, 2002). Deoxynivalenol does not always affect milk production, milk quality, feed intake or animal health when present in dairy cattle feeds (Kuldau and Woloshuk, 2002), but in some trials it has been shown to reduce performance when included in the ration for early
lactation cows (Ingalls, 1996) and mid-lactation cows (Charmley et al., 1993). Reduced performance was also observed when DON contaminated diets were fed to swine (Trenholm et al., 1984).

Management methods to reduce the spoilage of WBG during long-term and short-term storage have been studied extensively. In addition to promoting mold and mycotoxin growth, improper handling of this material results in high DM losses and a characteristic unpleasant odor, lowering the nutritional value and reducing palatability of this feedstuff.

Wet feedstuffs are often ensiled as a means of preservation. Ensiling and the resulting fermentation require the material to be compacted and sealed quickly to minimize losses associated with aerobic deterioration from undesirable bacteria and molds (Harrison, 1996); and rapid lactic acid production must occur to lower the pH, thus inhibiting clostridial growth (Allen and Stevenson, 1975). Ensiled feedstuffs should also be fed rapidly to prevent further deterioration.

Allen and Stevenson (1975) proposed that bacterial inoculants may improve ensilability of WBG, as they observed a rapid increase in the Lactobacilli population during the first 2 days of ensiling WBG, followed by a decline over the remaining 16 days of the experiment. Dixon and Combellas (1983) observed no benefit of NaCl or NaOH as preservatives for WBG under aerobic conditions. Allen et al. (1975) found that including either formic acid (0.20 and 0.40 %) or propionic acid (0.40 %) was effective in reducing subsurface deterioration, but had no effect on surface deterioration. However, a 0.40 % mixture of the two acids maintained the quality of WBG during the 14-day study. Schneider et al. (1995) found the addition of lactic acid bacteria was beneficial for
fermentation, as they aided in lowering the pH, increased the initial concentration of lactate and consequently, decreased the initial concentrations of acetate and butyrate. In addition, Schneider et al. (1995) found that including a high moisture grain inoculant and beet pulp pellets to WBG lowered pH, acetate, and NH$_3$N concentrations and increased the content of lactic acid under long-term storage. However, including a high moisture grain inoculant for short-term storage was not beneficial.

**Conclusion**

Brewers grains, a by-product of beer production, are often fed to livestock because they provide adequate amounts of protein, fiber and energy at a lower cost than other feedstuffs. The use of BG in livestock rations, like other by-product feeds from the human food industry, limits the disposal of these products into landfills. Brewers grains can be fed to livestock wet, as is the case in many dairy and some feedlot rations, or may be dried and included in a complete pelleted feed. The high moisture content of WBG makes it susceptible to rapid spoilage and deterioration of the nutrient content. However, ensiling WBG may not be worth the added cost or burden. Therefore, products that will decrease the deterioration of aerobically stored WBG are necessary.
CHAPTER II

MICROBIAL AND NUTRITIONAL CHANGES OF WET BREWERS GRAINS
INOCULATED WITH A COMMERCIAL PRESERVATIVE

Introduction

The concentrate portion of a diet is quite costly for most dairy producers; however, a number of by-products from food processing industries are available for use as animal feeds at a lower cost. By-product feeds are secondary products obtained during harvest or processing of a principal commodity that have little value as a human product, but have a high value as an animal feed. These feeds are often included in dairy cattle rations because of their high protein or energy content, stimulating effect on feed intake, and low cost (De Brabander et al., 1999). The use of by-product feeds lessens the need of feeding animals grains that could otherwise be used for human consumption and reduces the need for costly waste management programs (Grasser et al., 1995).

The choice of which by-product feed or feeds to incorporate into the ration may vary depending on cost, season or geographical location of the dairy. Brewers grains are a by-product used extensively as a protein supplement, in areas near breweries. Brewers grains consist of the material that remains after grains (primarily barley) have been fermented during the beer making process. Brewers grains can be fed to dairy cattle wet (20-30% DM), or can be heat treated and fed in the dried form (80-90% DM). The CP content of BG have been reported to range from 23.6 to 29.2% of DM (Westendorf and Wohlt, 2002; NRC, 2001; DePeters et al., 2000), with over 50% of the protein escaping
ruminal fermentation (Clark et al., 1987). Data suggest that WBG are equal to or have a higher nutritive value than DBG (Porter and Conrad, 1975; Conrad and Rogers, 1977; Murdock et al., 1981), and may be higher in TDN (DePeters et al., 2000), resulting in improved efficiency of milk production. Rogers et al. (1986) suggested that the heat-treatment of the drying process might be responsible for the nutritional differences between WBG and DBG.

Due to the high moisture content, WBG are susceptible to mold growth and spoilage. Ensiling WBG is a method used for long-term storage, however, due to the periodic delivery patterns in some areas, long-term storage is often not practical (Schneider et al., 1995). A number of researchers have compared inoculants and different storage methods in order to improve the preservation of ensiled wet brewers grains (Schneider et al., 1995; Lilly et al., 1980; Allen and Stevenson, 1975; Allen et al., 1975). However, limited data are available on the preservation of WBG stored in uncovered piles.

The objectives of the project were to compare the deterioration of WBG over time, when a commercial preservative (Silo-King GPX) was added to either covered or uncovered piles of wet brewers grain, versus no preservative; and to compare the DM and CP ruminal degradability of samples taken throughout a 28 day period, using the in situ procedures described in the NRC (2001).


Materials and Methods

Experiment 1

Treatments

On day zero of the study, approximately 3,000 kg of wet brewers grain were delivered to the Fairchild Dairy Teaching and Research Center. Seventy kg of fresh WBG were removed from the pile and frozen for use during the in-situ degradability trial (experiment 2). The remainder of the WBG was then divided into three separate piles weighing 960 kg, 977 kg and 986 kg, respectively, using a Knight feed mixer (Reel Auggie, model 3250, Broadhead, WI). A commercial preservative (Silo-King GPX, Agri-King, Fulton, IL) was added to the WBG at a rate of 0 (control), 0.45kg/900 kg (treatment 1) and 0.9kg/900 kg (treatment 2) prior to mixing. The WBG was allowed to mix for 5 min, and was then unloaded into one of three four-sided wooden bunks measuring 0.6 m high x 1.83 m long x 1.22 m wide. Each pile was left uncovered for 28 days to simulate on-farm storage practices.

The Silo-King GPX preservative is a dry, granular, free-flowing product containing the lactic acid producing bacteria *Lactobacillus plantarum*, *Enterococcus faecium* and *Pediococcus pentosaceus* and fermentation extracts from *Aspergillus oryzae*, *Trichoderma longibrachiatum* and *Bacillus subtilis*. This product also contains the preservative and anti-oxidant butylated hydroxytoluene (BHT), along with anti-fungal agents such as potassium sorbate, sodium benzoate, propionic acid, acetic acid, benzoic acid and sorbic acid. Monosodium phosphate is included as a nutrient and acidulant, while sodium silico aluminate acts as a moisture scavenger.
Sampling and Measurements

On days 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28, approximately 300 g samples were taken from each treatment bunk. All samples were taken 20.32 cm below the surface so as not to include the top layer of spoilage. Sample locations were chosen at random each sample day, but were similar for all samples taken on that day. Samples were refrigerated at 4°C and sent twice weekly (Mondays and Thursdays) to Agri-King (Fulton, IL) for wet chemistry and microbial analysis. Samples were analyzed for DM, CP, NDF, ADF, neutral detergent insoluble protein (NDIP), fat, ash, starch, NH3-N, pH, minerals and the presence of molds, yeast and mycotoxins according to the AOAC methods (1999). In addition, the concentrations of the volatile fatty acids (Cancalon, 1993) and clostridia (AOAC, 1998) were analyzed. A 1500 g sub-sample was taken from each of the three bunks on days 0, 7, 14, 21 and 28 for use in an in situ degradation trial. The core was then collapsed to prevent spoilage in the center. At the conclusion of the 28-day period, the material remaining in each of the treatment piles was weighed using 19 L plastic buckets to determine total DM loss throughout the study. Sample weights were recorded throughout the study and included in the calculation.

Statistical Analysis

Nutrient analysis data were analyzed using the MIXED procedure of SAS [Version 9.1 (2002); (SAS Institute, Inc., Cary, NC)] according to the following model:

\[ Y_{ijklm} = \mu + T_i + B_j + H_k + e_{ijk} \]

Where:

- \( Y_{ijklm} \) is the dependent, continuous variable,
- \( \mu \) is the overall mean,
$T_i$ is the fixed effect of the $i$th treatment ($i = 0, 1, 2$)

$B_j$ is the random effect of the $j$th box ($j = 1, 2, 3$)

$H_k$ is the fixed effect of the $k$th hour of storage, and

$e_{ijk}$ is the residual error.

Fixed effects included treatment and hour, while the random effect was box.

Residual errors were modeled using a first-order autoregressive covariance structure.

Bayesian information criterion was tested using the following three covariance structures: autoregressive (1), compound symmetry and unstructured (Littell et al., 1996).

Autoregressive (1) resulted in the bestfit Bayesian information criterion, and was therefore chosen for this model. Mold, yeast and clostridia concentrations were logarithmically transformed for inclusion in the model. pH was analyzed as the H ion concentration of each sample and was then converted back to pH. Linear and quadratic contrasts were fitted within each level of treatment. Results were expressed as least squares means with the lowest standard error. The DIFF option of SAS was used to test treatment differences among least squares means. Degrees of freedom were calculated using the Kenward-Roger option of the MIXED procedure. Significance was declared at $P \leq 0.05$ and a trend in the data was declared at $P \leq 0.1$.

The Univariate Procedure of SAS was used to determine outlier samples. Observations greater than 2.5 standard deviations from the mean for each item analyzed were considered outliers. The results of the outlier analysis indicated that samples taken on day 12 of the study were outliers and were removed from the final statistical analysis.
Experiment 2

Cows and Treatments

Ruminal degradation of DM and CP of the WBG were determined using the in situ technique described in the NRC (2001). Three multiparous non-lactating Holstein cows fitted with rumen cannulas were assigned to a 3 x 3 Latin square with cow and period as blocking factors. Cows were fed the standard herd diet and were supplemented with fresh WBG at a rate of 1 kg per day. A 14-d diet adjustment period was used before beginning the trial, during which time the cows were supplemented with fresh WBG.

Wet brewers grain samples taken from each of the treatment piles on days 0, 7, 14, 21 and 28 (experiment 1) were dried at 55°C using a forced air oven (VWR Scientific, West Chester, PA). All dried samples were ground through a Wiley Mill (Arthur H. Thomas, Philadelphia, PA) equipped with a 2-mm screen. Dacron bags measuring 10 x 20 cm and having a 52-μm pore size were used. Bags were labeled with an identification number using a black permanent marker. Bags were then placed in a forced air oven set at 55°C (VWR Scientific, West Chester, PA) for 48 h. Bags were removed from the oven and placed in desiccators to cool. The weight of each bag was recorded and then 5.3 g of sample were weighed and placed in each bag. Bags were heat sealed and tied with plastic fastening ties 2 cm below the top. Bags were soaked in 39°C distilled water for 15 min and were then placed in a mesh laundry bag, which was fastened to the end of a 100 cm piece of fishing line. Bags were incubated in the rumen for 0, 2, 4, 8, 12, 16, 24, and 48 h. Two bags representing each sample point were incubated. Bags were inserted in reverse order (ten bags per time point) and were

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retrieved beginning at 0 h, rinsed in cold tap water for 30-40 s per bag and washed in a washing machine (cold tap water for three cycles, followed by a final spin). Bags were dried in a forced air oven at 55°C for 48 h, weighed and sent to Agri-King (Fulton, IL) for DM and CP analysis of the residue (AOAC, 1991).

**Statistical Analysis**

Data from the in situ trial were analyzed as a 3 x 3 Latin Square of treatments. Effective ruminal degradability (ERD) and fractional degradation rates of DM and CP in the rumen were calculated using a nonlinear model. The Marquart method of SAS (2002) was used to fit a non-lag model for the degradation curves of DM and CP. The non-lag model equation is as follows:

\[ P = A + B \left( 1 - e^{-c t} \right) \]

Where:

- \( P \) is the disappearance of the feedstuff,
- \( A \) is the soluble fraction,
- \( B \) is the potentially degradable fraction,
- \( c \) is the fractional degradation rate (\%/h) and
- \( t \) is the incubation time (hours).

Effective ruminal degradability (ERD) of CP and DM were calculated (nonlag model) using the following equation:

\[ ERD = A + B \left[ \frac{c}{c + k} \right] \]

Where:

- \( ERD \) is the effective ruminal degradability of the feedstuff,
- \( A \) is the soluble fraction,
B is the potentially degradable fraction,

c is the fractional degradation rate (%/h) and,

k is the fractional passage rate (assumed to be 6%/h).

The Univariate Procedure of SAS was used to determine outliers. Observations greater than 2.5 standard deviations from the mean for each item analyzed were considered outliers and were removed from the final statistical model. The outlier analysis determined ten of the bags to be outliers, thus these bags were removed from the final statistical model.

Experiment 3

Treatments

On day zero of the study, approximately 5,500 kg of WBG were delivered to the Fairchild Dairy Teaching and Research Center. The WBG were then divided into six separate piles weighing 932 kg, 973 kg, 1005 kg, 1000 kg, 1000 kg and 1019 kg, respectively, using a Knight feed mixer (Reel Auggie, model 3250, Broadhead, WI). The same preservative and use rate as described in experiment 1 was added to the WBG, prior to mixing. The WBG were mixed for 5 min, and then unloaded into one of six four-sided wooden bunks measuring 0.6 m high x 1.83 m long x 1.22 m wide, similar to experiment 1. One pile per treatment was left uncovered for 28 days, to simulate on-farm storage practices; while the other box for that treatment was covered with a plastic tarp.

Sampling and Measurements

On days 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28, two samples weighing approximately 300 g were taken and analyzed from each treatment bunk, using the same procedures as described in experiment 1. The core was then collapsed to
prevent spoilage in the center. At the conclusion of the 28-d period, the remaining WBG in each of the treatment piles was weighed using five gallon plastic buckets to determine total dry matter loss throughout the study. Sample weights were recorded throughout the study and included in the calculation.

Statistical Analysis

Nutrient analysis data were analyzed using the MIXED procedure of SAS [Version 9.1 (2002); (SAS Institute, Inc., Cary, NC)] according to the following model:

\[ Y_{ijklm} = \mu + T_i + B_j + H_k + C_l + TH_{ik} + TC_{il} + e_{ijkl} \]

Where,

- \( Y_{ijklm} \) is the dependent, continuous variable,
- \( \mu \) is the overall mean,
- \( T_i \) is the fixed effect of the \( i \)th treatment \((i = 0, 1, 2)\),
- \( B_j \) is the random effect of the \( j \)th box \((j = 1, 2, 3)\),
- \( H_k \) is the fixed effect of the \( k \)th hour of storage,
- \( C_l \) is the fixed effect of the \( l \)th cover,
- \( TH_{ik} \) is the interaction between treatment \( i \) and the effect of hour \( k \),
- \( TC_{il} \) is the interaction between cover \( l \) and the effect of hour \( k \),
- and
- \( e_{ijkl} \) is the residual error.

Fixed effects included treatment, hour, and cover, while the random effect was box. Residual errors were also modeled using a first-order autoregressive covariance structure in this experiment. Bayesian information criterion was tested and again, autoregressive (1) resulted in the best fit Bayesian information criterion, and was therefore
also chosen for this model. Mold, yeast and clostridia concentrations were logarithmically transformed, as in experiment 1. Linear and quadratic contrasts were fitted within each level of treatment and each type of cover. Results were expressed as least squares means with the lowest standard error. The DIFF option of SAS was used to test treatment differences among least squares means. Degrees of freedom were calculated using the Kenward-Roger option of the MIXED procedure, as was done for experiment 1. Significance was declared at P ≤ 0.05 and a trend in the data was declared at P ≤ 0.1.

The Univariate Procedure of SAS was used to determine outlier samples. Observations greater than 2.5 standard deviations from the mean for each item analyzed were considered outliers. However, no samples were determined to be outliers for this experiment, thus no data were removed from the final model.

Results

Experiment 1

The chemical composition of the three treatment piles of WBG over the 28-d period for experiment 1 and the nutrient analysis of a pretreatment composite sample taken upon arrival prior to application of treatments is presented in Table 1.

There were no differences in DM, CP, NDF, ADF, NDIP, ash or Na among the 3 treatments. There tended to be higher P levels in the control treatment samples (P < 0.1). There was a quadratic increase between treatments for fat (P < 0.05), and quadratic decreases in the concentrations of starch (P < 0.05), Ca (P < 0.01), Mg (P < 0.01) and K (P < 0.05). The concentration of fat was the highest in the 0.9 kg/900 kg GPX treatment, followed by the 0.45 kg/900 kg treatment and then the 0 GPX treatment. The starch
content was highest in the control pile, followed by the 0.45 kg/900 kg GPX pile and was the lowest in the 0.9 kg/900 kg GPX pile. The same pattern was observed for the Ca and Mg concentrations. The 0.9 kg/900 kg treatment had the lowest K concentration, but similar concentrations for both the 0 and 0.45 kg/900 kg treatments.

The pH, VFA, lactic acid and NH$_3$N concentrations are presented in Table 2. There was a linear increase (P < 0.05) in pH and a linear decrease in the lactic acid concentration among treatments. The control treatment of WBG had the highest pH, followed by the 0.45 kg/900 kg treatment and then the 0.9 kg/900 kg treatment. A quadratic increase was observed for both acetic (P < 0.01) and butyric (P < 0.05) acids. The highest concentration of both acids was with the 0.9 kg/900 kg treatment and the lowest concentration was in the control pile. There were no differences among treatments for NH$_3$N concentration during the study.

There were both linear (P < 0.0001) and quadratic (P <0.0001) decreases in the logarithmically transformed number of CFUs of yeast, mold and clostridia (Table 3). The Log CFU concentration was higher in the control pile for all of these organisms and declined as the inclusion rate of the Silo-King GPX increased. Zearalenone concentration did not differ among treatments (Table 3.)

The trace mineral content of WBG is presented in Appendix B (Table 9). There were no differences in the Fe or Cu concentrations of the WBG across treatments. However, there were quadratic decreases in the levels of Mn (P < 0.05) and Zn (P < 0.01). The control piles of WBG had the highest levels of Mn and Zn and the concentration of these two minerals decreased as the amount of GPX treatment increased.
There were no differences in the weights of the WBG remaining in the boxes at the conclusion of the 28-d experimental period (data not shown). The three treatment piles weighed 593 kg, 609 kg and 626 kg, respectively.

**Experiment 2**

The ingredient composition of the diet fed during the in situ trial is presented in Table 4. The average particle separations for the TMR using the Penn State Shaker were 23.5 % on the top screen, 29.6 % on the middle screen and 46.9 % on the bottom screen. The orts averaged 35.8 % on the top screen, 30.0 % on the middle screen and 34.2 % on the bottom screen.

The results of the in situ DM and CP degradability of WBG samples taken from experiment 1 are presented in Table 5. There were no differences among treatments in the DM content of either the soluble A fraction or the insoluble potentially degradable B fraction of WBG, the rate of degradability (c) or the overall effective ruminal degradability (ERD). No differences were seen among treatments for A, B, c or ERD of the CP degradability of WBG.

**Experiment 3**

The results of the chemical composition of the experimental piles of WBG for experiment 3 are presented in Table 6. There were both linear and quadratic increases among treatments in the DM concentration of WBG (P < 0.001 and <0.01), concentration of starch (P < 0.001 and <0.001) and the concentration of K (P < 0.01 and <0.001). Dry matter values were lowest in the 0 GPX treatment piles and were highest in the 0.9
kg/900 kg piles of WBG. The presence of a cover did not effect the DM of the samples. Starch values were also lowest in the control piles of WBG and there was no effect of cover on the starch levels of the samples. Potassium concentration increased with increasing levels of GPX and were higher in covered piles compared with uncovered piles (P < 0.05). A linear decrease was observed for the CP concentration (P < 0.05), while there was a linear increase in the concentration of Ca (P < 0.05). Cover did not effect either CP or Ca concentrations. There were no differences among treatments for NDF; however, samples from the covered piles had higher values (P < 0.001) than samples from the uncovered piles. Samples taken from covered piles had higher ADF (P < 0.001) and 0.9 kg/900 kg GPX treatments tended (P < 0.1) to be higher in ADF. The concentration of NDIP in WBG increased quadratically with treatment (P < 0.01) with the control piles having lower NDIP than GPX treated piles, but samples from covered piles had higher (P < 0.001) NDIP concentrations than samples from uncovered piles. Similarly, there were quadratic increases between control and 0.9 kg/900 kg GPX treatments for fat (P < 0.05) and Na (P < 0.001), but cover did not effect either fat or Na concentrations. No effect of cover or treatment was observed for ash, P or Mg in experiment 3.

There was no effect of GPX treatment on the pH of WBG samples taken during experiment 3, but uncovered samples had higher pH than covered samples (P < 0.05) (Table 7). There was a positive linear response to treatment (P < 0.001) for lactic acid, but no effect of cover. Cover did not have an effect on acetic acid, but there were quadratic increases (P < 0.001) among treatments with the 0.9 kg/900 kg treated piles having the highest acetic acid concentrations and the control piles having the lowest.
Uncovered piles had higher concentrations of butyric acid (P < 0.05) and both linear (P < 0.001) and quadratic (P < 0.01) decreases in butyric acid concentrations were observed as the amount of GPX treatment increased. Ammonia-N concentrations did not differ in experiment 3, similar to experiment 1.

The log CFU yeast, mold and clostridia concentrations and zearalenone and deoxynivalenol concentrations of WBG samples taken during experiment 3 are presented in Table 8. There was a linear decrease (P < 0.05) among treatments for yeast (Log CFU) and there tended (P < 0.1) to be a quadratic decrease among treatments as the inclusion rate of Silo-King GPX increased. There was, however, no effect of cover on yeast in experiment 3. Mold (Log CFU) decreased both linearly (P < 0.05) and quadratically (P < 0.05) among treatments with the control samples having the highest Log CFU for mold and covered piles tending (P < 0.1) to have higher concentrations of mold than uncovered piles. Neither treatment nor cover had an effect on clostridia numbers, deoxynivalenol or zearalenone concentrations in experiment 3 (Table 8).

The trace mineral content of WBG samples taken during experiment 2 are presented in Appendix C (Table 10). There was no effect of treatment on the Fe, Cu or Mn concentration of the samples, but there tended (P < 0.1) to be a linear increase in the Zn levels of the samples. Covered samples had higher (P < 0.001) concentrations of Fe and tended (P < 0.1) to have higher concentrations of Zn than uncovered samples. However, concentrations of Cu were higher (P < 0.01) in uncovered samples. There were no differences observed in the Mn concentrations of either covered or uncovered samples.
There was a significant treatment x hour interaction for mean starch ($P < 0.05$) values throughout the 28-d period (Figure 1). Control samples had lower starch concentrations than both 0.45 kg/900 kg GPX and 0.9 kg/900 kg GPX treatments beginning 48 h after treatment and continuing throughout the remainder of the study. There were no differences between the two levels of GPX at the individual time points.

There was also a significant treatment x hour interaction for the mean butyric acid values ($P < 0.05$) (Figure 2). Samples taken from the 0 GPX treatment piles had higher ($P < 0.05$) or tended to have higher ($P < 0.1$) concentrations of butyric acid than both the 0.45 kg/900 kg or 0.9 kg/900 kg GPX treatments at 18, 20, 22, 24, 26 and 28 days after treatment. However, concentrations of butyric acid in the 0.45 kg/900 kg and 0.9 kg/900 kg treatments did not differ on these days.

There were no effects of treatment or cover on the weights of the WBG remaining in the boxes at the conclusion of the study, similar to experiment 1 (data not shown). Weights remaining uncovered boxes were 606 kg (control), 598 kg (treatment 1) and 612 kg (treatment 2), while the WBG remaining in the covered boxes weighed 620 kg, 617 kg and 609 kg, respectively.

Discussion

**Experiment 1**

Dry matter, CP, NDF, ADF, NDIP, ash, NH$_3$N and Na levels were not affected by treatment with Silo King GPX and are similar to values reported by Westendorf and Wohlt (2002) of samples of fresh WBG taken from four different breweries.
The concentration of fat was significantly lower in the control pile compared with the treatment piles, while the presence of molds, yeasts and clostridia were higher in the control samples, suggesting that these organisms used fat as an energy source throughout the experiment. Molds are obligate aerobic organisms and proliferate when in an environment with a stable temperature, free water, N and energy sources (Nelson, 1993). The same is true for yeasts and clostridia. The required nutrients were available in all three treatment piles, but the higher concentrations of these organisms within the control pile suggests that preservation agents within the GPX product were successful in inhibiting their growth. There were no differences among treatments in the concentrations of zearalenone throughout the experimental period and concentrations did not differ from the pretreatment sample.

Successful fermentation is characterized by the rapid reduction in pH (often to pH values below 4) as lactic acid bacteria convert soluble sugars to lactic acid and a smaller amount of acetic acid (Lowes et al., 2000). The pH values were highest in the control pile of WBG and decreased with increasing amounts of the GPX preservative, probably due to the presence of lactic acid bacteria in the product. The lower pH measurements in the 0.45 kg/900 kg and 0.9 kg/900 kg piles is consistent with the lower clostridia counts observed in these treatments because the growth of Clostridium spp. is inhibited by low pH (Lowes et al., 2000; Thomas, 1978).

Certain amylolytic lactic acid bacteria (ALAB), including Lactobacillus plantarum the major LAB present in Silo-King GPX, are capable of converting raw starch into lactic acid, which is essential for proper fermentation (Altam et al., 2006; Nguyen et al., 2007; Guyot et al., 2000). Starch concentrations in this experiment were
highest in control samples and decreased with increasing amounts of treatment. These results indicate that a portion of the starch content of WBG was used as a readily available carbohydrate source for LAB growth within the GPX treated WBG and agrees with the greater lactic acid concentrations of the GPX treated WBG compared with control WBG.

Both acetic and butyric acid concentrations were highest for the 0.9 kg/900 kg treatment. The high concentration of butyric acid in this treatment may be explained by the high fat and low starch concentrations of the 0.9 kg/900 kg treatment as butyric acid is often found in plant oils and requires starch for fermentation. The higher concentration of acetic acid for this treatment may be due to the high concentration of the LAB *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *Enterococcus faecium* within the GPX. These three organisms are facultative heterofermenters and produce mainly lactic acid from hexoses, but are also able to degrade some pentoses to lactic acid and acetic acid and/or ethanol (Oude Elferink et al. 1999).

There were higher concentrations of Ca, Mg, K, Mn and Zn in the control pile of WBG and P tended to be higher for this treatment also. However, there was no difference in concentrations of Na, Fe or Cu. The higher amounts of some minerals in the untreated pile may be explained by the loss of organic matter due to spoilage. McLaughlin et al. (2002) observed higher mineral concentrations in the top layer of untreated corn silage compared with the top layer of NaCl treated corn silage and theorized that the greater mineral content of the control treatment was the result of less organic matter in the untreated corn silage due to the increased spoilage observed.
Experiment 2

In situ DM degradability values for WBG were not different among treatments. However, length of storage decreased the DM degradability for fractions A and B, the rate of degradation and ERD, which may be due to the loss of organic matter and availability of nutrients seen as spoilage increases. These results are in agreement with Mills and Kung (2002) who reported higher deterioration and lower DM degradability values for untreated barley silage samples compared with propionic acid-based additive treated samples. The CP degradability values were not different among treatments and decreased as storage length increased, similar to DM values. Total ruminal degradability for the control, 0.45 and 0.9 kg/900 kg GPX treatments, were 97.2, 99.5 and 99.2 % of CP and the rates of degradation were 6.2, 5.9 and 6.0 %/h, respectively. The NRC (2001) summarized the results of 4 studies and reported total ruminal degradability to be 90.8 % of CP for WBG and 82.9 % of CP for DBG with degradation rates of 4.6 and 4.7 %/h, respectively. Armentano et al. (1986) observed ruminal digestion estimates of 70, 90 and 100 % of CP for DBG, WBG and SBM at rates of 4.2, 6.6 and 17.7 %/h.

Experiment 3

The mean DM values were higher in the 0.9 kg/900 kg treatment pile during experiment 3, but the presence or lack of a covering over the pile did not affect the DM percentage during this experiment. The DM values for these samples were higher than those reported in experiment 1, but are within the ranges reported by Westendorf and Wohlt (2002). Crude protein values were higher in untreated samples compared with the GPX treated samples, however, cover did not affect the CP levels. The higher amount of
CP in the control may have been a result of the higher presence of microorganisms in this treatment, particularly yeasts and molds. Neutral Detergent Fiber concentrations did not differ among treatments, as in to experiment 1; however, covered treatments were higher than uncovered. Covered treatments were also higher in ADF and NDIP. Silo-King GPX treated samples were higher in NDIP and tended to be higher in ADF.

Treatment of WBG with Silo-King GPX resulted in higher fat concentrations in experiment 3, similar to experiment 1, and cover did not effect fat concentration. Starch values were higher in samples taken from piles treated with the preservative in experiment 3, unlike what was observed in experiment 1. Control piles also had higher butyric acid concentrations in this experiment, suggesting that some of the starch in these piles may have been used for butyric acid fermentation. There were fewer microorganisms present in the treated piles of WBG in this experiment, as was observed in experiment 1. These results further suggest that the organisms may have been using starch as a fermentable carbohydrate source, thus lower starch concentrations were seen in the control samples.

Concentrations of P, Mg, Fe and Cu were not different among treatments, but Ca, K, Na and Zn concentrations were slightly higher in the treated piles. However, numerically mineral concentrations were similar across treatments and were comparable to the pretreatment values.

The pH did not differ among treatments in experiment 3, but covered samples had lower pH measurements that uncovered. These results indicate that the cover may have promoted anaerobic conditions, enabling the start of lactic acid fermentation.
Lactic acid concentrations were higher in the treated WBG piles in both experiments, possibly due to high amounts of lactic acid producing bacteria present in the Silo-King GPX. The highest acetic acid concentration was observed in the 0.9 kg/900 kg treatment, similar to experiment 1. Clostridia counts were not different among treatments in this experiment, therefore this result can be explained by either the presence of acetate producing bacteria within the material, the acetic acid supplied by the product, or perhaps a combination of these two factors. Butyric acid concentrations were significantly higher in uncovered, untreated WBG, indicating that the most spoilage occurred in this treatment. Visual appraisal of the piles would agree with this. Ammonia-N was similar among treatments in this experiment, as observed in experiment 1.

Yeast and mold counts were higher in control piles in this experiment as well as in experiment 1. Cover did not affect the log CFU of yeast. However, covering the pile tended to increase mold growth. Though there were no differences in DM levels of covered and uncovered samples, covered piles were slower to dry out following precipitation events, which may have promoted mold growth.

Conclusion

Wet brewers grains are often chosen to replace more expensive concentrate sources for livestock diets. However, their high moisture content (generally 22.0 – 28.0 % DM) make them susceptible to spoilage during hot, humid weather, limiting their use on small dairies. Ensiling WBG may be a viable option for dairies wanting to store WBG for an extended period; however, this method requires an anaerobic silo or a large enough area to store plastic ag-bags. Therefore, commercially available products to assist in the
preservation of WBG for a few weeks would be beneficial. The results of the three present experiments indicate that the addition of Silo-King GPX to WBG at the time of delivery may maintain the nutritive value of WBG for up to 14 days of storage, by reducing the spoilage and that covering WBG with a plastic tarp is not beneficial and in some cases may promote the growth of mold. In addition, these data suggest that treatment with 0.45 kg/900 kg of may be optimal.
Table 2. Nutrient content of experiment 1 wet brewers grains before and after treatment with 0, 0.45 or 0.9 kg/900 kg Silo-King GPX. All values expressed on a DM basis.

<table>
<thead>
<tr>
<th>Item</th>
<th>Day 0</th>
<th>0 GPX</th>
<th>0.45 kg GPX</th>
<th>0.9 kg GPX</th>
<th>SE</th>
<th>L</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>23.36</td>
<td>25.02</td>
<td>25.21</td>
<td>25.22</td>
<td>0.34</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CP, %</td>
<td>32.57</td>
<td>35.53</td>
<td>34.68</td>
<td>34.91</td>
<td>0.52</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>NDF, %</td>
<td>45.47</td>
<td>42.59</td>
<td>41.41</td>
<td>41.51</td>
<td>0.60</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ADF, %</td>
<td>21.20</td>
<td>21.68</td>
<td>20.96</td>
<td>21.14</td>
<td>0.45</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>NDIP, %</td>
<td>2.79</td>
<td>2.56</td>
<td>2.52</td>
<td>2.53</td>
<td>0.03</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Fat, %</td>
<td>7.98</td>
<td>7.93</td>
<td>8.23</td>
<td>8.41</td>
<td>0.13</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ash, %</td>
<td>4.03</td>
<td>5.92</td>
<td>5.22</td>
<td>5.66</td>
<td>0.30</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Starch, %</td>
<td>6.12</td>
<td>3.47</td>
<td>3.08</td>
<td>2.79</td>
<td>0.19</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.25</td>
<td>0.21</td>
<td>0.20</td>
<td>0.18</td>
<td>0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>P, %</td>
<td>0.58</td>
<td>0.64</td>
<td>0.62</td>
<td>0.61</td>
<td>0.01</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Mg, %</td>
<td>0.20</td>
<td>0.16</td>
<td>0.15</td>
<td>0.13</td>
<td>0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>K, %</td>
<td>0.08</td>
<td>0.07</td>
<td>0.07</td>
<td>0.06</td>
<td>0.003</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Na, %</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.002</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Material was treated with either 0, 0.45 or 0.9 kg/900 kg GPX (preservative), Agri-King, Fulton, IL. 0 GPX = control, 0 g/kg Silo-King GPX treatment, 0.45 kg GPX = 0.45 kg/900 kg Silo-King GPX treatment, 0.9 kg GPX = 0.9 kg/900 kg Silo-King GPX treatment.

2 L = Linear, Q = Quadratic.

3 Day 0 values were obtained from samples taken the day when wet brewers grains arrived prior to treatment.
Table 3. pH, average lactic, acetic and butyric acids and NH$_3$N concentration of experiment 1 wet brewers grains before and after treatment with 0, 0.45 or 0.9 kg/900 kg Silo-King GPX. All values expressed on a DM basis.

<table>
<thead>
<tr>
<th>Item</th>
<th>Day 0 $^3$</th>
<th>0 GPX</th>
<th>0.45 kg GPX</th>
<th>0.9 kg GPX</th>
<th>SE</th>
<th>Contrast $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.10</td>
<td>4.40</td>
<td>3.73</td>
<td>3.71</td>
<td>0.19</td>
<td>$&lt;0.05$ NS</td>
</tr>
<tr>
<td>Lactic, %</td>
<td>2.22</td>
<td>3.39</td>
<td>5.02</td>
<td>4.02</td>
<td>0.55</td>
<td>$&lt;0.05$ NS</td>
</tr>
<tr>
<td>Acetic, %</td>
<td>0.17</td>
<td>1.42</td>
<td>1.68</td>
<td>2.61</td>
<td>0.25</td>
<td>NS</td>
</tr>
<tr>
<td>Butyric, %</td>
<td>0.00</td>
<td>0.20</td>
<td>0.23</td>
<td>0.44</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>NH$_3$N, mg/kg</td>
<td>116</td>
<td>3643</td>
<td>2561</td>
<td>2046</td>
<td>723</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^1$ Material was treated with either 0, 0.45 or 0.9 kg/900 kg GPX (preservative), Agri-King, Fulton, IL. 0 GPX = control, 0 g/kg Silo-King GPX treatment, 0.45 kg GPX = 0.45 kg/900 kg Silo-King GPX treatment, 0.9 kg GPX = 0.9 kg/900 kg Silo-King GPX treatment.

$^2$ L = Linear, Q = Quadratic.

$^3$ Day 0 values were obtained from samples taken the day when wet brewers grains arrived prior to treatment.

Table 4. Logarithmically transformed yeast, mold and clostridia concentrations and zearalenone concentration of experiment 1 wet brewers grains before and after treatment with 0, 0.45 or 0.9 kg/900 kg Silo-King GPX. All values expressed on a DM basis.

<table>
<thead>
<tr>
<th>Item</th>
<th>Day 0 $^3$</th>
<th>0 GPX</th>
<th>0.45 kg GPX</th>
<th>0.9 kg GPX</th>
<th>SE</th>
<th>L</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast, Log CFU</td>
<td>7.53</td>
<td>5.62</td>
<td>4.75</td>
<td>4.58</td>
<td>0.10</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Mold, Log CFU</td>
<td>5.23</td>
<td>3.58</td>
<td>2.75</td>
<td>2.24</td>
<td>0.13</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Clostridia, Log CFU</td>
<td>1.60</td>
<td>5.10</td>
<td>3.82</td>
<td>3.79</td>
<td>0.16</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>ZEAR $^4$, µg/kg</td>
<td>214.0</td>
<td>170.00</td>
<td>187.93</td>
<td>210.27</td>
<td>19.68</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^1$ Material was treated with either 0, 0.45 or 0.9 kg/900 kg GPX (preservative), Agri-King, Fulton, IL. 0 GPX = control, 0 g/kg Silo-King GPX treatment, 0.45 kg GPX = 0.45 kg/900 kg Silo-King GPX treatment, 0.9 kg GPX = 0.9 kg/900 kg Silo-King GPX treatment.

$^2$ L = Linear, Q = Quadratic.

$^3$ Day 0 values were obtained from samples taken the day when wet brewers grains arrived prior to treatment.

$^4$ ZEAR = Zearalenone.
Table 5. Ingredient composition of the in situ trial diet.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>(% DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>32.1</td>
</tr>
<tr>
<td>Mixed, mostly grass silage</td>
<td>14.0</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>7.7</td>
</tr>
<tr>
<td>Corn, ground</td>
<td>13.8</td>
</tr>
<tr>
<td>Corn, steam flaked</td>
<td>5.6</td>
</tr>
<tr>
<td>Beet pulp</td>
<td>1.8</td>
</tr>
<tr>
<td>Citrus pulp</td>
<td>1.8</td>
</tr>
<tr>
<td>Soybean hulls</td>
<td>3.6</td>
</tr>
<tr>
<td>Soybean meal (48 % CP)</td>
<td>12.6</td>
</tr>
<tr>
<td>Urea</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>3.5</td>
</tr>
<tr>
<td>Wet Brewers Grains *</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Cows used for the in situ trial were fed the standard herd diet. Fresh wet brewers grains were applied to the TMR as a top dress at the time of feeding and were mixed in by hand.
Table 6. Average ruminal DM and CP degradation of wet brewers grains after treatment with 0, 0.45 or 0.9 kg/900 kg Silo-King GPX. All values expressed on a DM basis.

<table>
<thead>
<tr>
<th>Item³</th>
<th>Treatment¹</th>
<th>Contrast²</th>
<th>SE</th>
<th>L</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 GPX</td>
<td>0.45 kg GPX</td>
<td>0.9 kg GPX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>25.46</td>
<td>23.39</td>
<td>24.30</td>
<td>6.95</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>57.44</td>
<td>58.21</td>
<td>61.17</td>
<td>4.35</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>82.90</td>
<td>81.61</td>
<td>85.47</td>
<td>3.77</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>0.07</td>
<td>0.05</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>52.70</td>
<td>54.03</td>
<td>51.45</td>
<td>3.36</td>
<td>NS</td>
</tr>
<tr>
<td>CP</td>
<td>27.38</td>
<td>27.96</td>
<td>27.84</td>
<td>5.55</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>69.81</td>
<td>71.58</td>
<td>71.34</td>
<td>5.00</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>97.18</td>
<td>99.55</td>
<td>99.18</td>
<td>1.57</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>62.74</td>
<td>63.06</td>
<td>62.00</td>
<td>5.07</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹ Material was treated with either 0, 0.45 or 0.9 kg/900 kg GPX (preservative), Agri-King, Fulton, IL. 0 GPX = control, 0 g/kg Silo-King GPX treatment, 0.45 kg GPX = 0.45 kg/900 kg Silo-King GPX treatment, 0.9 kg GPX = 0.9 kg/900 kg Silo-King GPX treatment.

² L = Linear, Q = Quadratic.

³ A = soluble fraction (%), B = insoluble potentially degradable fraction (%), T = total potentially degradable fraction (%), A + B, kd = rate of degradation, ERD = estimated ruminal DM or CP degradation (%), ERD = A + [(B x kd)/(kd + kp)] where kp = ruminal rate of passage (6.0 % h).
Table 7. Nutrient content of experiment 3 wet brewers grains stored with or without a cover before and after treatment with a 0, 0.45 or 0.9 kg/900 kg Silo-King GPX. All values expressed on a DM basis.

<table>
<thead>
<tr>
<th>Item</th>
<th>Day 0&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Treatment</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>SE</th>
<th>C vs. U&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Contrast&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Covered</td>
<td>Uncovered</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 GPX</td>
<td>0.45 kg</td>
<td>0.9 kg</td>
<td>0</td>
<td>0.45 kg</td>
<td>0.9 kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>23.12</td>
<td>27.18</td>
<td>27.78</td>
<td>28.00</td>
<td>27.38</td>
<td>27.76</td>
<td>0.14</td>
<td>NS</td>
<td>&lt;0.001 &lt;0.01</td>
</tr>
<tr>
<td>CP, %</td>
<td>33.27</td>
<td>34.09</td>
<td>33.50</td>
<td>33.68</td>
<td>33.95</td>
<td>33.92</td>
<td>0.19</td>
<td>NS</td>
<td>&lt;0.05 NS</td>
</tr>
<tr>
<td>NDF, %</td>
<td>41.14</td>
<td>39.68</td>
<td>40.31</td>
<td>40.30</td>
<td>38.75</td>
<td>38.83</td>
<td>0.35</td>
<td>&lt;0.001 NS</td>
<td>NS NS</td>
</tr>
<tr>
<td>ADF, %</td>
<td>19.47</td>
<td>20.83</td>
<td>20.82</td>
<td>20.93</td>
<td>20.49</td>
<td>20.49</td>
<td>0.11</td>
<td>&lt;0.001 NS</td>
<td>NS 0.08</td>
</tr>
<tr>
<td>NDIP, %</td>
<td>2.64</td>
<td>2.71</td>
<td>2.73</td>
<td>2.75</td>
<td>2.64</td>
<td>2.71</td>
<td>0.02</td>
<td>&lt;0.001 NS</td>
<td>NS &lt;0.01</td>
</tr>
<tr>
<td>Fat, %</td>
<td>7.22</td>
<td>8.00</td>
<td>8.02</td>
<td>8.04</td>
<td>7.64</td>
<td>8.23</td>
<td>0.13</td>
<td>NS</td>
<td>NS &lt;0.05</td>
</tr>
<tr>
<td>Ash, %</td>
<td>5.98</td>
<td>6.14</td>
<td>5.92</td>
<td>5.91</td>
<td>5.63</td>
<td>6.06</td>
<td>0.10</td>
<td>NS</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Starch, %</td>
<td>7.57</td>
<td>2.81</td>
<td>4.47</td>
<td>4.56</td>
<td>2.42</td>
<td>4.37</td>
<td>0.12</td>
<td>NS</td>
<td>&lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.27</td>
<td>0.24</td>
<td>0.25</td>
<td>0.25</td>
<td>0.24</td>
<td>0.24</td>
<td>0.004</td>
<td>NS</td>
<td>&lt;0.05 NS</td>
</tr>
<tr>
<td>P, %</td>
<td>0.59</td>
<td>0.60</td>
<td>0.62</td>
<td>0.60</td>
<td>0.59</td>
<td>0.60</td>
<td>0.009</td>
<td>NS</td>
<td>NS NS</td>
</tr>
<tr>
<td>Mg, %</td>
<td>0.20</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.16</td>
<td>0.003</td>
<td>NS</td>
<td>NS NS</td>
</tr>
<tr>
<td>K, %</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.11</td>
<td>0.09</td>
<td>0.10</td>
<td>0.002</td>
<td>&lt;0.05 NS &lt;0.01 &lt;0.001</td>
<td>NS NS &lt;0.001</td>
</tr>
<tr>
<td>Na, %</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.002</td>
<td>NS</td>
<td>NS &lt;0.001</td>
</tr>
</tbody>
</table>

<sup>1</sup> Material was treated with either 0, 0.45 or 0.9 kg/900 kg GPX (preservative), Agri-King, Fulton, IL. 0 GPX = control, 0 g/kg Silo-King GPX treatment, 0.45 kg GPX = 0.45 kg/900 kg Silo-King GPX treatment, 0.9 kg GPX = 0.9 kg/900 kg Silo-King GPX treatment.

<sup>2</sup> L = Linear, Q = Quadratic.

<sup>3</sup> Day 0 values were obtained from samples taken the day when wet brewers grains arrived prior to treatment.

<sup>4</sup> C vs. U = Covered vs. Uncovered.
Table 8. pH, concentrations of lactic, acetic and butyric acids and NH$_3$N concentration of experiment 3 wet brewers grains stored with or without a cover before and after treatment with 0, 0.45 or 0.9 kg/900 kg Silo-King GPX. All values expressed on a DM basis.

<table>
<thead>
<tr>
<th>Item</th>
<th>Covered</th>
<th>Uncovered</th>
<th>SE</th>
<th>C vs. U $^4$</th>
<th>Contrast $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>0</td>
<td>0.45 kg</td>
<td>0.9 kg</td>
<td>0 GPX</td>
</tr>
<tr>
<td>pH</td>
<td>4.6</td>
<td>3.69</td>
<td>3.68</td>
<td>3.71</td>
<td>3.74</td>
</tr>
<tr>
<td>Lactic, %</td>
<td>1.77</td>
<td>5.66</td>
<td>6.64</td>
<td>6.41</td>
<td>5.91</td>
</tr>
<tr>
<td>Acetic, %</td>
<td>0.70</td>
<td>1.11</td>
<td>1.06</td>
<td>1.07</td>
<td>0.92</td>
</tr>
<tr>
<td>Butyric, %</td>
<td>0.00</td>
<td>0.16</td>
<td>0.02</td>
<td>0.02</td>
<td>0.29</td>
</tr>
<tr>
<td>NH$_3$N, mg/kg</td>
<td>225</td>
<td>141</td>
<td>1154</td>
<td>744</td>
<td>1235</td>
</tr>
</tbody>
</table>

$^1$ Material was treated with either 0, 0.45 or 0.9 kg/900 kg GPX (preservative), Agri-King, Fulton, IL. 0 GPX = control, 0 g/kg Silo-King GPX treatment, 0.45 kg GPX = 0.45 kg/900 kg Silo-King GPX treatment, 0.9 kg GPX = 0.9 kg/900 kg Silo-King GPX treatment.

$^2$ L = Linear, Q = Quadratic.

$^3$ Day 0 values were obtained from samples taken the day when wet brewers grains arrived prior to treatment.

$^4$ C vs. U = Covered vs. Uncovered.
Table 9. Logarithmically transformed yeast, mold and clostridia concentrations and zearalenone and deoxynivalanolo concentrations of experiment 3 wet brewers grains stored with or without a cover before and after treatment with 0, 0.45 or 0.9 kg/900 kg Silo-King GPX. All values expressed on a DM basis.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>Covered</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>C vs. U</th>
<th>Contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0 3</td>
<td>0 GPX</td>
<td>0.45 kg GPX</td>
<td>0.9 kg GPX</td>
<td>0 GPX</td>
<td>0.45 kg GPX</td>
<td>0.9 kg GPX</td>
<td>SE</td>
</tr>
<tr>
<td>Yeast, Log</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.70</td>
<td>3.32</td>
<td>2.81</td>
<td>2.76</td>
<td>3.20</td>
<td>2.65</td>
<td>2.46</td>
<td>0.25</td>
<td>NS</td>
</tr>
<tr>
<td>Mold, Log</td>
<td>3.00</td>
<td>2.18</td>
<td>1.92</td>
<td>1.83</td>
<td>1.99</td>
<td>1.79</td>
<td>1.70</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>Clost, Log</td>
<td>1.00</td>
<td>2.11</td>
<td>1.49</td>
<td>1.49</td>
<td>1.67</td>
<td>1.71</td>
<td>1.63</td>
<td>0.28</td>
<td>NS</td>
</tr>
<tr>
<td>DON 5, mg/kg</td>
<td>0.00</td>
<td>0.03</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.00</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>ZEAR 6, µg/kg</td>
<td>225.0</td>
<td>1419.07</td>
<td>1154.20</td>
<td>743.57</td>
<td>1267.30</td>
<td>1116.90</td>
<td>1519.53</td>
<td>548.10</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Material was treated with either 0, 0.45 or 0.9 kg/900 kg GPX (preservative), Agri-King, Fulton, IL. 0 GPX = control, 0 g/kg Silo-King GPX treatment, 0.45 kg GPX = 0.45 kg/900 kg Silo-King GPX treatment, 0.9 kg GPX = 0.9 kg/900 kg Silo-King GPX treatment.

2 L = Linear, Q = Quadratic.

3 Day 0 values were obtained from samples taken the day when wet brewers grains arrived prior to treatment.

4 C vs. U = Covered vs. Uncovered.

5 DON = Deoxynivalenol.

6 ZEAR = Zearalenone.
Figure 1: Starch (% DM) concentration changes of WBG over a 672 h period after treatment with 0, 0.45 or 0.9 kg/900 kg Silo-King GPX. 0 GPX = control, 0 g/kg Silo-King GPX treatment, 0.45 kg GPX = 0.45 kg/900 kg Silo-King GPX treatment, 0.9 kg GPX = 0.9 kg/900 kg Silo-King GPX treatment. The SEM was 0.32. * (P < 0.05) Control samples of WBG had lower starch concentrations than Silo-King treated samples.
Figure 2. Butyric acid (% DM) changes of wet brewers grains over a 672 h period after treatment with 0, 0.45 or 0.9 kg/900 kg Silo-King GPX. 0 GPX = control, 0 g/kg Silo-King GPX treatment, 0.45 kg GPX = 0.45 kg/900 kg Silo-King GPX treatment, 0.9 kg GPX = 0.9 kg/900 kg Silo-King GPX treatment. The SEM was 0.10. * (P < 0.05) Control samples of WBG had higher Butyric acid concentrations than Silo-King treated samples.
REFERENCES


July 8, 2005

Erickson, Peter
Animal & Nutritional Sciences
Ritzman Nutrition Lab
Durham, NH 03824

IACUC #: 050603
Approval Date: 06/29/2005
Review Level: D
Project: Enzyme Inoculants for Wet Brewer's Grain

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category D on Page 4 of the Application for Review of Vertebrate Animal Use in Research or Instruction - the research involves chronic maintenance of animals with a disease/functional deficit and/or procedures potentially inducing moderate pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Van Gould at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Roger E. Wells, D.V.M.
Vice Chair

cc: File

Research Conduct and Compliance Services, Office of Sponsored Research, Service Building,
51 College Road, Durham, NH 03824-3585 * Fax: 603-862-3564

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APPENDIX B

Table B-10. Trace mineral content of wet brewers grains used in experiment 1 before and after treatment with 0, 0.45 or 0.9 kg/900 kg Silo-King GPX. All values expressed on a DM basis.

<table>
<thead>
<tr>
<th>Item</th>
<th>Day 0</th>
<th>0 GPX</th>
<th>0.45 kg GPX</th>
<th>0.9 kg GPX</th>
<th>SE</th>
<th>L</th>
<th>Q</th>
<th>1 Material was treated with either 0, 0.45 or 0.9 kg/900 kg GPX (preservative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe, mg/kg</td>
<td>194</td>
<td>201</td>
<td>184</td>
<td>233</td>
<td>27.61</td>
<td>NS</td>
<td>NS</td>
<td>0 GPX = control, 0.45 kg GPX = 0.45 kg/900 kg Silo-King GPX treatment, 0.9 kg GPX = 0.9 kg/900 kg Silo-King GPX treatment.</td>
</tr>
<tr>
<td>Cu, mg/kg</td>
<td>5.00</td>
<td>5.93</td>
<td>6.40</td>
<td>5.40</td>
<td>0.49</td>
<td>NS</td>
<td>NS</td>
<td>0.05 Day 0 values were obtained from samples taken the day when wet brewers grains arrived prior to treatment.</td>
</tr>
<tr>
<td>Mn, mg/kg</td>
<td>57.00</td>
<td>50.47</td>
<td>48.33</td>
<td>46.07</td>
<td>1.33</td>
<td>NS</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Zn, mg/kg</td>
<td>99.00</td>
<td>85.33</td>
<td>82.47</td>
<td>75.20</td>
<td>2.65</td>
<td>NS</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

L = Linear, Q = Quadratic.
Table C-11. Trace mineral content of experiment 3 wet brewers grains stored with or without a cover before and after treatment with 0, 0.45 or 0.9 kg/900 kg Silo-King GPX. All values expressed on a DM basis.

<table>
<thead>
<tr>
<th>Item</th>
<th>Day 0</th>
<th>Covered</th>
<th>0 GPX</th>
<th>0.45 kg GPX</th>
<th>0.9 kg GPX</th>
<th>Uncovered</th>
<th>0 GPX</th>
<th>0.45 kg GPX</th>
<th>0.9 kg GPX</th>
<th>SE</th>
<th>C vs. U</th>
<th>L</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe, mg/kg</td>
<td>153.0</td>
<td>410.53</td>
<td>372.00</td>
<td>436.40</td>
<td>283.53</td>
<td>394.60</td>
<td>303.07</td>
<td>23.90</td>
<td>0.0001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cu, mg/kg</td>
<td>5.0</td>
<td>7.00</td>
<td>7.17</td>
<td>6.97</td>
<td>7.50</td>
<td>7.97</td>
<td>8.00</td>
<td>0.33</td>
<td>0.01</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mn, mg/kg</td>
<td>58.0</td>
<td>51.73</td>
<td>52.70</td>
<td>53.50</td>
<td>51.50</td>
<td>52.70</td>
<td>51.97</td>
<td>1.35</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Zn, ppm</td>
<td>109.0</td>
<td>90.73</td>
<td>94.90</td>
<td>94.30</td>
<td>89.53</td>
<td>91.57</td>
<td>90.67</td>
<td>1.79</td>
<td>0.06</td>
<td>0.09</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Material was treated with either 0, 0.45 or 0.9 kg/900 kg GPX (preservative), Agri-King, Fulton, IL. 0 GPX = control, 0 g/kg Silo-King GPX treatment, 0.45 kg GPX = 0.45 kg/900 kg Silo-King GPX treatment, 0.9 kg GPX = 0.9 kg/900 kg Silo-King GPX treatment.

2 L = Linear, Q = Quadratic.

3 Day 0 values were obtained from samples taken the day when wet brewers grains arrived prior to treatment.

4 C vs. U = Covered vs. Uncovered.