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Effect of Adding Chinese Wildrye or Alfalfa to Wet Corn Gluten Feed During Fermentation

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Abstract

The objective of this experiment was to determine the fermentation quality of wet corn gluten feed ensiled alone and treated with Chinese wildrye or alfalfa using different concentrations. Two experiments were performed to investigate the fermentation profile of wet corn gluten feed after 60 days of anaerobic storage in plastic model silos. Wet corn gluten feed was ensiled alone and mixed with Chinese wildrye (10%, 20% and 30%) or alfalfa (10%, 20% and 30%). The wet corn gluten feed ensiled alone was the control. Sampling was performed on the 60th day after ensiling for fermentation quality and chemical analysis. The results showed very low fermentation intensity, that high levels of lactic acid dominated the fermentation of wet corn gluten feed ensiled alone, and the pH increased during fermentation with Chinese wildrye or alfalfa at different rations. Ammonia-nitrogen was highest for wet corn gluten feed ensiled alone, decreasing as the Chinese wildrye concentration increased, and increasing as the alfalfa concentration increased. Adding of 30% Chinese wildrye to wet corn gluten feed or 10% alfalfa significantly increased acetic acid production, which improved aerobic stability and reduced the lactic acid: acetic acid ratio relative to the control. In conclusion, organic acid fermentation of wet corn gluten feed ensiled alone was low. Adding 30% Chinese wildrye to wet corn gluten feed is recommended to reduce dry matter loss and improve aerobic stability. Adding 10% alfalfa to wet corn gluten feed is recommended to improve aerobic stability.

Keywords: Wet corn gluten feed; Chinese wildrye; Alfalfa; Fermentation quality

Introduction

Increased pressure for land use, including for more domestic agriculture, has contributed to the loss of forage acres in China. As a result, nutritionists are pressured to devise strategies by which to control cost of feedstuff. Recently, numerous fibrous by-product feeds have been evaluated as potential feedstuff for ruminant livestock. By-product feed from the milling industry includes readily digestible NDF fraction and a combination of energy sources for ruminal microbes (Varga and Hoover, 1983). Wet corn gluten feed is co-produced in corn wet milling, wherein corn is soaked in a solution with water and sulfur dioxide. After steeping, the steep liquor is separated and concentrated. The corn germ is then removed, and the remaining portion of the kernel, starch and bran are screened and separated (Hoffman, 1991). The bran is typically then mixed with the steep liquor at a ratio of approximately two parts bran to one part condensed liquor, which yields wet corn gluten feed (Schroeder, 2003). The corn bran provides less energy than steeping (Scott *et al.*, 1997). However, the wet corn gluten feed may also contain varying levels of distiller soluble, germ meal, and kernel screening (Macken *et al.*,2004). It also includes low starch and fat concentrations as well as highly digestible fiber (Fieck *et al.*,1988; Belyea *et al.*, 1989). Additionally, the feed replaces starch with fiber, which may increase rumen pH and reduce the incidence of acidosis (Herold *et al.*, 1998; Sindt *et al.*,2002). Typically, wet corn gluten feed is a rapidly digestible, non-forage source of fiber and protein (Firkins, 1997; Boddugari *et al.*, 2001). The use of wet corn gluten feed has certain disadvantages, such as high moisture content (36–40% DM), causing certain storage and shelf life issues. The feed can spoil within a few days depending on the amount of oxygen exposure and ambient air temperature (Christensen *et al.*, 2010), and it typically changes the texture and smell, among other properties. Such changes cause the animals to reject the food (Doyle, 2007).

Certain ecologists believe that the changes, such as rancid smells from spoiled foods, are produced by microbial activity (Burkepile *et al.*, 2006; Sherratt *et al.*, 2006) because microbial activity can degrade nutrients and contribute to the strong odor from spoiled food (Ellis and Goodacre, 2006). Traditionally, it is often dried to approximately 90% DM for dry corn gluten feed, but the drying process wastes energy and can produce air quality problems (such a CO_2, SO_2 and high levels of smoke). A suitable storage method should be developed for a broader use of wet corn gluten feed. Generally, the three types of

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organisms that contribute to spoilage are bacteria, yeasts and molds (Gram *et al.,* 2002). Due to the high moisture content of wet corn gluten feed, it must be used rapidly or preserved through anaerobic storage (Droppo *et al.,*1985; Larson *et al.,*1983; Staples *et al.,* 1984).

Many studies have been conducted on feedstuff storage, which have high levels of moisture and easily spoil. Certain common storage and preservation practices include bagging storage, fresh piles, bunker storage and additives (Nelson *et al.,* 2009). Silage bags are commonly used for wet feeds storage and are considered to be effective, but deterioration was observed at the edges in the silo bags, and more pressure can result in bag bursting (Strohbehn *et al.,* 2008 a, b; Erickson *et al.,* 2008); further, once the silage bags were opened, rapid air infiltration produces non-enzymatic browning. Bagging is not the most adequate storage method. In addition, certain researcher noted that typical storage methods depend on the equipment and structure costs, among other considerations (Strohbehn *et al.,* 2008b; Waterbury *et al.,* 2008). Wet feed storage in bunkers is the most common method. Moreover, researchers found that wet feed should be stored properly in bunkers with forage because it has high moisture content that causes the pile to flow (Erickson *et al.,*2008; Baskett *et al.,* 2009). Wet feed additives for storage include biological and chemical preservatives. Biological additives for preservation include the following: incorporated grass hay, corn stalk, wheat straw and soybean hulls into wet feeds (Garcia and Kalscheur, 2004; Schingoethe *et al.,* 2006; Loy, 2008; Strohbehn *et al.,* 2008b). Chemical additives for preservation include chemical preservatives to wet feed. However, the potency of chemical preservatives is affected by chemical balance and composition (Drackley *et al.,* 2004; Kung, 2005;Walker and Forster, 2008; Sommerfeldt, 2011). Herein, Chinese wildrye or alfalfa is the most common dry forages for ruminants in northeast China. Research was conducted to determine the fermentation quality of wet corn gluten feed treated with Chinese wildrye or alfalfa after 60 days of anaerobic storage in a plastic model silo.

Materials and Methods

Ensiling

The Chinese wildrye and alfalfa were acquired from the Xiangfang Experimental Farm of the Northeast Agricultural University (Harbin, China); and the wet corn gluten feed was acquired from Cargill Biochemistry Co., Ltd (Songyuan, China). The Chinese wildrye and alfalfa were chopped to a theoretical length of 5-6 cm using a crop chopper. The following experimental silages were generated: wet corn gluten feed was ensiled alone and mixed with Chinese wildrye (10%, 20% and 30%) or alfalfa (10, 20 and 30). Approximately 2.75 kg (wet mass) from each treatment was added to the plastic model silo(airtight sealing, air-valves and volume: 0.00275 m^3), and each silo was covered and stored for 60 days at the ambient temperature (approximately 20±5℃). Each treatment was ensiled in three experiment plastic model silos. After the ensiling time, three silos per treatment were opened. The silages were randomly sampled from different positions, and then mixed to generate a composite sample for chemical analysis. The applied treatments were as follows.

Experiment 1: Density of 1000 kg/m^3

Control group: ensiled wet corn gluten feed alone including 2.75 kg wet corn gluten feed.

Trial group: mixed with Chinese wildrye (90:10, 80:20 and 70:30).

90:10 group, including 2.48 kg wet corn gluten feed and 0.28 kg Chinese wildrye

80:20 group, including 2.20 kg wet corn gluten feed and 0.55 kg Chinese wildrye.

70:30 group, including 1.93 kg wet corn gluten feed and 0.83 kg Chinese wildrye

Experiment 2: Density of 1100 kg/m^3

Control group: ensiled alone including 3 kg wet corn gluten (fiber) feed

Trial group: mixed with alfalfa (90:10, 80:20 and 70:30).

90:10 group, including 2.7 kg wet corn gluten feed and 0.3 kg alfalfa.

80:20 group, including 2.4 kg wet corn gluten feed and 0.6 kg alfalfa.

70:30 group, including 2.1 kg wet corn gluten feed and 0.9 kg alfalfa.

Chemical Analysis

The silage samples were dried at 60±5℃ and analyzed for DM in accordance with AOAC (1990) procedures. The nitrogen (N) content was measured using the Kjeldahl method (AOAC, 1990). The crude protein (CP) was calculated as N×6.25. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) values were analyzed in accordance with the procedures in Van Soest *et al.* (1991) using the Ankom system (Ankom 220 fiber analyzer; Ankom) with heat-stable α-amylase. A 20 g silage sample was homogenized in 180 mL of distilled water and stored for 24 h at 4℃ in a refrigerator (Nishino and Uchida, 1999). The slurry mixture was then filtered through four layers of cheesecloth (Xing *et al.,* 2009). The filtrate was used for pH, ammonia-N, lactic acid and VFA determination. The pH was directly measured using a pH meter (Sartorius Basic pH Meter, Germany). The ammonia-N (NH_3-N) concentration was determined using an ammonia-sensing electrode (Expandable Ion Analyzer EA 940, Orion, USA). The samples for VFA analysis were prepared as described by Li and Meng (2006).

The concentrations of a volatile fatty acid (VFA) were analyzed through gas-liquid chromatography (GC 2010, Tokyo, Japan) with a flame-ionization detector and a FFAP capillary column (HP-INNOWAX, 30 m ×

0.250 mm×0.25 um). The lactic acid levels were determined using high-performance liquid chromatography (Waters 600, Tokyo, Japan) and the procedure in Muck and Dickerson (1988). The chemical analyses were performed in triplicate and expressed as dry weight, except for the DM content (% fresh matter) and NH_3-N (% total nitrogen (TN).

Statistical Analysis

The data from the experiments were analyzed through one-way ANOVA with the GLM procedure from SAS (SAS, 1989). If the variances were significant, the differences between mean values were determined using Duncan's multiple range test method. The standards error for the means was calculated from the residual mean square in the analysis of variance.

Results

The pH and Ammonia Nitrogen Content after the Ensiling Time

The silage pH and ammonia nitrogen concentration for the different proportions of Chinese wildrye or alfalfa in wet corn gluten feed are shown in Table 1 and Table 2. After ensiling, the pH was low for the wet corn gluten feed ensiled alone ($P<0.05$). The pH increased when the wet corn gluten feed was mixed with Chinese wildrye or alfalfa at different rations ($P<0.05$ and $P<0.05$, respectively). The pH values for experiment 1 and experiment 2 were below 4.1 or 4.5, respectively. The ammonia-N was lowest for the wet corn gluten feed ensiled alone and increased as the Chinese wildrye or alfalfa concentration increased ($P<0.05$ and $P<0.05$, respectively).

Organic Acids Content after the Ensiling Time

The silage organic concentration for different proportions of Chinese wildrye or alfalfa in wet corn gluten feed is shown in Table 1 and Table 2. Acetic acid increased as the Chinese wildrye concentration increased ($P<0.05$) in experiment 1, but acetic acid decreased as alfalfa concentration increased ($P<0.05$) in experiment 2. After ensiling, the lactic acid levels were highest for wet corn gluten feed and decreased as Chinese wildrye or alfalfa concentration increased ($P<0.05$). Adding 30% Chinese wildrye or 10% alfalfa to the wet corn gluten feed significantly increased the acetic acid production and reduced the lactic acid:acetic acid (L:A) ratio relative to the control. The L: A ration was appropriate (Experiment 1, L: A =9.91, Experiment 2, L: A =11.21) for the control. After the ensiling period, butyric acid was the fermentation end-product.

Chemical Composition and DM Losses 60-Day Silages

The level of wet corn gluten feed ensiled alone and mixed with Chinese wildrye (10%, 20% and 30%) or alfalfa (10%, 20% and 30%) affected the chemical composition of silage including DM, CP, NDF, ADF and DM loss (Table 3 and 4). For experiment 1 and experiment 2, wet corn gluten feed and 30% Chinese wildrye or alfalfa had the highest DM and ADF ($P<0.05$). The addition of different levels of Chinese wildrye or alfalfa to wet corn gluten feed had no significant effect on NDF ($P>0.05$). The 30% Chinese wildrye reduced the DM loss compared the other groups ($P<0.05$), but the wet corn gluten feed and 10% alfalfa had the greatest DM loss than the other groups ($P<0.05$).

Aerobic Stability of Silage after 60 Days of Ensiling

The aerobic stability of silage after 60 days of ensiling is shown in Fig. 1 and 2. The aerobic stability was measured as the number of hours for the temperature in the feed to increase 2^oC above ambient temperature. The aerobic stability of the control group was low. For Fig. 1, wet corn gluten feed and 30% Chinese wildrye had the greatest aerobic stability for 302 h. For Fig. 2, wet corn gluten feed and 10% alfalfa had the greatest aerobic stability for 314 h.

Discussion

The pH is considered to be one of the crucial factors in evaluating the fermentation quality of silage (Muck, 1988). After ensiling, the low pH is likely due to the sulfuric acid addition, which halts fermentation towards the end products of the starch plant, and it is sufficiently acidic to preserve the wet corn gluten feed during anaerobic conditions. The pH increased when the wet corn gluten feed was mixed with Chinese wildrye or alfalfa at different rations, because Chinese wildrye or alfalfa has a high original pH, depending on the buffering effect. The pH below 4.1 or 4.5 is considered a good index of silage quality. The 30% Chinese wildrye or alfalfa in wet corn gluten feed had the highest level of ammonia-N likely produced the highest level of proteolysis, and the ethanol was likely produced by the hetero-fermentative type organisms in the presence of available fermentation substrates. Although ethanol was not measured herein, a similar observation was reported previously by Orosz and Kapás (2010), who found that the wet corn gluten feed generated low fermentation intensity, and lactic acid dominated the fermentation with a high ethanol concentration. Many researchers reported an increase in NH_3-N when another feed type was added to the wet corn gluten feed (Mills and Grant, 2002). Kalscheur *et al.*, (2003) also found that forages

Table 1: The effect of different levels of Chinese wildrye on the fermentation quality of wet corn gluten feed

Item	D[1]	Y10[2]	Y20[3]	Y30[4]
pH	3.85±0.02c	4.02±0.11b	4.06±0.01b	4.14±0.03a
Ammonia-N,% of total N	2.85±0.14d	3.09±0.10c	3.22±0.06b	3.33±0.02a
Organic acids (% of DM)				
Lactic acid	2.14±0.01a	1.89±0.04d	1.97±0.08c	2.04±0.01b
Acetic acid	0.22±0.01d	0.65±0.06c	0.68±0.02b	0.71±0.03a
Propionic acid	0.06	0.03	0.06	-
Butyric acid	0	0	0	0
L:A[5]	9.91±0.19a	2.92±0.11b	2.91±0.06b	2.88±0.02b

[1]D= Wet corn gluten feed ensiled alone. [2]Y10= Addition of 10% Chinese wildrye to wet corn gluten feed. [3]Y20= Addition of 20% Chinese wildrye to wet corn gluten feed. [4]Y30= Addition of 30% Chinese wildrye to wet corn gluten feed.[5]L: A=Lactic acid: acetic acid
Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05)

Table 2: The effect of different levels of alfalfa on the fermentation quality of wet corn gluten feed

Item	D[1]	M10[2]	M20[3]	M30[4]
pH	3.85±0.03d	4.16±0.01b	4.33±0.12b	4.41±0.07a
Ammonia-N,% of total N	3.00±0.24d	3.12±0.08c	3.85±0.02b	4.45±0.22a
Organic acids (% of DM)				
Lactic acid	2.32±0.05a	2.12±0.02b	2.02±0.02c	1.90±0.02d
Acetic acid	0.21±0.01d	0.79±0.01a	0.71±0.03b	0.65±0.01c
Propionic acid	0.04	-	-	-
Butyric acid	0	0	0	0
L:A5	11.21±0.50a	2.71±0.06b	2.86±0.25b	2.91±0.01b

[1]D= Wet corn gluten feed ensiled alone. [2]Y10= Addition of 10% alfalfa to wet corn gluten feed.[3]Y20= Addition of 20% alfalfa to wet corn gluten feed. [4]Y30= Addition of 30% alfalfa to wet corn gluten feed. [5]L: A=Lactic acid: acetic acid
Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05)

Table 3: The effect of different levels of Chinese wildrye addition on chemical composition and DM loss in wet corn gluten feed

Item	D[1]	Y10[2]	Y20[3]	Y30[4]
DM	38.02±0.09d	42.29±0.41c	47.58±0.23b	52.16±0.36a
CP (%DM)	17.10±0.29a	16.64±0.31b	14.87±0.04c	13.20±0.41d
NDF (%DM)	46.66±0.32a	51.69±0.18a	53.39±0.36a	50.65±0.29a
ADF (%DM)	13.60±0.15d	17.31±0.29c	18.48±0.18b	21.26±0.14a
DML (%)	3.17±0.18c	4.82±0.11a	4.00±0.09b	2.96±0.18d

[1]D= Wet corn gluten feed ensiled alone. [2]Y10= Addition of 10% Chinese wildrye to wet corn gluten feed. [3]Y20= Addition of 20% Chinese wildrye to wet corn gluten feed. [4]Y30= Addition of 30% Chinese wildrye to wet corn gluten feed
Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05)

Table 4: The effect of different levels of alfalfa addition on chemical composition and DM loss in wet corn gluten feed

Item	D[1]	M10[2]	M20[3]	M30[4]
DM	37.54±0.42d	41.38±0.21c	45.73±0.14b	50.87±0.32a
CP (%DM)	17.10±0.21a	17.22±0.08c	17.56±0.15b	17.97±0.11a
NDF (%DM)	46.66±0.56a	47.95±0.24a	47.74±0.31a	48.55±0.48a
ADF (%DM)	13.60±0.09d	18.62±0.15c	20.23±0.14b	23.48±0.45a
DML (%)	3.57±0.09b	4.59±0.14a	3.17±0.32c	2.10±0.15d

[1]D= Wet corn gluten feed ensiled alone. [2]Y10= Addition of 10% alfalfa in wet corn gluten feed.[3]Y20= Addition of 20% alfalfa in wet corn gluten feed. [4]Y30= Addition of 30% alfalfa in wet corn gluten feed
Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05)

were successfully ensiled together with wet distillers grains (WDG); the ammonia-nitrogen increased over time (P<0.05) for all silages.

Acetic acid is a good antifungal component in naturally fermented silages and improves aerobic stability (Danner *et al.,* 2003; Holzer *et al.,* 2003) due to its capacity to inhibit yeast and mold growth (Moon, 1983). During fermentation in experiment 1, acetate was likely produced from the activity of hetero-fermentative bacteria competing with lactobacilli for available fermentation sugars. Low levels of acetate were observed in wet corn gluten feed ensiled alone, which can be explained by the relative absence of sugars. After ensiling, the lactic acid levels were highest for wet corn gluten feed and decreased as Chinese wildrye or alfalfa concentration increased likely, because the steep liquor in wet corn gluten feed contains high levels of lactate (Krehbiel *et al.,*1995), which improves lactic acid

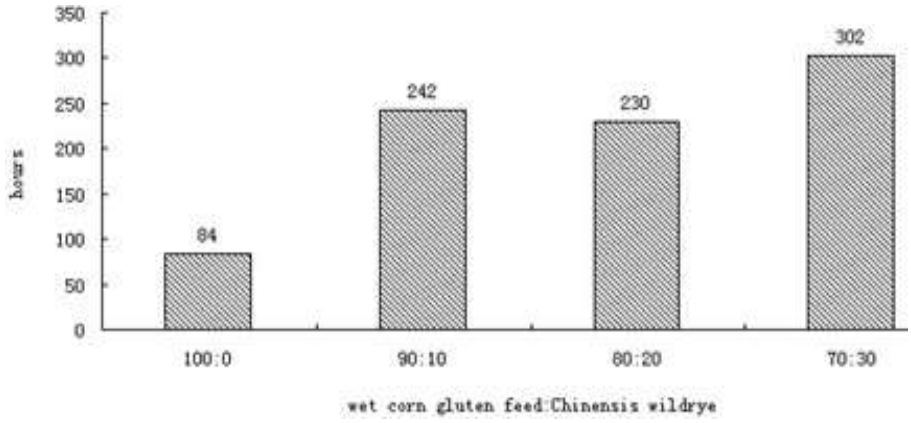

Fig. 1: Aerobic stability (hours for the feed temperature to increase 2℃ above ambient temperature) in wet corn gluten feed ensiled with Chinese wildrye

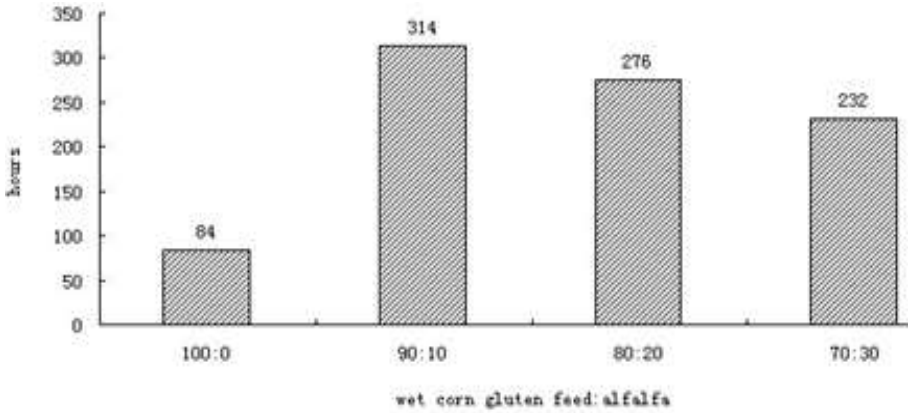

Fig. 2: Aerobic stability (hours for the feed temperature to increase 2℃ above ambient temperature) in wet corn gluten feed ensiled with alfalfa

metabolism (Fron *et al.,* 1995). After the ensiling period, butyric acid was the fermentation end-product, which often deteriorates the silage quality (McDonald *et al.,* 1991); further, it was identified as a factor that inhibits fungi growth (Muck, 1988). For both the experiments, butyric acid was not observed in treatments, which is an indicator of poor fermentation quality (Kalscheur *et al.,* 2003)

The group with Chinese wildrye (10%, 20% and 30%) or alfalfa (10%, 20% and 30%) had a significantly higher DM content than wet corn gluten feed was ensiled alone, and significant differences in DM loss were observed between the silages. Oude Elferick *et al.* (1999a) reported that extra DM loss would result from CO_2 emission, which was formed during the conversion of lactic acid to acetic acid and 1, 2-propanediol. Therefore, the DM loss could be considered to be a good reflection of acetic acid metabolism during fermentation.

Aerobic stability is a term that nutritionists use to define the length of time that silage remains cool and does not spoil after exposure to air (Kleinschmit *et al.,*2005). A high acetate concentration was likely responsible for the greater aerobic stability observed in the 70:30 blend, and for figure 2, a high acetate concentration was likely responsible for the greater aerobic stability observed in the 90:10 blend.

In conclusion, given the fermentation results, the plastic model silo is recommended for wet corn gluten feed storage. In conclusion, wet corn gluten feed ensiled alone generated low levels of organic acid fermentation. Chinese wildrye at 30% is a recommended addition to wet corn gluten feed to reduce the DML and improve aerobic stability and alfalfa at 10% is a recommended addition to wet corn gluten feed to improve aerobic stability.

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