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Conversion of Distiller's Grain into Fuel Alcohol and a Higher-Value Animal Feed by Dilute-Acid Pretreatment

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Abstract

Over the past three decades ethanol production in the United States has increased more than 10-fold, to approx 2.9 billion gal/yr (mid-2003), with ethanol production expected to reach 5 billion gal/yr by 2005. The simultaneous coproduction of 7 million t/yr of distiller's grain (DG) may potentially drive down the price of DG as a cattle feed supplement. The sale of residual DG for animal feed is an important part of corn dry-grind ethanol production economics; therefore, dry-grind ethanol producers are seeking ways to improve the quality of DG to increase market penetration and help stabilize prices. One possible improvement is to increase the protein content of DG by converting the residual starch and fiber into ethanol. We have developed methods for steam explosion, SO_2, and dilute–sulfuric acid pretreatment of DG for evaluation as a feedstock for ethanol production. The highest soluble sugar yields (~77% of available carbohydrate) were obtained by pretreatment of DG at 140°C for 20 min with 3.27 wt% H_2SO_4. Fermentation protocols for pretreated DG were developed at the bench scale and scaled to a working volume of 809 L for production of hydrolyzed distiller's grain (HDG) for feeding trials. The pretreated DG was fermented with *Saccharomyces cerevisiae* D_5A, with ethanol yields of 73% of theoretical from available glucans.

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The HDG was air-dried and used for turkey-feeding trials. The inclusion of HDG into turkey poult (as a model non-ruminant animal) diets at 5 and 10% levels, replacing corn and soybean meal, showed weight gains in the birds similar to controls, whereas 15 and 20% inclusion levels showed slight decreases (–6%) in weight gain. At the conclusion of the trial, no negative effects on internal organs or morphology, and no mortality among the poults, was found. The high protein levels (58–61%) available in HDG show promising economics for incorporation of this process into corn dry-grind ethanol plants.

Index Entries: Distiller's grain; corn dry-grind; pretreatment; enzymatic hydrolysis; ethanol; animal feed.

Introduction

Seventy-four ethanol plants were in operation in the United States in mid-2003, with nearly sixteen more expected to come on line by the end of 2004 *(1)*. The majority of these new plants are corn dry-grind ethanol plants. Approximately 2.5–2.7 gal of ethanol, 17.5 lb of dried distiller's grain (DDG), and 17 lb of carbon dioxide are produced from each bushel of corn processed through a corn dry mill *(2)*. Since 1980, process improvements in enzymes, thermal-tolerant yeasts, molecular sieves, and cogeneration have achieved a 50% reduction in the energy required to produce ethanol from corn *(2)*. Further improvements in efficiencies and reductions in production costs can be expected in the future.

Currently 3.8 million t of distiller's grain (DG) per year is produced as a coproduct of corn dry mill ethanol production. This amount is expected to rise to 7 million t/yr when ethanol production reaches 5 billion gal/yr in 2005 *(3)*. The present DDG and distiller's dried grain with solubles (DDGS) animal feed markets are not expected to absorb these increases without erosions of price and profit margins. The resultant loss in income will affect the economic viability of both current and future dry mills. The primary use of DG residues (DDG and DDGS) is in feed formulations for dairy and beef cattle. However, a higher-protein and lower-fiber-content DG residue would allow the market to expand by penetrating the swine and poultry feed markets. Converting the residual starch and fiber into DG for ethanol production would raise ethanol yields and result in hydrolyzed distiller's grain (HDG) residues with a higher protein content. HDG would compete with soybean meal in animal feed markets, provided that the high protein content is of a high-quality, digestible protein. The increase in ethanol yield is also a significant benefit to corn dry-grind operators because corn costs represent a significant portion of the production costs for a gallon of ethanol.

Acceptance of DDG and DDGS products as animal feed supplements is limited by the variable "quality" of DDG and DDGS among ethanol plants and the relatively low crude protein content *(4)*. This variability is caused by differing amounts of residual starch and crude protein in DG products. Crude protein content depends in part on preserving protein during the drying step. A study conducted by the Department of Animal

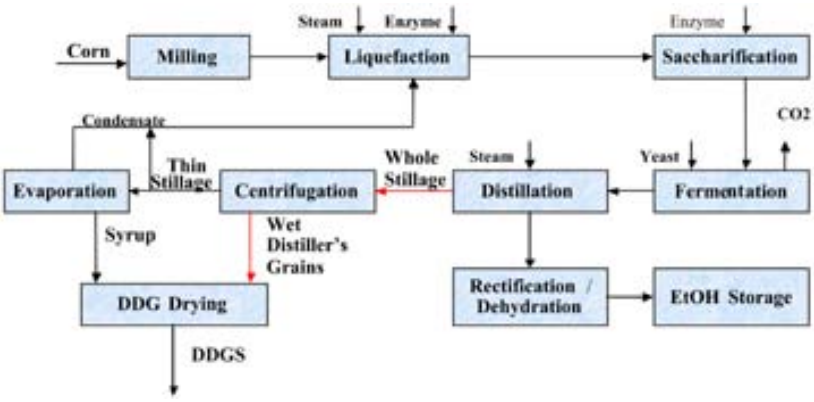

Fig. 1. Process flow diagram for typical dry-grind ethanol process.

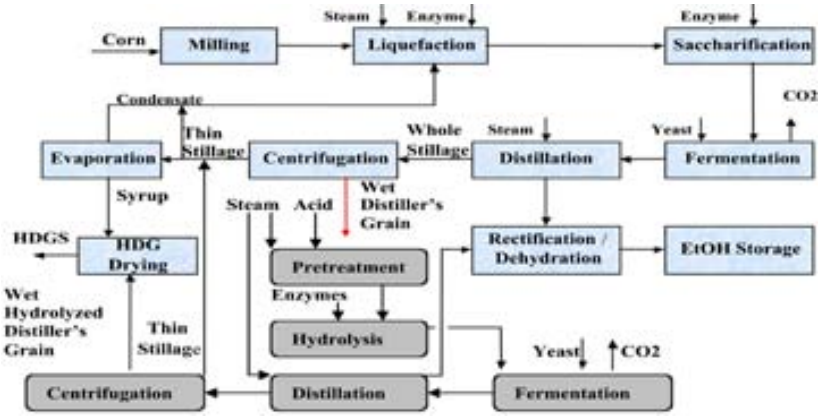

Fig. 2. Dry-grind process with pretreatment option for production of HDG.

Sciences at the University of Minnesota comparing six Minnesota dry mill ethanol plants concluded that crude protein content of DDG and DDGS ranged from 28.7 to 31.6 % *(4)*. Additional findings from this study confirm that the lysine, phosphorous, and fat content of DDG or DDGS vary considerably among the corn dry-grind ethanol producers. Crude protein content is one of the currently accepted baseline indicators for essential amino acid content *(4)*. As a result, quality control (QC) programs for DG residues that include protein content have begun to be implemented in several dry mills. Programs and services, such as those at the University of Minnesota DDGS QC program, offer certification to dry-grind ethanol plants that produce DG residues that meet established standards *(5)*. Certification allows dry-grind ethanol plants to command a higher price for their DG residue.

Figure 1 shows an elementary schematic diagram for process flows within a typical dry mill operation. A proposed process for converting the residual starch and fiber in wet DG into ethanol and HDG and hydrolyzed distiller's grain with solubles (HDGS) is shown schematically in Fig. 2.

Modifications to an existing dry mill plant include a pretreatment section added after the first centrifugation step, in which dilute acid and steam are used to convert the residual starch and fiber in wet DG into fermentable sugars. Next, an enzymatic hydrolysis step is inserted to convert oligomeric sugars freed by pretreatment to fermentable monomeric sugars, which are sent to a secondary fermentation step. Whole stillage from the second distillation column is sent to a second centrifugation step for separation into concentrated HDG and thin stillage. Finally, concentrated HDG is sent to the dryer and combined with evaporated thin stillage syrup to form the dried, higher-protein-content HDGS.

Producing higher-quality HDG and HDGS products requires overcoming several technical challenges. A major challenge is pretreatment with sufficient severity to allow solubilization of the carbohydrate fraction from both the residual starch and the fiber while limiting protein degradation and acid catalyst consumption. Neutralization of the acid in the fermentation section results in salt formation, which can be detrimental to animals fed these residues. The most difficult hurdle for producing high-quality HDGS is minimizing deleterious Maillard reactions between amino acids and reducing sugars during pretreatment *(6)*. Pretreatment of corn fiber residues from corn wet-milling operations has been found to generate fewer Maillard reactions as a consequence of the lower protein content in corn fiber residues *(7–13)*. Maillard reactions are known to lead to sugar and essential amino acid losses, produce enzyme inhibitors and degradation products, cause undesirable color changes in products, and impart off flavors that some animals (especially poultry) are sensitive to *(14–16)*. Several strategies can be employed to reduce Maillard reactions in pretreatment of DG feedstocks, including reducing the protein content of wet DG feedstocks prior to pretreatment *(17)*, reducing pretreatment severity by lowering the concentration of acid catalyst, reducing reaction temperature and residence times, and sulfation of the sugars with SO_2 during pretreatment. Opportunities also exist for reducing browning and charring during the drying step by incorporating new filter technologies to replace energy-intensive dryers (S. Benesi, personal communication, April 30, 2003).

Pretreatment of DG followed by fermentation was shown to increase ethanol yield and may be of economic benefit for a corn dry-grind ethanol producer. The resulting HDG was shown to be low in fiber with increased digestible protein content, which may increase markets for this residue. Preserving the "high-quality" protein in the residues in pretreated DG is the major challenge facing the incorporation of HDG and HDGS production into the typical dry mill. Several of the unit operations proposed in the modified dry mill (Fig. 2) process were tested at the bench or pilot scale, and the results are reported herein. Animal feeding trials with turkey poults were also conducted to determine the effects of pretreatment on residual protein "quality" in HDG in relation to growth and other performance parameters. We have shown that increases in ethanol yield are possible

from pretreatment of DG, and that a lower-fiber, high-protein residue is produced that can be successfully incorporated into nonruminant animal feeds. The turkey poults were used as a model animal, and the feeding trial results suggest that HDG may be used as a high-protein feed supplement in other nonruminant animals.

Materials and Methods

Feedstock

Three 55-gal drums of wet DG (28.7 wt% total solids) were received at the National Renewable Energy Laboratory (NREL) via overnight shipment from a centrifuge in the production line of a dry-grind ethanol plant. The material was spread as soon as received on rubber-coated tarpaulins in a cool (~10°C) enclosed building and partially air-dried for 4 d using fans. The spread wet DG was mixed twice per day to promote even drying to a final concentration of approx 74.5 wt% solids. The partially air-dried DG was sieved to pass through a 6-mm screen with the large pieces broken up before screening. Thorough mixing of the screened material was achieved by the method of cone and quarter mixing three times prior to acid impregnation and pretreatment. The partially air-dried DG was stored at 4°C for a few days prior to completing acid impregnation. Pretreatment was completed within 2 wk of receiving the wet DG. The results of the solids compositional analysis of the mixed, partially air-dried feedstock are given in Table 1. The drying and sieving of DG was performed to ensure even acid distribution in the acid impregnation step, described next. Note that drying DG may not be necessary for acid impregnation using industrial high-speed mixers such as pug mill mixers.

Acid Impregnation

Impregnation of DG with SO_2 was accomplished by injecting a known weight of SO_2 gas into a sealed polyethylene bag containing DG. For dilute-H_2SO_4 impregnation, the acid solution was sprayed on 3 kg of partially air-dried DG while mixing in a 30-qt bread dough mixer. Tests using a red dye and salt tracer with the bread dough mixer were promising, showing an even distribution of both the dye and salt tracer. Preparing acid-impregnated feedstock for the poultry feeding trial required mixing 42 individual batches with acid using the bread dough mixer, with all batches combined and mixed by the method of cone and quarter mixing before pretreatment. Approximately 176 kg of feedstock at approx 50 wt% solids and 1.9 wt% H_2SO_4 in the liquid fraction was obtained. H_2SO_4 concentrations used in the pretreatment experiments given in Table 2 were determined by titration with NIST traceable NaOH solutions.

Pretreatment

Pretreatment screening experiments of partially air-dried DG were carried out in both a 4-L steam explosion reactor *(18,19)* and a 4-L

Table 1
Survey by NREL of Composition of Corn Dry-Grind DG (wt%)

Sample	Crude protein	Total carbohydrates	Nonstarch glucan	Starch	Galactan	Mannan	Xylan	Arabinan	Extractives	Acetyl	Total ash	Acid insoluble residue
Plant A	35.2	39.0	12.4	5.60	2.2	1.5	10.1	7.2	18.5	1.6	2.6	3.2
Plant B	34.0	38.2	10.6	5.80	2.2	1.6	10.5	7.4	18.4	1.6	2.4	3.2
Plant B [a]	32.0	41.0	11.0	7.30	2.3	1.5	11.2	7.7	20.0	1.5	2.6	3.2
Plant C	31.2	41.2	11.5	6.20	2.4	1.4	11.7	7.9	15.7	1.7	2.8	3.2
Plant D	31.9	41.2	12.1	6.30	2.3	1.6	11.0	7.7	13.8	1.5	2.5	4.3
Air Dried DG [b]	33.9	42.7	12.1	8.40	2.2	7.4	10.2	7.4	20.3	1.4	2.7	3.1
Pretreated DG	42.8	30.3	15.7	2.80	1.4	2.4	6.9	2.4	ND [c]	ND [c]	0.5	20.1
HDG [d]	60.6	14.0	4.5	0.82	1.0	1.8	4.6	1.8	22.6	0.6	1.2	12.6

[a] Sample taken at same plant months later.
[b] Partially dried to 74.5% solids.
[c] ND, not detected.
[d] Used for feeding trials.

Table 2
DG Pretreatment Conditions, Yields, and Conversions

Experiment	Reactor[a]	Catalyst (wt%)	Time (min)	Temp (°C)	CS[b]	Glucose yield (%)[c]	Xylose yield (%)[c]	Total soluble sugar yield (% total carbohydrates in DG)[c]
1	SG	Steam	20	160	2.35	14.1 (2.6)	17.1 (2.1)	20.4 (8.7)
2	SG	SO_2 (2%)	15	160	2.42	25.8 (4.7)	35.9 (4.3)	36.2 (15.5)
3	SG	H_2SO_4 (1.1%)	5	185	2.35	41.5 (7.2)	57.4 (6.5)	50.1 (20.4)
4	ZC	H_2SO_4 (3.27%)	20	140	2.17	65.1 (13.0)	93.4 (11.7)	77.2 (35.0)
5	ZC	H_2SO_4 (3.27%)	30	140	2.34	65.1 (11.2)	77.3 (8.7)	68.9 (28.0)
6	ZC	H_2SO_4 (3.27%)	40	140	2.76	47.6 (9.5)	50.7 (6.4)	49.0 (22.2)
7	ZC	H_2SO_4 (3.27%)	12	150	2.23	59.1 (11.8)	90.2 (11.3)	70.1 (31.7)
8	ZC	H_2SO_4 (3.27%)	16	150	2.35	59.5 (11.9)	86.3 (10.8)	71.3 (32.3)
9	ZC	H_2SO_4 (3.27%)	20	150	2.42	59.0 (11.8)	75.7 (9.5)	65.9 (29.8)
10	SG	H_2SO_4 (3.27%)	12	150	2.35	59.3 (11.8)	85.9 (10.8)	73.2 (33.2)
11	SG	H_2SO_4 (3.27%)	16	150	2.23	55.2 (11.0)	76.5 (9.6)	64.5 (29.2)
Production	SG	H_2SO_4 (1.9%)	8	160	2.10	47.5 (9.8)	57.2 (5.8)	54.7 (24.1)

[a] SG, 4-L steam explosion reactor (steam gun); ZC, 4-L Zipperclave stirred reactor.
[b] $CS = \text{Log}_{10}(R_o) - \text{pH}$; $R_o = t_r \cdot \exp[(T_r - 100)/14.75]$.
[c] Parentheses indicate g of soluble sugar yield/100 g of dry input feedstock.

Zipperclave® stirred reactor (Autoclave Engineers, Erie, PA). The Zipperclave reactor was equipped with an anchor-type mixer and a 2.5-L Hastelloy® pail to reduce condensate accumulation in the pretreated slurry. The pretreatment screening conditions used in the two reactors are presented in Table 2. Comparisons of sugar yield obtained from various pretreatment conditions are simplified using the severity concept of combining time and temperature *(20,21)*. The combined severity factor (CS) used in our study also includes acid concentration and is represented as $CS = \log_{10}(R_o) - pH$ *(22)*, with reaction ordinate (R_o) defined as $R_o = t_r \cdot \exp[(T_r - 100)/14.75]$. The factor t_r is defined as reaction time (min), and T_r is the reaction temperature (°C). In our study, the value of pH used in the CS calculation is from the measurement of pH in the pretreated slurry after pretreatment. No attempt was made to estimate the pH during the reaction time course within the feedstock at temperature because the dynamics of acid dilution with steam condensate and activity of the acid within the feedstock during pretreatment were not known. Producing HDG for conducting the poultry-feeding trials required the pretreatment of 104 individual 1.7-kg acid-impregnated batches using the 4-L steam explosion reactor. All pretreated batches were mixed together prior to fermentation.

The H_2SO_4 concentration was decreased to 1.9 wt% for the HDG production run to further reduce the amount of salt formed during neutralization of acid in the fermentation step. This was done because animals (especially poultry) become distressed if they are given feed containing high salt concentrations (S. Noll, personal communication, 2002). In addition to lowering the acid loading, the CS of the production experiment was lowered from the optimum found during the pretreatment screening experiments to reduce the possibility of excessive protein hydrolysis, and protein as well as sugar losses through Maillard degradation reactions.

Fermentation

An initial set of fermentation screening experiments was carried out using pretreated DG (Table 2, experiment 4) to test for fermentation inhibitors, the efficacy of various commercial enzymes in hydrolyzing the oligomeric sugars formed during pretreatment, and the benefits of coculture fermentation. Fermentation screening experiments consisted of duplicate 100-mL working volume flasks with bubble traps and solids loading of 10 wt% pretreated DG. A cellulase enzyme loading of approx 15 filter paper units (FPU)/g of cellulose using Iogen cellulase enzyme preparation (lot no. BRC191095; Iogen, Ottawa, Canada) was added to the pretreated DG residue. This was supplemented with four commercially available glucoamylase and xylanase enzymes at 20 times the manufacturer's recommended dosage to ensure that the level of enzyme loading was not limiting. Xylanase MX® and Xylanase XL® preparations from Genencor (Richmond, CA) and UHP Xylanase® preparation from Iogen were used to test the efficacy of these enzymes on residual xylan in pretreated DG. The glucoamylase preparation, Distillase L-400® (Genencor), was used to hydrolyze residual starch

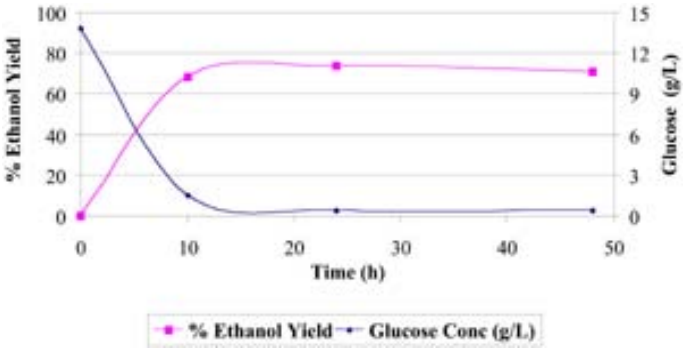

Fig. 3. Ethanol yield from pilot-scale fermentation of pretreated DG. Starch and cellulose in the pretreated DG slurry (10% [w/w]) were saccharified using glucoamylase and cellulose enzymes for 24 h prior to fermentation to ethanol using *S. cerevisiae* D_5A yeast at 32°C, pH 5.0, and 75-rpm agitation.

and amylodextrins. Prior to inoculation of the flasks with yeasts, enzyme cocktails were added and incubated with the solids at 48°C for 24 h and 150 rpm in a shaking incubator to hydrolyze residual carbohydrates in the flasks. Three sets of flasks were run as cocultures using the yeasts *Saccharomyces cerevisiae* D_5A (ATCC 200062) and *Pichia stipitis* strain NPw9 (ATCC PTA-3717) *(23)*, and a D_5A control. The flasks were supplemented after saccharification with corn steep liquor (CSL) to 1 wt% and then inoculated with yeasts (10% [v/v]) at an optical density at 600 nm (OD_{600nm}) of 0.50 for D_5A and 0.48 for NPw9. The fermentation conditions for the screening experiments were 32°C, pH 5.0, and 150 rpm in the shake flasks.

Pilot-Scale Fermentation

HDG was obtained from an 809-L fermentation of pretreated DG. The pretreated DG was diluted in a 1450-L fermentation vessel to a 10 wt% slurry. Enzymatic saccharification of the pretreated DG slurry was performed at 48°C for 24 h using an enzyme cocktail with a cellulase (Iogen) loading of 15 FPU/g of cellulose and a glucoamylase (Genencor Distillase L-400) loading of 0.08 times the weight of starch in the solids (Table 1). After saccharification, the 809-L fermentation was cooled to 32°C and CSL was added to 1 wt%. The yeast *S. cerevisiae* D_5A was inoculated using an 80-L culture at an OD_{600nm} of 0.50 (~10% [v/v] final). Fermentation was carried out at 32°C and pH 5.0, with agitation set at 75 rpm, and air overlay at an airflow rate of 50 L/min, with backpressure set to 0.1 bar (10 kPa). The results of the pilot-scale fermentation are presented in Fig. 3. Samples for enzymatic saccharification steps were taken at 0 and 24 h, and postinoculation fermentation samples were taken at 0, 10, 24, and 48 h. Samples were analyzed for sugars, ethanol, and organic acids using NREL's Laboratory

Table 3
Protein Mass Balance for HPG Production

Operation	Protein (wt%)[a]	Protein balance (% recovery)
Feedstock	33.9 [b]	100.0
Pretreatment	41.7	108.0 [c]
Fermentation	42.8	99.9
Centrifuged solids	55.6	53.1 [d]
Supernatant	23.2	25.4
Final feed	60.6	56.0 [e]

[a] Protein was measured using the crude protein method *(25)*.
[b] Analysis of the percentage of protein may be low, leading to mass balance error.
[c] This amount is used as 100% protein mass from feedstock for subsequent calculations.
[d] This mass includes solids from Pneumapress filter and Boch centrifuge.
[e] The amount shown here is larger than that from the centrifuge because we processed the supernatant twice through the centrifuge.

Analytical Procedures *(24)*. Crude protein analyses on the combined solid/liquid fraction of the 0- and 48-h postinoculation samples were performed using carbon–hydrogen–nitrogen analysis *(25)* with a protein conversion factor of 6.25 × nitrogen content.

Solid/Liquid Separation

The spent fermentation broth from the pilot-scale fermentation was separated in a Bock centrifugal extractor, model 775 (Bock, Toledo, OH) operating at 1725 rpm (~1600*g*). Approximately 86 kg of solids (36 wt% solids) was collected. The supernatant was recycled through the centrifuge again to remove additional solids. A small quantity of spent broth was filtered using a Pneumapress® model 3-C-276 automatic pressure filter (Pneumapress Filter, Richmond, CA) to test the benefits of new filtration technologies. Solids collected using the Pneumapress filter (~50% moisture) were mixed with the centrifuged solids and dried (~93 wt% solids) in a pan-type forced hot-air oven by an outside laboratory (Hazen Research, Golden, CO) using air heated to a maximum of 45°C. The low-temperature air-drying step was specified to reduce Maillard reactions.

The dried fermentation residues were broken up to pass through a 6-mm screen and mixed three times by the method of cone and quarter mixing. The dried and screened HDG (~32 kg) was available for poultry-feeding trials (Table 3). The supernatant from the centrifugation step and filtrate from the filter belt were not evaporated to form concentrated syrup to be added to the dried HDG to form HDGS because of lack of pilot-scale equipment to accomplish the evaporation step.

Table 4
Comparison of NRC (1994) Corn and Corn Coproducts vs NREL HDG[a]

Parameter	Corn (4-02-935)[b]	DDG (5-28-235)[b]	DDGS (5-28-236)[b]	HDG (NREL)
DM (%)	89.0	97.0	93.0	95.86
ME (kcal/kg)	3350	1972	2480	2008
TME (kcal/kg)	3470	—	3097	2566
Protein (%)	8.5	27.8	27.4	57.8
Ether extract (%)	3.8	9.2	9.0	14.6
Linoleic acid (%)	2.2	—	4.55	—
Crude fiber (%)	2.2	12.0	9.10	3.90
Calcium (%)	0.02	0.10	0.17	0.02
Total phosphorus (%)	0.28	0.40	0.72	0.22
Nonphytate phosphorus (%)	0.08	0.39	0.39	0.11
Potassium (%)	0.30	0.17	0.65	0.19
Chlorine (%)	0.04	0.07	0.17	0.03
Sodium (%)	0.02	0.09	0.48	0.16
Magnesium (%)	0.12	0.25	0.19	0.07
Manganese (%)	0.0002	0.0022	0.0024	0.00
Aluminum (%)	—	—	—	0.00
Iron (%)	0.0045	0.0300	0.028	0.04
Zinc (%)	0.0018	0.0055	0.008	0.00
Copper (%)	—	—	—	0.00
Ash (%)	—	—	—	1.43
Starch (%)	—	—	—	1.60
Sugars (%)	—	—	—	<2.0

[a] Data from NCR reports for standardized poultry rations *(26).*
[b] International feed no.

Poultry-Feeding Trials

Poultry-feeding trials were carried out in two phases as follows.

Phase 1

Initial feed ingredient evaluation and compositional characterization were carried out for HDG using proximate analyses by an outside laboratory for protein, fat, fiber, ash, moisture, mineral composition (major and trace elements), starch, and sugars and were compared to standard reference feed materials (National Research Center [NRC], 1994 methods) *(26)* (Table 4). An amino acid profile was obtained on HDG and compared to standard reference corn and corn coproduct feeds to determine the digestible amino acids present in the HDG feed (Table 5). In vivo metabolizable energy (ME) and digestible amino acids were determined by feeding a known quantity of HDG to eight cecatomized roosters (Table 4). The feces were quantitatively collected for a 48-h time period after feeding, and amino acids and digestibility were based on quantitative measurement of input feed and output feces. Endogenous secretions were corrected for by having eight nonfeed roosters. True metabolizable energy (TME) was similar to ME, except that intact turkeys were used (Table 4). Excreta were freeze-dried, ground, and analyzed for nitrogen and gross energy content.

Table 5
Amino Acid and Digestible Amino Acid Composition of Soybean Meal, NRC Feeds, and HDG

	Amino acid as % of protein					Disgetible amino acid as % of protein			
	Composition (NRC 1994)					Composition (NRC 1994)			
	Soybean meal (5-04-612)	Corn (4-02-935)	DDG (5-28-235)	DDGS (5-28-236)	NREL HDG Analyzed [a]	Soybean meal (5-04-612)	Corn (4-02-935)	DDGS (5-28-236)	NREL HDG Analyzed [a]
Aspartic acid					5.20				3.75
Threonine	3.94	3.41	1.76	3.38	3.28	3.46	2.87	2.44	2.47
Serine	5.22	4.35	2.52	5.92	3.56	11.70			
Glutamic acid					14.87				11.70
Proline					7.60				6.14
Glycine	4.32	3.88	1.76	2.10	2.99				
Alanine					6.44				5.27
Cysteine	1.52	2.12	0.86	1.47	2.00	1.24	1.80	1.13	1.56
Valine	4.67	4.71	4.24	4.78	4.76	4.25	4.14	3.87	3.66
Methionine	1.41	2.12	1.44	2.21	2.08	1.30	1.93	1.85	1.78
Isoleucine	4.46	3.41	3.56	3.68	3.79	4.15	3.00	3.09	2.94
Leucine	7.87	11.76	10.83	8.09	12.15	7.24	10.94	7.20	10.08
Tyrosine	4.11	3.53	3.02	2.72	4.19				3.61
Phenylalanine	4.93	4.47	3.38	0.74	5.29	4.53	4.07	0.65	4.46
Histidine	2.69	2.71	2.23	2.43	2.26	2.37	2.54	1.82	1.74
Lysine	6.23	3.06	2.81	2.76	1.99	5.67	2.48	1.79	1.35
Arginine	7.33	4.47	3.49	3.60	2.66	6.74	3.98	2.27	2.10
Tryptophan	1.56	0.71	0.72	0.70	0.39				0.25

[a] International feed numbers are given in parentheses.

Table 6
Composition of Poult Diets

Ingredient (%)	Incorporation of HDG				
	Control (%)	5%	10%	15%	20%
Corn, ground	39.49	38.32	37.10	35.88	34.65
Soybean meal[a]	50.38	45.92	41.46	37.01	32.56
Poultry byproduct meal	4.0	4.0	4.0	4.0	4.0
HDG	0.0	5.0	10.0	15.0	20.0
Dicalcium phosphate[b]	2.35	2.35	2.35	2.35	2.35
Calium carbonate	1.14	1.14	1.14	1.14	1.14
Sesquicarb (sodium bicarbonate)[b]	0.152	0.157	0.179	0.200	0.222
Salt	0.250	0.229	0.194	0.160	0.125
99% DL-Methionine	0.175	0.138	0.101	0.064	0.027
L-Lysine HCl	0.000	0.067	0.177	0.287	0.396
Starter vitamin mix	0.35	0.35	0.35	0.35	0.35
Prestarter vitamin mix	0.08	0.08	0.08	0.08	0.08
Trace mineral mix	0.12	0.12	0.12	0.12	0.12
60% Choline chloride	0.175	0.175	0.175	0.175	0.175
Animal fat	1.34	1.96	2.57	3.19	3.80
Total	100.00	100.00	100.00	100.00	100.00

[a] Protein content of soybean meal is 47 wt%.
[b] Dicalcium phosphate, calcium carbonate, sodium carbonate, trace mineral, and vitamins.

Phase 2

Feed ingredient evaluation was carried out at low levels of HDG inclusion to test the effects of this protein source on viability, organ weight gains, and other measures of turkey performance. A corn-soybean meal–based diet with some meat and bone meal was used as the control. HDG was incorporated into the diet (replacing corn and soybean meal) at levels of 5, 10, 15, and 20%. Diets were formulated to provide similar levels of ME, lysine, methionine, calcium, and phosphorus. In addition, all of the poult diets contained the necessary vitamins and trace minerals, salt, and added fat (choice white grease or tallow). Major ingredients (corn, soybean meal, and meat bone meal) were analyzed prior to the start of the trial. Mixed diets were analyzed for protein content. The composition of feed rations fed to the turkey poults are given in Table 6.

Prior to the start of the feeding trial, newly hatched poults (420 male commercial turkey poults) were randomly placed into 42 cage battery brooder units. All poults received the control starter diet up until 3 d of age. At d 3 each poult was wing banded, weighed, and approx 20% of the poults (heaviest and lightest) was discarded. The remaining 350 poults were sorted into 50 cages (7 poults/cage) with each bird of approximately equivalent body weight. Each diet was fed to 10 replicate cage units from 3 to 21 d of age. Body weights were taken at 3, 7, 14, and 16 d of age. Feed intake records for each pen were kept. No mortality was found. At the end of the trial, two poults per pen were randomly selected, weighed, and euthanized using

approved procedures, and the weights of internal organs (spleen, heart, liver, gastrointestinal tract, bursa) were recorded. Analysis of variance was conducted to determine the effect of HDG inclusion in the feed on the probability of treatment differences for each of the measured criteria on poult performance and organ characteristics.

Results and Discussion

Composition

The compositional variation of samples of wet DG received from a number of different corn dry mill ethanol plants is given in Table 1. Two of the entries show the variation between samples of wet DG removed from the production line centrifuge of a dry-grind ethanol plant several months apart. The crude protein content was found to vary among the different plants from 31.2 to 35.2%. These results can be compared with those of a study of six Minnesota dry-grind ethanol plants conducted by the Department of Animal Sciences at the University of Minnesota that found crude protein content of DDG and DDGS from these plants ranging from 28.7 to 31.6 % *(4)*. This variation is of some concern to animal feedlot operators.

Table 3 provides the increase in protein content as a result of the pretreatment of DG with dilute H_2SO_4, followed by enzymatic hydrolysis and fermentation of the pretreated DG. The residual protein content in the HDG was found to increase from approx 33.9 to 60.6 wt%. Analysis by an independent laboratory places the crude protein content of the HDG produced at 57.8% (Table 4).

Pretreatment

The sugar yields and sugar conversion recoveries for the dilute-acid-pretreated DG are given in Table 2. Sugar yields and conversions were low in steam pretreatment (experiment 1) without the addition of acid catalysts, indicating a recalcitrant form of starch and fiber in the DG. However, when SO_2 and dilute-acid impregnation were not homogeneous throughout the feedstock before pretreatment, soluble sugar yields and conversions suffered, as shown for experiments 2 and 3. Visual investigation of the pretreated slurries from experiments 2 and 3 clearly showed areas of partial pretreatment, indicating nonhomogeneous acid impregnation. Higher yields and conversions were found in pretreatment experiments 4 through 11 and the production experiment when a bread dough mixer was used for acid impregnation.

Gravimetric mass balance closure around each pretreatment experiment in Table 2 ranged from 95 to 100%, with an average of 96.7 ± 1.9% (data not shown), indicating losses owing to unaccounted for volatile components. The Zipperclave reactor showed the highest level of solubilization of residual carbohydrates, suggesting that internal mixing and direct steam injection enhances pretreatment kinetics within the Zipperclave reactor. Less mixing of the acid-impregnated feedstock with steam within the steam

explosion reactor was expected because it lacks an impeller mixer such as that found in the Zipperclave, thus decreasing performance. The highest total soluble sugar yield was found using the Zipperclave reactor, where approx 77% of the carbohydrates available in the DG was solubilized (experiment 4). The soluble xylose yield from the hemicellulosic fraction in experiment 4 was found to be 93.4%, while 65% of the available glucan was solubilized during this pretreatment. Approximately 35 g of total soluble sugar was produced/100 g of dry DG under these pretreatment conditions. The steam explosion reactor gave slightly lower total soluble sugar yields. At the maximum, approx 73% of available carbohydrate was solubilized with pretreatment using this reactor (experiment 10). The soluble xylose and glucose yields were slightly lower in the steam explosion reactor for experiment 10, compared with the stirred Zipperclave reactor, giving 85.9 and 59.3%, respectively. The steam explosion reactor produced a maximum of 33.2 g of total soluble sugar/100 g of dry DG. However, the mechanical simplicity of the steam explosion reactor, compared with the Zipperclave reactor with mixer, is of economic advantage because of lower capital and operating costs associated with this design.

Pretreating wet DG with SO_2 (experiment 2) resulted in higher solubilization of residual carbohydrates than uncatalyzed steam explosion (experiment 1), however, higher yields and conversions are possible if homogeneous absorption of the acid gas can be accomplished. Careful examination of the SO_2-pretreated residue indicated nonhomogeneous pretreatment because the SO_2 appears to have been absorbed only at the surface of the particles and did not penetrate far into the interior. However, less expensive pretreatment reactors are possible if SO_2 is used as the catalyst, as opposed to dilute-acid pretreatment, because exotic alloys may not be needed.

Consistent steam explosion reactor performance was found during the 104-batch production experiment, with uniform temperatures measured throughout the production run. However, DG feedstock baked onto the internal walls of the reactor and charring occurred over the extended period of time needed to produce the required quantities of pretreated DG. The baked-on material was collected and analyzed separately for mass. Overall gravimetric mass balance for the production run was lower, 93.3%, as a result of this charring, compared to the screening experiments, in which approx 97% mass recovery was found. Removing corn oil prior to pretreatment may decrease this tendency of the feedstock to stick to, and bake on, the walls of the pretreatment reactor. Total soluble sugar recoveries of 24.1 g/100 g of dry input material were found for the production experiment. These recoveries were less than those found in the screening experiments for both the Zipperclave and steam gun reactors (Table 2). This may be owing to the less severe pretreatment conditions chosen to reduce protein hydrolysis and Maillard reactions, nonhomogeneous acid impregnation, and nonuniform heat transfer effects caused by charring on the walls of the reactor. Homogeneous acid impregnation is one of the technical challenges limiting yields for the conversion of DG.

Fermentation

Fermentation screening studies using two different yeasts showed that pretreated DG does not contain significant levels of inhibitors (data not shown). Screening shake-flask fermentations were complete in 17 h, with ethanol yields reaching 82% from all available hexose sugars in the pretreated DG. Crude protein levels increased from 43 to a high of 58 wt% (data not shown). The combined cellulase and glucoamylase enzymes were effective in increasing the amount of soluble glucose, while the three commercial xylanases were ineffective in solubilizing residual xylan with this pretreated material (high-performance liquid chromatography data not shown).

The NREL *Pichia* strain NPw9 was equivalent to the D_5A yeast in ethanol production; however, it did not utilize xylose under the anaerobic conditions used in the screening experiments (data not shown). However, the *Pichia* strain NPw9 has been shown to ferment xylose in toxic pretreated softwood hydrolysates under microaerophilic conditions *(23)*.

Saccharification of the residual carbohydrates in the pretreated DG slurry for the pilot-scale production experiment increased the glucose concentration from 4.4 to 15.1 g/L in 24 h at 48°C. Following inoculation with D_5A yeast, the initial glucose was consumed in 10 h with ethanol concentration reaching 8.81 g/L at 24 h (Fig. 3) with 73% ethanol yield from glucan and starch. Mannose, galactose, arabinose, and xylose were not utilized by *S. cerevisiae* D_5A in this fermentation (data not shown), thus lowering the ethanol yield from available hexose and pentose sugars. A different microorganism would be needed to utilize these sugars and increase ethanol yields. The ethanol concentration decreased slightly after 48 h, suggesting that the residual cellulose in the HDG was not digestible by the cellulase enzyme after the initial saccharification period.

Feeding Trial

Analysis of the international NRC standardized (1994) *(26)* corn (4-02-935), DDG (5-28-235), and DDGS (5-28-236) feeds, and NREL HDG indicates that HDG had the highest crude protein content, with high levels of glutamine, alanine, proline, and asparagine (Table 4). With the exception of lysine, the amino acid content of HDG was in the range reported for the NRC standardized corn, DDG, and DDGS feeds *(26)*. More important, the in vivo metabolically determined relative digestibility and percentage of digestible amino acids indicate that amino acid digestibility is in the range reported for international corn, DDG, and DDGS feeds. The exception is lysine, for which the percentage of composition in the crude protein was reduced along with the availability. Typical amino acid digestibility coefficients for soybean meal are 95%. This reduction in availability may have occurred during pretreatment in the steam gun, which was conducted at 160°C for 8 min. Other essential amino acids (e.g., histidine, arginine, cysteine, methionine, and tryptophan) demonstrated reduced digestibility. However, factoring in the amount and percentage of availability of these amino acids in HDG, the range of values is equal to or greater than those

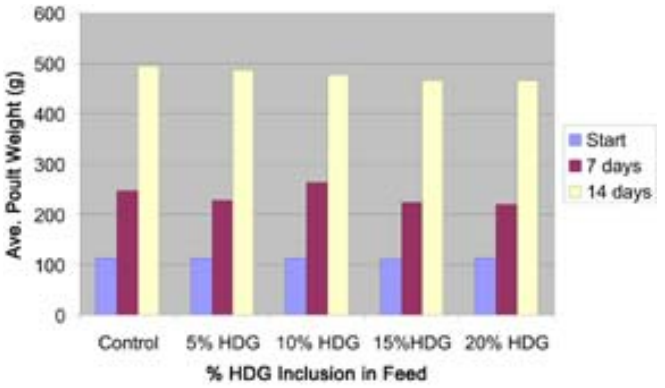

Fig. 4. Average turkey poult weight gain over 14 d with inclusion of HDG in diet.

reported for the NRC feeds. We speculate that including nutrients from evaporated supernatant from the solid-liquid separation step to produce HDGS may reduce the amino acid compositional differences between HDG and the NRC feeds reported in Tables 4 and 5.

The preliminary results reported in Fig. 4 from the 16-d feeding trial of poults are promising. These poults consumed the blended material without separating out the HDG from the base feed ration, their weight gain tracked the control group, there was no mortality in the group, there was no toxicity of HDG to the poults. Small (–6%), but significant differences in the total weight gain between the control group and the experimental group incorporating HDG in the feed at the 15 and 20% level were found (Fig. 4).

Economic Analysis

A preliminary economic analysis performed early in the project before pretreatment testing began showed that if the protein content and type, and hence value, of the hydrolyzed DG was comparable to soybean meal (estimated at 52 wt% with a value of $200/dry t), and the ethanol yield was at least 92% of all hexose sugars available in the DG feed, the payback period for the additional process equipment needed could be as short as 2 yr. The effects of adding additional capital equipment for conversion of DG to an existing corn dry-grind ethanol plant are presented in Table 7. Three preliminary process scenarios are presented that include new equipment for dilute-acid pretreatment, fermentation, distillation, and cellulase enzyme costs. A hot water pretreatment process scenario is also included to show the effects of issues regarding pretreatment reactor materials of construction on costs of the overall process. Adding additional solid/liquid separation equipment increases the costs. The pilot-scale fermentation achieved 73% theoretical yield from available hexoses, lower than the 92% target; however, the overall initial results are very promising and suggest that additional research could result in an economically viable process to convert DG into ethanol and a higher-value

Table 7
Effects of Additional Ethanol Production on Minimum Ethanol Selling Price for Various Pretreatment and Process Options on Existing 25 Million Gallon Per Year Dry-Grind Ethanol Plant

Process	Capital investment ($million)	Annualized capital cost ($) (capital charge factor, 0.17)	Minimum ethanol selling price ($/gal ethanol) Conversion of hexose to EtOH[a] 80%	84%	88%	92%
Basic using hot water pretreatment[b]	10.5	1,784,142	0.86	0.84	0.83	0.81
Basic using dilute-acid pretreatment	10.9	1,860,225	0.90	0.88	0.87	0.85
Basic using dilute-acid pretreatment and solid/liquid separation	11.5	1,954,394	0.94	0.92	0.90	0.88
Additional ethanol (gal/yr)			2,060,606	2,499,186	2,560,200	2,638,917

[a] Conversion of all hexose monomers, meaning glucose, galactose, and mannose.
[b] Basic processing refers to DG pretreatment, simultaneous saccharification and fermentation with cellulase enzyme and *Saccharomyes*, and ethanol recovery via distillation.

animal feed. The annual production of ethanol could increase an additional 2.0–2.6 million gal/yr.

Conclusion

Pretreatment of wet DG with dilute H_2SO_4 was shown to solubilize significant (~77%) amounts of carbohydrates from the wet DG. Pretreatment with SO_2 may give equivalent results if homogeneous absorption of the acid gas can be accomplished. Less expensive reactors are possible when using SO_2 as the catalyst. Homogeneous acid impregnation of wet DG feedstocks was found to be a showstopper requirement for obtaining high soluble sugar yields and recoveries.

Fermentation of pretreated DG slurries is readily accomplished because of the low toxicity of the pretreated slurries. Soluble monomeric sugars (mostly glucose) were fermented to ethanol by the yeasts *S. cerevisiae* D_5A and *P. stipitis* NPw9; however, the soluble oligomeric sugars were not converted by the added enzymes and decreased ethanol yields to 82% of theoretical. The use of cellulase enzymes in the saccharification and fermentation step decreased the nonstarch glucan from ~12 to ~4% in the pretreated DG residue, indicating that the pretreatment enhanced the cellulose digestibility.

The 14-d feeding trial using turkey poults as a model nonruminant animal is promising. These poults consumed the blended material without separating out the HDG from the base feed ration, their weight gain tracked the control group, there was no mortality in the group, no toxicity of the HDG to the poults was observed, and there were no significant differences between the morphology of the control or any of the experimental groups after necropsy. However, small (–6%) but significant differences in the total weight gain between the control group and the experimental group receiving the HDG mix at the inclusion level of 15 and 20% level were found.

Our study successfully demonstrated that increases in ethanol production and increases in protein level of HDG can be achieved with less severe pretreatment of DG and subsequent fermentation of the pretreated DG to produce HDG. The HDG residue had a crude protein content near 61 wt%, comparing favorably with soybean meal, which has a range of 44–52 wt%. The total amount of digestible amino acids available to turkey poults in HDG was comparable to NRC feeds *(26)*, with the exception of the levels of lysine.

Preliminary economic analysis of the process indicates economically feasible scenarios for the incorporation of pretreatment and fermentation of wet DG into existing corn dry-grind ethanol plants. The production of high-quality, high-protein animal feed and the additional production of 2.0–2.6 million gal of ethanol could lead to a payback period of 2 yr. The payback period and projected rate of return depend heavily on the "quality" of the high-protein animal feed and the price per ton that the market might be willing to pay for this new product. Other factors that can affect the economics include ethanol yield and whether all the hexose and pentose sugars can be utilized in fermentation.

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