Hydrolyzed Distiller's Grain Production, Fermentation and Animal Feeding Trials

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1. Abstract

•Ethanol production in the United States has increased to more than 2.3 billion gallons per year (2002) and expected to reach five billion gallons per year in the United States by 2012. The simultaneous co-production of 4.5 million tons per year of distiller's grain (DG) is expected to drive down the price of DG as a cattle feed supplement. To increase market penetration and help stabilize prices, dry mill ethanol producers are seeking ways to improve the quality of DG. One possible improvement is to increase the protein content of DG by converting the residual starch and fiber to ethanol.

•Methods were developed for steam explosion, SO_2, and dilute sulfuric acid pretreatment of DG as a feedstock for ethanol production and animal feeding trials. Pretreating DG at 140°C for 20 min, with 3.27% H_2SO_4, solubilized approximately 77% of the available carbohydrate, 65% of available glucan, and 93% of available xylan. Fermentation protocols for pretreated DG were developed at the bench-scale and scaled up to an 800-L fermentation.

•The air dried hydrolyzed distiller's grain (HDG) was provided to the Animal Science Laboratory at the University of Minnesota for turkey feeding trials. Including HDG in turkey poult diets at 5% and 10% levels (replacing corn and soybean meal), showed weight gains in the birds similar to controls, while 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 amongst the poults was found.

•The high protein levels (~57%) available in HDG show promising economics for incorporating this process into corn dry mill ethanol plants.

2. Introduction

- In 2001, 56 ethanol plants were in production in the U.S. with nearly a dozen more expected on line by end of 2002.
- Cornstarch fermentation to EtOH represents ~95% of current production in U.S., with two-thirds using the corn dry mill process.
- Each bushel of corn produces an estimated 2.5–2.7 gallons of ethanol, 17.5 pounds of dried distiller's grain (DDG), and 17 pounds of CO_2 in corn dry mill process.
- Current production of ~2 million tons DG/year expected to rise to 4.5 million tons/year.
- Present DDG and DDGS animal feed markets are not expected to absorb increases in DG production without certain price erosions.
- Use of starch and fiber in DG would increase ethanol production, and result in higher protein content hydrolyzed distiller's grain (HDG) residues.
- In order to compete with soybean meal in animal feed markets, high protein content HDG will need to be shown to contain high quality, digestible protein as well.
- Incorporation of new filter technologies to replace expensive dryers can be expected in the near future to lower energy costs.

3. Materials and Methods

- Dilute-acid impregnation of DG with bread dough mixer
- DG Pretreatment screening experiments
  - With ZipperClave and Steam Gun reactors
  - Steam, SO_2, and dil. H_2SO_4 (1.1%–3.3%)
  - 140°C – 185°C
  - 5 min–40 min residence times
- Production of HDG for turkey feeding trial
  - Wet distiller's grain (DG) obtained from 50 MM gal/year corn dry mill plant
  - Pretreated at 160°C, 1.9% H_2SO_4, 8 min for production of pretreated DG
  - 800-L fermentation of pretreated DG with cellulase, glucoamylase and *S. cerevisiae* D_5A followed by centrifugation to produce HDG
  - Hydrolyzed DG air dried at 45°C
  - Hydrolyzed DG fed to turkeys

4. Materials and Methods

Turkey Feeding Trial

Phase 1

- Characterization of HDG using proximate analyses for protein, fat, fiber, ash,moisture, minerals (major and trace elements), starch and sugars.
- HDG amino acid profile determined.
- *In vivo* metabolizable energy (ME) and digestible amino acids is determined by feeding a known quantity of ingredient to 8 cecatomized roosters, collecting and measuring feces.
- Endogenous secretions are corrected for by having 8 non-feed roosters.
- *In vivo* true metabolizable energy (TME) determined using intact turkeys. Excreta are freeze-dried, ground and analyzed for nitrogen and gross energy content.

Phase 2

- Feed ingredient evaluation is carried out at low levels of HDG inclusion to test this protein source on viability, organ weight gains, and other effects on turkey performance.
- A corn-soybean meal based diet with some meat and bone meal is used as the control.
- HDG was incorporated into the diets (replacing corn and soybean meal) at levels of 5, 10, 15, and 20%.
- Diets were formulated to provide similar levels of metabolizable energy, lysine, methionine, calcium, phosphorus, 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.

Figure 1. Dry Mill Process with Wet Distiller's Grain Conversion

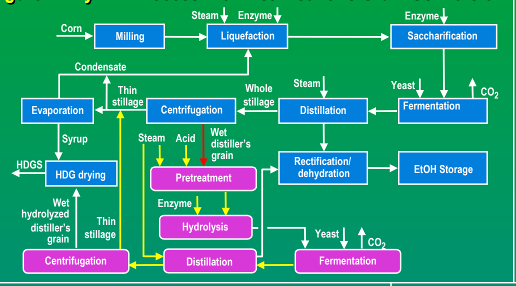


Table 1. Wet DG Composition

Component	% Wt
Glucan	18.47
Xylan	9.00
Galactan	1.96
Arabinan	6.5
Mannan	2.18
Acid Soluble Fiber	20.31
Acid Insoluble Fiber	3.09
Starch	8.41
Ash	2.7
Acetic Acid	1.44
Protein	33.8
Mass Balance	99.52

*Glucan includes starch

Figure 2. Acid Impregnation — Key Pretreatment Parameter


30 qt. bread dough mixer


DDG sprayed with red dye

Figure 3. Pretreatment Reactors


4-1 Zipperclave


4-1 Steam Gun

Table 2. Wet DG Pretreatment Yield and Conversions

Exp.	Reactor	Catalyst Concentration (wt-%)	Time (min)	Temp. (°C)	Combined Severity (CS)*	Glucose Yield (%)**	Xylose Yield (%)**	Total Soluble Sugar Yield (% sugars available in DG)**
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)
Prod.	SG	H_2SO_4 (1.9%)	8	160	2.10	47.5 (9.8)	57.2 (5.8)	54.7 (24.1)

*CS = $\log_{10}(Ro) - pH$; $Ro = t_r \cdot \exp[(T_r - 100)/14.75]$ **Parenthesis indicates g soluble sugar yield per 100 g dry input feedstock
+4-L steam explosion reactor (steam gun) ++4-L Zipperclave stirred reactor

Figure 4. Ethanol and HDG Production Using Pretreated DG

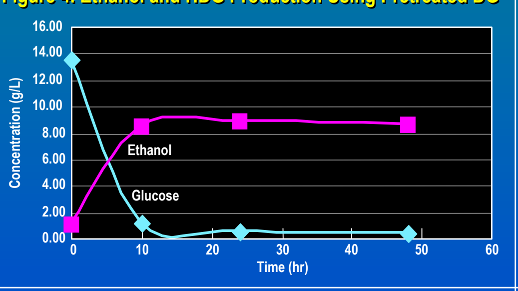


Table 3. Amino Acid Profile of Pretreated DG

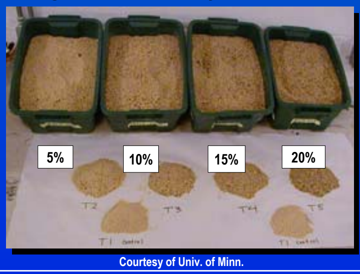
	Amino Acid (%)	HDG % ODW	#2 Digestibility coeff. (% by AA)	Digestible amino acid %ODW	%N in AA	Weighted %N
1	Asp	3.1	72.1	2.3	10.52	0.24
2	Thr	2.0	75.2	1.5	11.76	0.17
3	Ser	2.1	75.2	1.6	13.33	0.21
4	Glu	9.0	78.7	7.1	9.52	0.67
5	Pro	4.6	80.8	3.7	12.17	0.45
6	Gly	1.8		0.0	18.66	0.00
7	Ala	3.9	81.9	3.2	15.72	0.50
8	Cys	1.2	78.3	0.9	11.56	0.11
9	Val	2.9	76.9	2.2	11.96	0.26
10	Met	1.3	85.9	1.1	9.39	0.10
11	Ile	2.3	77.6	1.8	10.68	0.19
12	Leu	7.3	82.9	6.1	10.68	0.65
13	Tyr	2.5	86.2	2.2	7.73	0.17
14	Phe	3.2	84.3	2.7	8.48	0.23
15	His	1.4	77.1	1.0	27.08	0.28
16	Lys	1.2	68.1	0.8	19.16	0.16
17	Arg	1.6	79.0	1.3	32.16	0.41
18	Trp	0.2	64.0	0.2	13.72	0.02
Average			77.9			
St dev			6.8			
Total		51.5		39.5		4.8

Table 4. Composition of Feed Rations

Ingredient (%)	Treatment Control	5% HDG	10% HDG	15% HDG	20% HDG
CORN	39.4878	38.3225	37.0999	35.8772	34.6545
SOY013	50.3782	45.9162	41.4631	37.01	32.5569
meat	4	4	4	4	4
HDG	0	5	10	15	20
dical.phos*	2.35	2.35	2.35	2.35	2.35
ca.carb*	1.14	1.14	1.14	1.14	1.14
scarb*	0.1521	0.1567	0.1785	0.2002	0.222
salt	0.2503	0.2289	0.1942	0.1596	0.1249
dl.meth.99*	0.175	0.1379	0.1009	0.064	0.027
l.lys.hcl*	0	0.0673	0.1769	0.2865	0.3962
fse129.st*	0.35	0.35	0.35	0.35	0.35
fse141.st*	0.08	0.08	0.08	0.08	0.08
fse.tm.pm	0.12	0.12	0.12	0.12	0.12
choline60	0.175	0.175	0.175	0.175	0.175
ani fat	1.3416	1.9555	2.5715	3.1875	3.8035
Total	100	100	100	100	100

*Di-calcium phosphate, calcium carbonate, sodium carbonate, trace minerals and vitamins.

Figure 5. HDG Turkey Feed Rations



Courtesy of Univ. of Minn.

Table 5. Weight Gain of Turkeys During Feed Trial

Dietary Treatment	Body weight (g) at: Start	7 days	14 days
1. Control	113	246	495
2. As 1 incorporating 5% HDG	113	228	487
3. As 1 incorporating 10% HDG	113	236	478
4. As 1 incorporating 15% HDG	112	225	466
5. As 1 incorporating 20% HDG	113	219	466
Pvalue for Treatment Effect	0.67	0.02	0.18
LSD (P<.05)	2	16.5	29.3

5. Results

- Figure 1 shows schematic diagram for corn dry mill process with HDG production and wet DG conversion.
- Figure 2 shows homogenous mixing using bread dough mixer.
- Table 1 shows solids composition of wet DG used in this study.
- Table 2 shows soluble glucose, xylose, and total soluble sugar yields and conversions using steam, SO_2, and H_2SO_4 pretreatment of DG.
- Table 3 lists the amino acid profile, digestibility (in turkeys), and availability of amino acids in pretreated DG
- Figure 4 shows ethanol and HDG production from fermentation of pretreated DG.
- Table 4 lists composition of feed rations fed to turkey poults.
- Table 5 shows weight gains of turkey poults at 2 weeks fed with inclusion of 5%, 10%, 15%, and 20% HDG.
- No mortality or negative effects on the internal organs of poults.

Table 6. Effect of Additional Ethanol Production on MESP*

Processing Extent	Process Scenario Number	Capital Investment ($MM)	Annualized Capital Cost (capital charge factor 0.17)	Base Case* 80% EtOH yield	Base Case 84% EtOH yield	Base Case 88% EtOH yield
Existing 25MM gal/yr EtOH plant			Additional gal/yr	2,060,606	2,499,186	2,560,200
				Cost per Gallon	Cost per Gallon	Cost per Gallon
Dilute Acid PT, Ferm, Cellulase, Dist	1a	$10.9	$1,860,225	$0.90	$0.88	$0.87
Hot Water PT, Ferm, Cellulase, Dist, S/L Sep	2a	$11.0	$1,878,311	$0.90	$0.88	$0.86
Dilute Acid PT, Ferm, Cellulase, Dist, S/L Sep	3a	$11.5	$1,954,394	$0.94	$0.92	$0.90

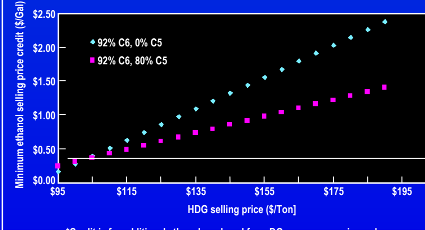
1a New capital to include new equipment for acid pretreatment, fermentation, distillation, and cellulase enzyme cost.

2a New capital cost for HW pretreatment, fermentation, distillation, cellulase an additional solid/liquid separation equipment.

3a 2a substitution of acid pretreatment for HW pretreatment capital cost.

*Minimum ethanol selling price

Figure 6. Minimum Ethanol Selling Price Credit* vs. HDG Selling Price



*Credit is for additional ethanol produced from DG sugar conversion only

6. Conclusions

- Pretreatment of wet DG with steam, SO_2, and H_2SO_4 results in high soluble sugar yields and conversions of available carbohydrate
- Fermentation not inhibited by pretreated DG slurry.
- High protein content (>57%) in HDG
- Low residual starch and fiber in HDG
- Little difference in weight gain of turkey poults with inclusion of 5% and 10% HDG in diet versus control
- Inclusion of 15% and 20% HDG in diet shows slight (~6%) difference in weight gain versus control
- Preliminary process economics indicate that pretreatment of HDG can reduce MESP
- Production of higher quality HDG, with elevated protein content can command higher selling price

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