Ethanol yields and elevated amino acids in distillers dried grains with solubles from maize grain with higher concentrations of essential amino acids

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Abstract

Concentrations of the essential amino acids, lysine and tryptophan, present in corn distillers dried grains with solubles (DDGS) are insufficient to fully meet the nutritional requirements in non-ruminant feeds. Mutations known to increase lysine and tryptophan concentrations were

combined by breeding into two maize hybrid backgrounds that differ for grain protein concentration (FR1064 x Mo17 normal protein, FR1064 x IRHP1 high protein). These mutant hybrids were compared to their non-mutant control isolines for field performance and ethanol and DDGS processing using laboratory scale procedures: conventional dry grind process and modified dry grind process using granular starch hydrolyzing enzyme (GSHE).

The mutant hybrid grain contained higher concentrations of a number of amino acids, with notable increases to at least 0.55% w/w lysine and 0.18% w/w tryptophan. Due to lower grain yield and starch concentrations, final ethanol yields of FR1064 x Mo17: *o2; asaC28* hybrid were reduced compared to control for both the conventional and modified dry grind processes. However, with the modified process, ethanol yield for FR1064 x IRHP1: *o2; asa2-C28* hybrid was similar to its control isoline. DDGS yields increased for both mutant hybrids compared to controls. Although amino acid profiles were similar for DDGS recovered from both the conventional and modified processes, DDGS from the mutant hybrids contained higher concentrations of lysine (1.48-1.70% w/w) and tryptophan (0.32-0.39% w/w) compared to controls (1.09% w/w lysine, 0.22% w/w tryptophan). Using these mutant corn hybrids at an ethanol plant resulted in lower ethanol yields; however, this loss can be recovered with the higher DDGS yield and increased nutritional value of the DDGS.

1. Introduction

In 2015, total ethanol and animal feed production were 14.7 billion gallons and 40 million metric tons, respectively (RFA 2016). Majority of the DDGS produced in the US is used as an ingredient in diets pertaining to dairy and beef cattle (76%) owing to its high fiber content; whereas, only a small percentage is used in poultry (8%) and swine (15%) diets (RFA 2016).

Improvements in the fuel ethanol production processes are adapted by ethanol plants to expand the existing markets for DDGS (Rausch and Belyea, 2006). For example, fractionation technologies, elusieve process, enzymatic milling (E-mill), quick germ quick fiber (QGQF) processes have been developed to recover additional co-products and increase the nutritional value of DDGS (Singh et al., 1999 and Radhakrishnan et al., 2005). Martinez-Amezcu et al. (2007) compared nutritional value of DDGS recovered from conventional dry grind process to modified processes such as elusieve and QGQF processes. Crude protein content of DDGS samples from modified processes were higher compared to conventional DDGS. However, amino acid contents (lysine, threonine and tryptophan) for all the DDGS samples were similar. DDGS properties from a modified process (E-mill process) were evaluated and compared to conventional DDGS (Kim et al., 2010). It was reported that E-mill DDGS had higher amino acid contents, amino acid digestibilities and true metabolizable energy (TME) compared to the conventional DDGS (Kim et al., 2010). Jacela et al. (2014) investigated the nutritional value of high protein DDGS (recovered from dry fractionation process) for swine diet formulations. It was reported that both amino acid concentrations and amino acid digestibility were higher for high protein DDGS compared to traditional DDGS. Low oil DDGS produced after extracting oil from DDGS is another valuable co-product with higher crude protein content and lower oil content (Saunders and Rosentrater, 2009).

Using specialty corn hybrids in the dry grind process is another approach to increase the profitability of ethanol plants. Sharma et al. (2007) investigated the effects of using different corn types such as waxy corn, high amylose corn and normal dent corn in the dry grind process. Waxy corn resulted in the highest ethanol yields and fastest fermentation rates; whereas, high amylose corn gave lowest ethanol yields. This was attributable to the high amount of resistant

starch due to the high percentage of amylose in high amylose corn (Sharma et al., 2007 and 2010). Another example of a specialty corn hybrid is the Quality Protein Maize (QPM, reviewed in Vasal, 2000) or high protein quality corn (HPQC) which was developed to improve the protein quality of corn. Lysine and tryptophan are the two most limiting essential amino acids in corn grain. These amino acids are added as supplements to animal feeds (Lysine in broiler diets and both lysine and tryptophan in swine diets) produced using bacterial fermentations which is expensive (Prasanna et al., 2001). There are few reported studies on the effect of using such corn hybrids in the dry grind process. Wu (1986) compared the fermentation properties of using a high lysine corn hybrid (SL 75) to normal dent corn in the dry grind process. Compared to high lysine corn, dent corn resulted in higher ethanol yields. High lysine fermentation products (distillers grains; distillers grains with solubles) contained higher lysine and tryptophan contents compared to the corresponding dent corn fermentation products.

The main goal of this study was to evaluate the potential of using certain mutant corn hybrids with high lysine and tryptophan contents in the dry grind process. The hybrids selected were double mutants containing the *opaque-2* mutation (*o2*) and a feedback-insensitive form of the alpha subunit of anthranilate synthase (*asa2-C28*). The *o2* mutation was initially described by Mertz et al. (1964) as reducing accumulation of zein proteins, leading to higher amounts of available lysine and tryptophan, and is the main genetic factor contributing to Quality Protein Maize and other HPQC genotypes. The *asa2-C28* mutation was first identified in a screen for maize tissue cultures capable of growing in the presence of the toxic tryptophan analog 5-methyltryptophan, which were subsequently regenerated into plants with elevated levels of free tryptophan in seeds and reduced feedback inhibition of anthranilate synthase by Trp (Hibberd et al., 1986). Later work identified a single nucleotide change in the ASA2 gene encoding the alpha

subunit of anthranilate synthase that conditions the same methionine to lysine mutation associated with loss of feedback inhibition of anthranilate synthase in a number of organisms (Anderson et al., 2000).

One of the concerns with using conventional dry grind process was the high temperature liquefaction treatment ($> 82^{\circ}\text{C}$) which might result in destruction of lysine. Therefore, the hybrids were also processed using a modified dry grind process using granular starch hydrolyzing enzyme (GSHE). This is a one-step fermentation process where GSHE is added along with the yeast. GSHE converts starch to dextrins at low temperatures $\leq 48^{\circ}\text{C}$ and hydrolyzes dextrins to sugars to be converted into ethanol. Specific objectives of this study were to evaluate the fermentation properties (rates and ethanol yields) of these mutant corn hybrids using conventional and modified dry grind processes and to evaluate the nutritional value of DDGS recovered from both the processes and compare them with their control isolines.

2. Materials and methods

2.1. Genetic materials and grain yield measurements

The *o2-R* mutant allele (Schmidt et al., 1987) was introgressed via backcrossing into the B73 and Mo17 inbred lines followed by selfing to homozygosity by Robert J. Lambert at the University of Illinois. The B73: *o2* inbred line was used as the donor for introgression into the FR1064 inbred line (Illinois Foundation Seeds) by backcrossing six times followed by selfing to homozygosity of *o2*, where the presence of *o2* allele was tracked by observation of the mutant phenotype among kernels of self-pollinated ears from the same plants used as male parents in each backcross generation. Similarly, the Mo17: *o2* inbred line was used as the donor for introgression into the Illinois Reverse High Protein1 (IRHP1) inbred line described in Uribe-larrea et al. (2004). The *asa2-C28* mutation was obtained from seed initially deposited with

In Vitro International (subsequently transferred to ATCC but no longer maintained) and introgressed into each of the FR1064, IRHP1, and Mo17 inbred lines via at least six backcrosses followed by selfing to homozygosity. The *asa2-C28* allele was tracked during the breeding program using the molecular marker assay described in Anderson et al. (2000). To generate double mutants for *o2* and *asa2-C28*, the converted inbred lines for each individual mutation were crossed to each other, self-pollinated, and progeny plants homozygous for both mutations identified using visual selection for *o2* and the molecular marker assay for *asa2-C28*. The double mutant inbred lines were then crossed to each other to generate hybrid seeds for field evaluation.

A set of four hybrids were evaluated in this study: FR1064 x Mo17: *o2*; *asa2-C28* (FMo2C28) and its control non-mutant FR1064 x Mo17 isoline (FM), as well as FR1064 x IRHP1: *o2*; *asa2-C28* (FRHo2C28) and its control isoline (FRH). The four hybrids were grown in each of three replicated field plots during 2012 at the Crop Sciences Research and Education Center located near the University of Illinois campus in Urbana, IL. Each plot was 5.3 meters long by 3 meters wide, in four rows with 0.76 meter spacing, and received pre-plant applications of 200 kg N fertilizer per hectare (as 28% urea ammonia nitrate) and GuardsmanMax herbicide (dimethenamid and atrazine, BASF, Florham Park, NJ). Plots were planted on May 5 at 77,800 plants per hectare with in-furrow tefluthrin soil insectide (ForceG, Syngenta, Greensboro, NC). They received 2.5 cm of overhead irrigation just prior to flowering, and five ears were harvested by hand from the middle portion of the center two rows of each plot on October 5 (14% grain moisture). Bulkied shelled grain from each of the four hybrids were hand cleaned using a 12/64" (4.8 mm) sieve by removing the broken corn and foreign material (BCFM). Cleaned corn samples were ground using a hammer mill (1100W, model MHM4, Glen Mills, Clifton, NJ) equipped with 0.5 mm sieve. Moisture content of ground corn samples were determined using a

standard two stage oven method (Approved Method 44-19, AACC International 2000). Corn samples were processed using two laboratory scale processes: conventional dry grind procedure and modified dry grind procedure using granular starch hydrolyzing enzyme.

2.2. Enzymes and other materials

All the enzymes used in this study were obtained from Dupont Industrial Biosciences (Palo Alto, CA, U.S.A). In the conventional procedure, Spezyme CL, Distillase and Fermgen enzymes were used as alpha amylase, glucoamylase and protease enzymes, respectively. Spezyme CL consists of a thermostable alpha amylase and was obtained from a genetically modified strain of *Bacillus licheniformis*. It had an activity of 15,225 AAU/g (AAU = Alpha Amylase Units), a specific gravity (sg) of 1.17 g/mL and an optimum pH range of 5.5 to 6.5. Distillase consists of amylase (1,4- α -D-105 glucan glucanohydrolase - EC 3.2.1.1), glucoamylase (1,4- α -D-glucan hydrolase – E.C. 3.2.1.3) and Aspergillopepsin I (EC 3.4.23.18). These enzymes were obtained from genetically modified strains of *Trichoderma reesei*. It had an activity of 380 GAU/g (GAU = Glucomylase Units), a sg of 1.10 to 1.14 g/mL and an optimum pH range of 4.0 to 4.5. Fermgen (sg = 1.12 to 1.20 g/mL) was an acid protease enzyme obtained from *Trichoderma reesei* with a declared activity of 1000 SAPU/g (SAPU = Spectrophotometric Acid Protease Units). Optimum pH range was 4.0 to 5.0. In the modified process, GSHE Stargen 002 along with protease enzyme, Fermgen, were used. Stargen 002 consists of alpha amylase from *Aspergillus kawachi* and a glucoamylase from *Trichoderma reesei* and had an activity of 570 GAU/g. Specific gravity and optimum pH range were 1.13 to 1.16 g/mL and 4.0 to 4.5, respectively.

Active dry yeast (Ethanol Red, Lesaffre Yeast Corp., Milwaukee, WI) was used for fermentation. Urea stock solution (50% w/v from 99.6% ACS grade) obtained from Fisher Scientific (Fair Lawn, NJ) was prepared and used as a nitrogen source for yeast.

2.3. Conventional dry grind process

Ground corn (100 g dry basis, db) was mixed with water to obtain a 30% slurry. Sulfuric acid (1N) was added to adjust pH to 5.8 for liquefaction process. The slurry was incubated at 85°C for 90 min for liquefaction with Spezyme CL (25 µL/100 g db). Before simultaneous saccharification and fermentation (SSF), pH of liquefied mash samples was adjusted to 4.3 using sulfuric acid (1N). Distillase enzyme (85 µL/100 g db), fermgen enzyme (150 µL/100 g db), urea (0.4 mL) and yeast inoculum (2 mL) were added subsequently. Yeast inoculum was prepared by dispersing 1 g active dry yeast (ADY) in 5 mL of water and incubating at 32°C and 30 min. SSF was carried out at 32°C and 120 rpm for 72 h in a shaking water bath (Innova 42, Eppendorf, Enfield, CT). Fermentation process was monitored by withdrawing 1 mL samples at 0, 2, 4, 8, 24, 48 and 72 h, which were then analyzed using HPLC (details of HPLC analyses are given below). Post fermentation, ethanol was evaporated from fermented mash at 90°C for 3 hr. DDGS was produced from the remaining material by subsequent drying in a convection oven at 49°C for 72 hr. DDGS was ground into a fine powder in a kitchen blender and moisture content of ground DDGS was determined (Approved Method 44-19, AACC International 2000). Each experiment was conducted in triplicates.

2.4. Modified dry grind process

Ground corn samples (100 g, db) were mixed with water to form 30% slurry and the pH of slurry was set to 4.0. Stargen 002 (0.5 mL/100 g db), fermgen (150 µL/100 g db), yeast

inoculum (2 mL) and urea (0.4 mL) were added. Slurry was incubated at 32°C and 120 rpm for 72 h in a shaking water bath (Innova 42, Eppendorf, Enfield, CT). Samples (1 mL) were taken at 0, 2, 4, 8, 24, 48 and 72 hr and analyzed using HPLC (details of HPLC analyses are given below). Ethanol evaporation and DDGS preparation was the same as the conventional process. Each experiment was conducted in triplicates.

2.5. Composition analyses

Corn and DDGS samples were analyzed for crude protein content (Method: 990.03, AOAC 2003), crude fat (Method: 920.39, AOAC 2003), and neutral detergent fiber (Van Soest et al., 1991) in a commercial analytical laboratory (Illinois Crop Improvement Association, Champaign, IL). Starch content was determined using acid hydrolysis method (Vidal et al., 2009). Amino acid profiles of corn and DDGS samples were analyzed at a university laboratory (Agriculture Experiment Station, University of Missouri, Columbia, MO) using official methods (Method: 982.30 E [a, b, c], AOAC 2006). All samples were analyzed in duplicates and results are reported in % dry basis (db).

2.6. DDGS pricing

Commercial least cost software was used for a corn-soybean meal broiler starter diet formulation containing 8% DDGS for control (FM) and mutant (FMo2C28) hybrids. The total analyzed amino acid levels in the control and mutant hybrids were multiplied by the digestibility values in the poultry (NRC 1994) for DDGS to calculate the digestible amino acid concentrations in the DDGS from the two hybrids. Thus, it was assumed that digestibility of the amino acids is similar for the DDGS from control and mutant hybrids. These digestible amino acid values were then used to formulate two broiler starter diets, one containing 8% control DDGS (FM) and one

containing 8% DDGS from the mutant hybrid (FMo2C28). Current industry prices for corn, soybean meal, DDGS feed ingredients and methionine, lysine and threonine amino acid supplements were used. DDGS value from the FMo2C28 mutant hybrid was estimated by setting the price of DDGS from the FM control hybrid to the current 130\$/ton and then setting the price of DDGS from the FMo2C28 mutant hybrid to a high value of 200\$/ton so that it would not come into the feed formula. The computer software then generated a shadow price for the DDGS from the mutant hybrid, which is the price at which this ingredient would economically come into the formula in place of the DDGS from the control hybrid. This shadow price indicates the relative economic value of the DDGS from the mutant hybrid relative to the economic value of the DDGS from the conventional hybrid. In addition, the total cost for two broiler diets formulated using 8% DDGS from control and mutant hybrids was estimated by using the price of both types of DDGS samples set at 130\$/ton.

2.7. HPLC analyses

Samples collected after SSF were centrifuged at $9,279 \times g$ (Model 5415 D, Brinkmann - Eppendorf, Hamburg, Germany) for 5 min and supernatant liquid was passed through 0.2 μm syringe filters (Nylon Acrodisc WAT200834, Pall Life Sciences, Ann Arbor, MI) into 150 μL vials. The resulting filtrates were analyzed to determine carbohydrate and ethanol concentration by HPLC, using an ion exclusion column (Aminex HPX-87H, Bio-Rad, Hercules, CA) as the stationary phase maintained at 50°C. Glucose, ethanol, oligosaccharides and organic acids, such as acetic and lactic acid, were eluted from the column using 5 mM sulfuric acid as a mobile phase with flow rate of 0.6 mL/min. Separated components were detected by a refractive index detector (model 2414, Waters Corporation, Milford, MA). Standard solutions for maltodextrins (DP4+) (0.44% w/v), maltotriose (DP3) (0.50% w/v), maltose (2.0% w/v), glucose (2% w/v),

fructose (1% w/v), succinic acid (0.50% w/v), lactic acid (1.0% w/v), glycerol (2% w/v), acetic acid (0.5% v/v), methanol (1% v/v) and ethanol (20% v/v) were prepared and used to calibrate the column.

2.8. Data analyses

Fermentation rates were determined using the linear region (8 to 24 hr) of fermentation profiles. Analysis of variance (ANOVA) and Fisher's least significant difference test (SAS 9.4 version) were used to compare means of ethanol concentrations, glucose concentrations and composition analyses (protein, starch, oil, fiber and amino acid contents). The level selected to show the statistical significance in all the cases were 5% ($P < 0.05$).

3. Results and discussion

3.1. Ethanol concentrations and fermentation rates from control and mutant hybrids

Final ethanol concentration for mutant hybrid FMo2C28 was 16.7 and 16.1% v/v with conventional and modified processes, respectively. These were lower than their control isoline (FM), 18.5 and 17.8% v/v with conventional and modified processes, respectively. For the FRH hybrid background, final ethanol concentration (16.3% v/v) for the FRHo2C28 mutant hybrid was lower than its control isoline (FRH, 17.4% v/v) with conventional process; however, they were similar with modified process (Fig 1 and Fig 2). Lower ethanol concentrations for the FMo2C28 hybrid can be attributed to the lower starch content compared to its control isoline (Table 1). As expected from prior work (Uribe-larrea et al., 2004), the FRH hybrid produced higher grain protein concentration than the FM hybrid, with only minor reductions in starch concentration. However, unlike the FM background, the *o2* and *asa2-C28* mutations did not change grain protein concentration in the FRH background and only slightly reduced starch

concentrations, which is also reflected in a much smaller reduction in grain yield for the mutants in the FRH compared to FM background (Table 2). These results suggest that although higher protein genotypes may show decreased yields compared to current commercial hybrids (represented by the FM background), they may not be as sensitive to the reductions in grain and ethanol yields often observed for improvements in nutritional quality traits.

It was expected that the higher amino acid levels in mutant corn hybrids might act as nutrition source for yeast and result in faster fermentation rates compared to the control hybrids. However, fermentation rates for mutant isolines (0.72 and 0.70% v/v/hr for FMo2C28 and FRHo2C28, respectively) were similar to their control isolines (0.75 and 0.70% v/v/hr for FM and FRH, respectively) with conventional process. Mutant isolines had lower fermentation rates (0.46 and 0.50% v/v/hr for FMo2C28 and FRHo2C28, respectively) compared to their control isolines (0.52 and 0.58% v/v/hr for FM and FRH, respectively) with modified process (Fig 1 and 2). Residual glucose concentrations were negligible at the end of fermentation (72 hr) for all hybrids, suggesting that the fermentation was complete (Figs 3 and 4).

Processes had an effect on final ethanol concentrations for corn hybrids, FM and FMo2C28 (Fig 1). Conventional process resulted in higher ethanol concentration compared to modified process. For the FRH control and mutant hybrids, both conventional and modified processes resulted in similar final ethanol concentrations. Fermentation rates for all the hybrids were higher with conventional process compared to modified process. The reason for the faster fermentation rates and ethanol concentrations with conventional process compared to modified process is not clear. It could be due to specific starch structural features such as amylose/amylopectin ratios or starch-protein matrix governing the accessibility of starch to enzymes. It is known that conventional process result in higher ethanol concentrations and faster

fermentation rates compared to GSHE process for samples containing higher amylose content (Sharma et al., 2007). High temperature treatment hydrolyzes the hydrogen bonds and makes amylose more accessible to enzymes to be hydrolyzed to sugars (Sharma et al., 2007). Glucose profiles were different for conventional and modified processes (Fig 3 and 4). This was due to the different enzymatic action of GSHE enzyme compared to conventional dry grind enzymes.

Overall ethanol yields/acre were lower for mutant isolines (1146 and 1023 L/acre for FMo2C28 and FRHo2C28, respectively with conventional process) compared to their respective control isolines (1467 and 1165 L/acre for FM and FRH, respectively). Similar results were obtained with the modified process (Table 2). This was mainly due to the lower overall grain yield for mutant hybrids compared to their respective control isolines. However, we point out that the hybrids evaluated here are experimental genotypes to demonstrate proof of concept, and that ongoing breeding of the *o2* and *asa2-C28* mutations into more current elite germplasm is likely to increase yields of the mutant hybrids. None of the hybrids evaluated in this study contain any transgenic traits for herbicide or insect resistance, which may increase yields, but also may be an advantage for either non-GMO or organic markets. Furthermore, the 2012 growing season was exceptionally hot and dry, although irrigation was used to supplement water availability, yields for all hybrids were significantly reduced relative to what has been observed for these hybrids in more typical conditions (150-180 bushels per acre).

3.2. Amino acid profiles of corn and DDGS

Total lysine and tryptophan contents in the FMo2C28 corn hybrid were 0.62 and 0.24% w/w, respectively. For the FRHo2C28, total lysine and tryptophan contents were 0.55 and 0.18% w/w. These were higher than their respective control isolines (Table 3). Crude protein content of DDGS from the FMo2C28 hybrid was higher compared to its FM control with conventional and

modified processes (Table 4). Higher crude protein content was indicative of higher amino acid contents in DDGS from FMo2C28 compared to FM. Total lysine contents in DDGS from FMo2C28 were 1.69 and 1.70% w/w with conventional and modified processes, respectively and were higher compared to their control isolines (1.09% w/w) (Table 5). Similarly, total tryptophan contents in DDGS from FMo2C28 were 0.39 and 0.32% w/w with conventional and modified processes, respectively and were higher than their respective FM control (0.22 and 0.20% w/w with conventional and modified processes, respectively) (Table 5). Amino acids, valine, arginine, histidine, aspartic acid, threonine, isoleucine, tyrosine, glycine and cysteine were also higher in DDGS from FMo2C28 compared to its FM control. Total lysine and tryptophan contents were higher in the FRHo2C28 mutant compared to the FRH control (Table 6). However, overall crude protein content of the FRHo2C28 hybrid was not different from FRH as other amino acids such as glutamic acid, leucine, phenylalanine and methionine were lower in the FRHo2C28 mutant hybrid compared to the FRH control (Table 6). Wu (1986) reported similar results where they found higher lysine levels in different fermentation products from high lysine corn (SL 75) compared to dent corn. Amounts of lysine in distillers grains (DG), centrifuged solids from thin stillage (CS) and distillers grains with solubles (DGS) were reported to be 4.2, 6.1 and 4.4 g/16 g of nitrogen from high lysine corn. For dent corn, lysine amounts were 2.9, 5.5 and 3.1 g/16 g of nitrogen in DG, CS and DGS, respectively (Wu 1986).

Processes did not have an effect on crude protein content of FM (30.3 and 29.6% with conventional and modified processes, respectively) or FMo2C28 (37.0 and 37.6% with conventional and modified processes, respectively). Protein contents of DDGS for the FRH and FRHo2C28 hybrids were significantly different with modified process compared to conventional process; however, the actual difference was quite small ($\leq 2.3\%$). Total lysine and tryptophan

contents in DDGS samples for each hybrid were similar with conventional or modified processes. This indicated that the high temperature liquefaction treatment in the conventional dry grind process did not affect the lysine levels in corn.

3.3. DDGS yields

DDGS yields from mutant FMo2C28 and FRHo2C28, were 37.5 and 36.0% db, respectively with conventional process and 36.6 and 34.1% db, respectively with modified process. These were higher than their respective control isolines (Conventional process: FM – 31.2% db, FRH – 32.9% db; Modified process: FM – 30.8% db, FRH – 31.9% db) (Table 4). Higher DDGS yields from the mutant hybrids was due to the lower starch content and higher amount of non-fermentable substrates (protein or neutral detergent fiber) present initially in these corn samples (Table 1).

3.4. Economic analyses at a 100 million gallon per year (MGY) ethanol plant

Overall ethanol and DDGS yields at a 100 MGY ethanol plant from FM and FRH hybrids using the conventional dry grind process were calculated to evaluate the feasibility of using mutant hybrids (FMo2C28 and FRHo2C28) in the plant. Using mutant hybrid FMo2C28 compared to its FM control isoline would result in an ethanol yield loss of 10 MGY and a gain in DDGS yield of approximately 58,700 metric tons/yr (Table 7). Similarly, an ethanol yield loss of 11 MGY and a gain in DDGS yield of approximately 29,180 metric tons/yr was found with mutant hybrid FRHo2C28 compared to its control isoline FRH (Table 7). From the computer software calculations, DDGS from mutant hybrids, FMo2C28 and FRHo2C28 were valued at 168 and 156\$/ton, respectively. These were higher compared to DDGS from FM (by 38\$/ton) and FRH (by 26\$/ton) control hybrids. Based on DDGS value, ethanol and DDGS yields, net

gain in income at the ethanol plant was estimated to be around 6 and 2 million \$/yr for FMo2C28 and FRHo2C28 hybrids, respectively (Table 7).

Using the price of 130\$/ton for DDGS from both control and mutant isolines, total cost of broiler diet formulation for poultry companies was estimated. Total savings of 2.84 and 1.90\$/ton were determined for companies using DDGS from FMo2C28 and FRHo2C28, respectively compared to their control isolines (FM and FRH).

4. Conclusions

Total lysine and tryptophan contents in mutant corn hybrids, FMo2C28 and FRHo2C28 were higher (≥ 1.7 times) than their control isolines. Ethanol concentration of the FMo2C28 mutant hybrid was lower than its control FM isoline from both conventional and modified dry grind processes. For the FRH background, the ethanol concentration of the FRHo2C28 mutant was lower than its FRH control isoline with conventional process. DDGS yields and total lysine and tryptophan contents were higher for mutant hybrids compared to their control isolines. DDGS value from FMo2C28 and FRHo2C28 mutant hybrids were priced 38 and 26\$/ton higher than their control isolines. Using mutant hybrids resulted in a loss in ethanol yield mainly due to lower grain yield compared to their control isolines. However, DDGS value and yields from the mutant hybrids were higher resulting in an overall gain in income at an ethanol plant. This shows promising potential for these mutant hybrids to be used at dry grind ethanol plants.

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Table 1

Composition of corn samples^a

Sample	Starch content (% db)	Protein content (% db)	Oil content (% db)	NDF content (% db)
FM	70.8 a	8.83 b	5.08 a	11.0 a
FMo2C28	63.8 b	13.4 a	4.08 b	11.1 a
FRH	69.9 a	10.0 b	4.02 b	8.86 b
FRHo2C28	66.1 a	10.5 a	4.31 a	11.1 a

^a Composition of control and mutant isolines within each corn hybrid were compared; means followed by same letter in a column are not different ($P < 0.05$).

Table 2

Grain yields and ethanol yields per acre of corn samples

Process	Sample	Grain yield (bu/acre)	Ethanol yield (L/acre)
Conventional Process	FM	140	1467
	FMo2C28	121	1146
	FRH	118	1165
	FRHo2C28	111	1023
Modified Process	FM	140	1412
	FMo2C28	121	1104
	FRH	118	1167
	FRHo2C28	111	1075

Table 3

Amino acid profiles of corn samples^a

	FM	FMo2C28	FRH	FRHo2C28
Taurine	0.00 a	0.07 a	0.00 a	0.00 a
Hydroxyproline	0.03 a	0.03 a	0.03 a	0.03 a
Aspartic Acid	0.66 b	1.19 a	0.66 b	0.88 a
Threonine	0.32 b	0.47 a	0.35 b	0.39 a
Serine	0.37 b	0.50 a	0.42 a	0.41 a
Glutamic Acid	1.56 b	2.09 a	1.93 a	1.56 b
Proline	0.75 b	0.97 a	0.88 a	0.85 a
Lanthionine	0.00 a	0.00 a	0.00 a	0.00 a
Glycine	0.42 b	0.64 a	0.39 b	0.57 a
Alanine	0.63 b	0.81 a	0.79 a	0.67 b
Cysteine	0.22 b	0.30 a	0.20 b	0.24 a
Valine	0.46 b	0.70 a	0.50 b	0.59 a
Methionine	0.17 b	0.22 a	0.22 a	0.20 a
Isoleucine	0.35 b	0.47 a	0.40 a	0.39 a
Leucine	0.98 b	1.16 a	1.33 a	0.89 b
Tyrosine	0.18 b	0.23 a	0.18 b	0.21 a
Phenylalanine	0.42 b	0.57 a	0.52 a	0.46 b
Hydroxylysine	0.01 a	0.02 a	0.01 a	0.01 a
Ornithine	0.01 a	0.02 a	0.01 a	0.01 a
Lysine^b	0.37 b	0.62 a	0.32 b	0.55 a
Histidine	0.29 b	0.46 a	0.28 b	0.34 a
Arginine	0.47 b	0.82 a	0.38 b	0.62 a
Tryptophan^c	0.10 b	0.24 a	0.09 b	0.18 a
Total	8.76	12.6	9.88	10.0

^a Values presented are in % w/w, dry basis.

^{b, c} Amino acids of control and mutant isolines within each corn hybrid were compared; means followed by same letter in a row are not different ($P < 0.05$).

Table 4Yields and composition of DDGS samples^a

Process	Sample	DDGS yield (% db)	Starch content (% db)	Protein content (% db)	Oil content (% db)	NDF content (% db)
Conventional Process	FM	31.2 b	9.44 a	30.3 b	11.9 a	27.2 a
	FMo2C28	37.5 a	6.06 b	37.0 a	9.90 a	24.2 a
	FRH	32.9 b	9.58 a	32.2 a	12.3 b	27.0 a
	FRHo2C28	36.0 a	6.65 b	30.5 a	12.7 a	30.2 a
Modified Process	FM	30.8 b	9.93 a	29.6 b	11.9 a	27.7 a
	FMo2C28	36.6 a	4.36 b	37.6 a	11.3 a	24.1 b
	FRH	31.9 b	7.98 a	33.5 a	13.0 a	24.2 b
	FRHo2C28	34.1 a	3.69 b	32.8 a	13.2 a	31.5 a

^a DDGS yields and composition of control and mutant isolines within each corn hybrid were compared; Means followed by same letter in a column are not different ($P < 0.05$).

Table 5Amino acid profiles of DDGS samples (Hybrids FM and FMo2C28)^a

	Conventional Process		Modified process	
	FM	FMo2C28	FM	FMo2C28
Taurine	0.05 a	0.05 a	0.05 a	0.05 a
Hydroxyproline	0.07 a	0.07 a	0.07 a	0.06 a
Aspartic Acid	1.93 b	2.45 a	1.89 b	2.53 a
Threonine	1.15 b	1.42 a	1.15 b	1.48 a
Serine	1.34 a	1.54 a	1.30 a	1.59 a
Glutamic Acid	4.27 a	4.65 a	4.14 b	4.71 a
Proline	2.38 a	2.50 a	2.26 b	2.51 a
Lanthionine	0.00 a	0.00 a	0.00 a	0.00 a
Glycine	1.21 b	1.73 a	1.20 b	1.82 a
Alanine	2.11 a	2.28 a	2.08 a	2.30 a
Cysteine	0.61 b	0.77 a	0.59 b	0.77 a
Valine	1.53 b	1.96 a	1.44 b	1.97 a
Methionine	0.55 a	0.58 a	0.52 a	0.55 a
Isoleucine	1.25 b	1.39 a	1.19 b	1.41 a
Leucine	3.51 a	3.19 b	3.22 a	3.06 a
Tyrosine	0.99 b	1.13 a	0.96 b	1.12 a
Phenylalanine	1.49 a	1.59 a	1.36 a	1.52 a
Hydroxylysine	0.17 a	0.26 a	0.15 a	0.28 a
Ornithine	0.05 a	0.08 a	0.07 a	0.08 a
Lysine^b	1.09 b	1.69 a	1.09 b	1.70 a
Histidine	0.86 b	1.17 a	0.82 b	1.22 a
Arginine	1.34 b	2.23 a	1.28 b	2.28 a
Tryptophan^c	0.22 b	0.39 a	0.20 b	0.39 a
Total	28.2	33.1	27.0	33.4

^a Values presented are in %w/w, dry basis.^{b, c} Amino acids of control and mutant isolines within each corn hybrid were compared; means followed by same letter in a row are not different ($P < 0.05$).

Table 6Amino acid profiles of DDGS samples (Hybrids FRH and FRHo2C28)^a

	Conventional process		Modified process	
	FRH	FRHo2C28	FRH	FRHo2C28
Taurine	0.05 a	0.05 a	0.03 a	0.06 a
Hydroxyproline	0.06 a	0.09 a	0.08 a	0.09 a
Aspartic Acid	2.05 a	2.19 a	2.13 a	2.28 a
Threonine	1.23 a	1.27 a	1.27 a	1.33 a
Serine	1.49 a	1.34 a	1.53 a	1.40 a
Glutamic Acid	4.92 a	3.64 b	5.08 a	3.77 b
Proline	2.51 a	2.28 a	2.56 a	2.35 a
Lanthionine	0.00 a	0.00 a	0.00 a	0.00 a
Glycine	1.22 a	1.62 a	1.25 b	1.67 a
Alanine	2.40 a	1.98 a	2.46 a	2.05 b
Cysteine	0.60 a	0.63 a	0.61 a	0.65 a
Valine	1.60 a	1.71 a	1.64 a	1.77 a
Methionine	0.64 a	0.53 b	0.67 a	0.53 b
Isoleucine	1.33 a	1.22 a	1.37 a	1.28 a
Leucine	4.08 a	2.50 b	4.14 a	2.56 b
Tyrosine	1.17 a	0.95 a	1.19 a	0.98 a
Phenylalanine	1.66 a	1.31 b	1.69 a	1.35 b
Hydroxylysine	0.16 a	0.21 a	0.15 a	0.25 a
Ornithine	0.04 a	0.06 a	0.05 a	0.07 a
Lysine^b	1.05 b	1.48 a	1.09 b	1.50 a
Histidine	0.81 a	0.88 a	0.85 a	0.94 a
Arginine	1.30 b	1.73 a	1.34 b	1.80 a
Tryptophan^c	0.22 b	0.32 a	0.23 b	0.34 a
Total	30.6	28.0	31.4	29.0

^a Values presented are in %w/w, dry basis.^{b, c} Amino acids of control and mutant isolines within each corn hybrid were compared; means followed by same letter in a row are not different ($P < 0.05$).**Table 7**

Economic analyses at a 100 MGY ethanol plant

Hybrid	Ethanol (million gal/yr)^a	Income (million \$/yr)^b	Loss (million \$/yr)	DDGS (tons/yr)^c	DDGS value (\$/ton)^d	Income (million \$/yr)	Gain (million \$/yr)
FM	100	154	15	285,168	130	37	21
FMo2C28	90	139		343,852	168	58	
FRH	100	154	11	321,031	130	42	13
FRHo2C28	93	143		350,215	156	55	

^a Ethanol from processing 36.1 and 38.3 million bu of corn/yr for FM and FRH hybrids, respectively: Calculated considering ethanol yield/acre from Table 2.

^b Ethanol selling price: 1.54\$/gal.

^c DDGS yield from processing 36.1 and 38.3 million bu of corn/yr for FM and FRH hybrids, respectively.

^d Value predicted using amino acid levels in DDGS and current industry prices (broiler diet containing 8% DDGS).