JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Molecular Structure and Metabolic Characteristics of the Proteins and Energy in Triticale Grains and Dried Distillers Grains with Solubles for Dairy Cattle

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ABSTRACT: To our knowledge, there is no research on the molecular structure of triticale grain in comparison with other types of cereal grains and metabolic characteristics of the protein and energy in this grain and its coproducts, called dried distillers grains with solubles (DDGS), for dairy cattle. The objective of this study was to identify differences in molecular structures of proteins among grains and their DDGS using a molecular spectroscopy technique, namely, DRIFT, and to determine the nutrient profile and supply to dairy cattle. The protein molecular structure studies showed a difference ($P < 0.01$) in the amide I to amide II ratio and the α-helix to β-sheet ratio between grains and their DDGS. The energy content was similar for triticale grain and DDGS. There were differences in the protein and carbohydrate subfractions ($P < 0.05$) and the ruminal degradability of DM, CP, and NDF ($P < 0.01$) between triticale grain and DDGS. Triticale grain and DDGS had similar intestinal digestibility of rumen undegraded CP. However, triticale DDGS had higher ($P < 0.01$) predicted total metabolizable protein and degraded protein balance than triticale, indicating that triticale DDGS is a superior protein source for dairy cattle as compared with triticale grain. Bioethanol processing induced changes in the protein molecular structure.

KEYWORDS: *protein molecular structure, metabolic characteristics of protein, bioprocessing, molecular spectroscopy, triticale, DDGS*

INTRODUCTION

Recent studies have evaluated triticale dried distillers grains with solubles (DDGS) as a feed ingredient for ruminants.[1−5] These studies confirm that inclusion of triticale DDGS does not affect livestock performance. However, information with respect to protein structure on a molecular basis, metabolic characteristics of protein, and digestive behaviors of the protein in triticale grain and DDGS is lacking. This situation is an obstacle to improving the quality of triticale DDGS and to properly formulating animal diets using triticale DDGS.

A database containing molecular structure and nutritional values for triticale grain and DDGS would be helpful to reveal the variation in its nutritional value, help develop improved processing methods, and consequently produce a higher quality of triticale DDGS. In addition, knowledge of rumen undegraded protein (RUP) content, predicted intestinal protein digestibility, and predicted degraded protein balance is crucial to determining the quality of DDGS and should be included in any nutritional evaluation. Expected performance based on modeling animal requirements would be of tremendous value for feed evaluation. The Dutch DVE/OEB system[6] and the NRC-2001 model[7] were developed for such an evaluation with dairy cattle.

Diffuse reflectance infrared Fourier transform (DRIFT) molecular spectroscopy is able to detect structure features or heat-induced changes of inherent structures of biological materials on a molecular basis.[40,41] The hypotheses of this study were that bioethanol processing changed the molecular structure of the proteins in DDGS as compared with the original cereal grain and that these changes in protein could be detected by a molecular spectroscopy technique—DRIFT—as has been previously reported in other cereal.[39,41] In addition, bioethanol processing changes the nutrient content and protein digestive characteristics of triticale DDGS relative to that of triticale grain

The objectives of this study were (1) to identify differences in molecular structures of proteins between triticale grain and DDGS in comparison with two other common cereal grains (wheat and corn) and their DDGS (wheat DDGS, corn DDGS) using molecular spectroscopy, namely, DRIFT; (2) to investigate differences in chemical profiles as well as protein and carbohydrate fractions between triticale grain and DDGS; (3) to determine the ruminal degradation kinetics of various nutrients in triticale DDGS and triticale grain; (4) to detect the effect of bioethanol production on the predicted intestinal availability of the protein in triticale DDGS in comparison with triticale grain; and (5) to estimate the amount of truly absorbable protein in the small intestine using the DVE/OEB system and the NRC-2001 model in triticale and triticale DDGS.

MATERIALS AND METHODS

Triticale Grain and DDGS. Three varieties of spring triticale (Pronghorn, AC Alta, and AC Ultima) and three batches of triticale DDGS (Pronghorn, AC Alta, and AC Ultima) samples were obtained from Dr. T. McAllister, Alberta Lethbridge Research Centre; Dr. M. Oba, University of Alberta; and Dr. G. McLeod, Agriculture and Agri-food Canada. AC Alta triticale was harvested in 2006, and the Pronghorn and AC Ultima triticales were harvested in 2008. The three

Received: June 4, 2012
Revised: September 7, 2012
Accepted: September 11, 2012
Published: September 12, 2012

dx.doi.org/10.1021/jf302382b | J. Agric. Food Chem. 2012, 60, 10064−10074

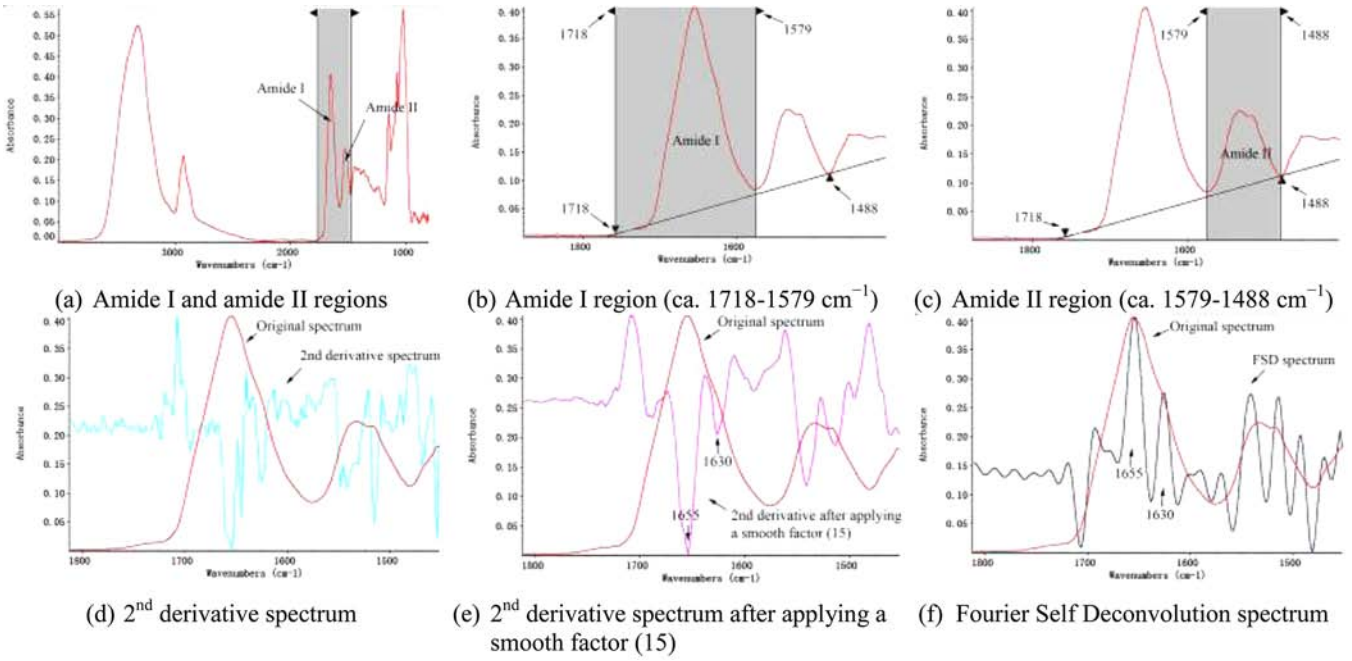

Figure 1. Typical synchrotron-based Fourier transform infrared microspectroscopy spectrum and its second derivative and Fourier self-deconvolution spectra for triticale in the amide I and amide II regions (ca. 1718−1488 cm^{-1}).

batches of triticale DDGS were produced by Alberta Distillers Limited (Calgary, AB) during 2006, 2007, and 2009, respectively. The wheat (n = 3), wheat DDGS (n = 3), corn (n = 3), corn DDGS (n = 5), and blend DDGS (n = 3) were used as references for protein molecular structure study in comparison with triticale and triticale DDGS. The detailed descriptions were reported previously.[28,33,39]

DRIFT Molecular Spectroscopy Data Collection and Analysis. DRIFT molecular spectroscopy was performed using a Bio-Rad FTS-40 with a ceramic infrared source and a mercury cadmium telluride detector (Bio-Rad Laboratories, Hercules, CA) at the Saskatchewan Structural Sciences Center (SSSC, Saskatoon, SK). All samples were ground through a 0.25 mm screen twice with a Retsch Grinder ZM100 (Brinkmann Instruments Ltd., Mississauga, ON) and then mixed in a 2 mL centrifuge tube with potassium bromide powder in a ratio of 1:4 and vortexed for 1 min.[40] Each feed sample was scanned five times. Data were collected using Win-IR software installed in the coupled computer system. Spectra were generated from the Mid-IR (ca. 4000−800 cm^{-1}) portion of the electromagnetic spectrum with 256 scans coadded and a spectral resolution of 4 cm^{-1}. The collection of background spectra (potassium bromide power) was performed prior to the sample spectra collection using the same settings. Spectral analysis was conducted using OMNIC 7.3 Software (Thermo Nicolet, Madison, WI). Baseline correction was conducted for all spectra prior to further interpretation.

Univariate Spectral Analysis. The protein molecular structure is usually determined from two primary bands in the spectra, namely, the amide I and amide II region. The amide I region contained C═O stretching, C−N, and N−H and was identified in this study in the range of ca. 1718−1579 cm^{-1}. The amide II region consisted of C−N stretching and N−H bending vibrations and was found in the range of ca. 1579−1488 cm^{-1}. Both amide I and amide II regions are used in protein molecular structure studies, although compared with amide I, amide II is less useful because of the involvement of multiple functional groups, which lead to complex vibrations.[39,42,43] The amide I and amide II peak area absorption intensities and their ratios were calculated. Using Fourier self-deconvolution or the second derivative functions in the OMNIC software, amide I was further resolved into several multicomponent peaks where α-helices (center at ca. 1655 cm^{-1}) and β-sheets (center at ca. 1630 cm^{-1}) were identified (Figure 1). The intensities of the peak heights of the α-helix and β-sheet and their ratios were also calculated.

Animal and Diets. Three dry Holstein cows, fitted with a rumen cannula with an internal diameter of 10 cm (Bar Diamond, Parma, ID), were used for this work. The cows were cared for according to the guidelines of the Canadian Council on Animal Care.[8] The cows were given ad libitum access to water and individually fed 15 kg (as fed) of a ration formulated to meet or exceed NRC Nutrient Requirements[7] twice daily (7.5 kg per feeding) at 0800 and 1600. The ration consisted of 56.82% barley silage, 10.23% alfalfa hay, 4.54% dehydrated alfalfa pellets, and 28.41% concentrates (containing barley, wheat, oats, canola meal, soybean meal, wheat DDGS, corn gluten meal, molasses, golden flakes, canola oil, minerals, and vitamins) as described in a previous study.[9]

Rumen Incubation Procedures. Rumen degradation kinetics were determined using an in situ method.[10] For triticale grain, samples were first coarsely rolled using a Sven Roller Mill (Apolo Machine and Products Ltd., Saskatoon, SK) with a roller gap of 0.203 mm (industry practice). The in situ experiment was designed as a randomized complete block design with two experimental runs as a block effect. The experiment was randomly carried out in three cows using two runs for each incubation time. All of the bags were randomly assigned to the three cows.

Seven grams of each feed sample was placed into number-marked nylon bags (Nitex 03-41/31 monofilament open mash fabric, Screentec Corp., Mississauga, ON) and tied. The bags were 10 cm × 20 cm with a pore size of 41 μm. The nylon bags were placed into a polyester mesh bag (45 cm × 45 cm attached to a 90 cm length of rope) and suspended in the rumen. Bags were added into the rumen according to a "gradual addition/all out" schedule and were incubated for 48, 24, 12, 8, 4, 2, and 0 h.[10] The number of bags increased with the length of incubation to ensure that sufficient residue was available for analysis. After incubation, the bags were collected from the rumen and washed under a cold water stream without detergent to remove rumen fluid. Washed bags were dried in a forced air oven at 55 °C for 48 h. The dried samples were subsequently kept in a refrigerated room (4 °C) until needed for chemical analysis.

Chemical Analyses. The residues collected from the nylon bags were transferred into labeled containers and ground through a 1 mm screen (Retsch ZM-1; Brinkmann Instruments, Mississauga, ON) for analysis, with the exception of the starch analysis where samples were ground through a 0.5 mm screen. Samples were analyzed for dry matter (DM, AOAC official method 930.15), ash (AOAC official

method 942.05), ether extract (EE, AOAC official method 954.02), and crude protein (CP, AOAC official method 984.13) content according to the AOAC.[11] Starch was analyzed using the Megazyme Total Starch Assay Kit (Megazyme International Ltd., Bray, Wicklow, Ireland) and by the α-amylase/amyloglucosidase method.[12] Acid (ADF) and neutral detergent fiber (NDF) and acid detergent lignin (ADL) were analyzed.[13] Sodium sulfite was added prior to neutral detergent extraction. The N adjusted NDF (NDFn) was calculated as NDF − neutral detergent insoluble protein (NDICP). The acid (ADIN) and neutral detergent insoluble N (NDIN) values were determined.[14] The nonprotein nitrogen (NPN) content was analyzed by precipitating true protein with tungstic acid (samples were soaked in water with 0.3 M Na_2WO_4 for 30 min) and calculated as the difference between the total N and the N content of the residue after filtration.[14] Soluble crude protein (SCP) was determined by incubating the sample with borate phosphate buffer and filtering through Whatman #54 filter paper.[15] The nonstructural carbohydrates including starch, sugars, organic acids, and other reserve carbohydrates such as fructan were estimated by nonfiber carbohydrates (NFC) and calculated using the NRC.[7] The total carbohydrate (CHO), true protein, hemicellulose, and cellulose contents were also calculated.[7,13]

Protein and Carbohydrate Subfractions. The CP and carbohydrate subfractions were partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS).[16,17] For the protein fractions, the total CP pool was partitioned into three categories by this system as fractions PA, PB, and PC. Furthermore, PB (true protein) was sequentially divided into three subfractions named PB1, PB2, and PB3 according to their different degradation rates in the rumen.[17] Fraction PA is NPN with a hypothesized infinite degradation rate, and fraction PC is the unavailable protein fraction, which is acid detergent insoluble crude protein (ADICP), having an assumed degradation rate of zero because of its high degradation resistance. PB1 is the rapidly degradable fraction of protein with a degradation rate of 120−400% h^{-1}, and it is soluble in borate phosphate buffer similar to PA and is calculated as SCP − NPN. PB3 is the plant cell wall-associated protein fraction with a degradation rate of 0.06−0.55% h^{-1} and is calculated as NDICP − ADICP. It is insoluble in neutral detergent solution but soluble in acid detergent solution. It is believed that a large proportion of PB3 can bypass rumen degradation and is available for intestinal digestion. PB2 is calculated as CP − the sum of PA, PB1, PB3, and PC. It is insoluble in borate phosphate buffer but soluble in neutral detergent solution. PB2 has a lower degradation rate (3−16% h^{-1}) in the rumen than the borate phosphate buffer-soluble fractions (PA and PB1); thus, some PB2 fraction escapes from the rumen into the intestine.

Carbohydrate was partitioned into the rapidly degradable fraction (CA), which has a degradation rate of 300% h^{-1}, composed of sugars and organic acids; the intermediately degradable fraction (CB1), which is starch and pectin, having an intermediate degradation rate of 20−50% h^{-1}; the slowly degradable fraction (CB2), which is the available cell wall with a degradation rate of 2−10% h^{-1}; and an unfermentable fraction (CC), which is the unavailable cell wall. CC is calculated as 0.024 times ADL, CB1 is calculated as NDFn − CC, and CA is calculated as NFC − CB1.

Energy Content. The estimated energy content was determined using a summative approach[18] obtained from the dairy NRC.[7] Total digestible nutrients at maintenance (TDN_{1X}) and digestible energy at maintenance (DE_{1X}) were calculated from total digestible CP (tdCP), fatty acid (tdFA), NDF (tdNDF), and NFC (tdNFC). The change caused by different intake levels was adjusted by a discount factor.[7] On the basis of the DE_{1X} value and the discount variable, digestible energy (DE_{3X}), metabolizable energy (ME_{3X}), and net energy for lactation (NE_{L3X}) at three times maintenance were calculated. The net energy for maintenance (NE_m) and net energy for growth (NE_g) were determined using the beef NRC.[19] Both the dairy NRC and the beef NRC use the same formula to estimate NE_m and NE_g.

Rumen Degradation Kinetic Model. In situ degradation kinetics for DM, CP, and NDF were determined using the first-order kinetics equation[20] modified by Robinson, Fadel, and Tamminga[21] and Dhanoa[22] to include lag time:

$$R(t) = \mathrm{U} + \mathrm{D} \times e_d^{-K\times(t-T0)}$$

where $R(t)$ = residue present at t h of incubation (%), U = undegradable fraction (%), D = degradable fraction (%), $T0$ = lag time (h), and K_d = degradation rate (% h^{-1}). The parameters were calculated using the NLIN (nonlinear) procedure of SAS (SAS Institute, Cary, NC)[23] with iterative least-squares regression (Gauss−Newton method).

The degradation model for starch was different in that lag time and U are assumed to be zero in the DVE/OEB system.[6] Therefore,

$$R(t) = (100 - \mathrm{S}) \times e_d^{-K\times t}$$

The effective degradability (ED) of DM, CP, and NDF was calculated according to the following equation:

$$\mathrm{ED}\ (\%) = \mathrm{S} + \mathrm{D} \times K_d/(K_p + K_d)$$

where S = soluble fraction (%) and K_p = passage rate (% h^{-1}) and was considered to be 6% h^{-1}.[6]

The rumen undegradable fractions (RU) of DM, CP, NDF, and starch (St) were calculated as:

$$\mathrm{RUDM, P, NDF}\ (\%) = \mathrm{U} + (\mathrm{D} \times K_p)/(K_p + K_d)$$

$$\mathrm{RUSt}\ (\%) = \mathrm{S} \times 0.1 + (\mathrm{D} \times K_p)/(K_p + K_d)$$

where D = 100 − S − U (%); K_p is the estimated rate of outflow of digesta from the rumen (% h^{-1}) and was assumed to be 6% h^{-1} in the DVE/OEB system for concentrate feedstuffs[6] and the factor 0.1 is a compensation factor between in situ and in vivo starch results, indicating that 10% of the S fraction of starch escapes rumen degradation.[6,10,24]

Intestinal Digestion of RUP. The estimation of intestinal digestion of RUP was determined by a modification[25] of the three-step in vitro procedure. Briefly, dried ground residues containing 15 mg of N after a 12 h ruminal preincubation[6,10] were exposed for 1 h in 10 mL of 0.1 M HCl solution containing 1 mg of pepsin. The pH was neutralized with 0.5 mL of 1 M NaOH and 13.5 mL of pH 7.8 phosphate buffer containing 37.5 mg of pancreatin (Sigma-Aldrich, St. Louis, MO) added to the solution and incubated at 38 °C for 24 h.[25] After incubation, 3 mL of a 100% (w/v) trichloroacetic acid (TCA) solution was added to stop enzymatic activity and precipitate undigested proteins. Samples were centrifuged, and the supernatant (soluble N) was analyzed for N (Kjeldahl method, AOAC 984.13). Intestinal digestion of protein was calculated as TCA-soluble N divided by the amount of N in the rumen residue sample.

Metabolizable Protein Supply to Dairy Cattle by Non-TDN-Based Model. The DVE/OEB system has been described in detail in a previous study.[6] This model features two important values, the DVE value, which is the truly absorbed feed protein in the small intestine, and the OEB value, which indicates the balance between potential microbial protein (MCP) synthesized based on rumen degraded protein (RDP) and the potential microbial protein synthesized based on energy derived from organic matter fermented in the rumen. The following brief description is provided to understand the concept and prediction of the ruminant nutrient supply.

Fermented Organic Matter (FOM). The FOM was used to estimate microbial protein (MCP) synthesis. FOM was calculated as

$$\begin{aligned}\mathrm{FOM}\ (\mathrm{g\,kg^{-1}\,DM}) = {} & \mathrm{DOM}\ (\mathrm{g\,kg^{-1}\,DM}) - \mathrm{EE}\ (\mathrm{g\,kg^{-1}\,DM}) \\ & - \mathrm{RUP}\ (\mathrm{g\,kg^{-1}\,DM}) - \mathrm{RUSt}\ (\mathrm{g\,kg^{-1}\,DM}) \\ & - \mathrm{FP}\ (\mathrm{g\,kg^{-1}\,DM})\end{aligned}$$

where, DOM is digestible OM, estimated after 48 h of incubation, RUSt = rumen undegraded starch, assumed to be zero for the *in situ* residue of DDGS, and FP = end products of fermentation in ensiled forages that are assumed to be zero for concentrates.

Microbial Crude Protein (MCP) Synthesis and Truly Absorbed Rumen Microbial Protein. MCP synthesis was calculated based on FOM as follows:

$$MCP_{FOM}{}^{DVE}\ (g\,kg^{-1}\,DM) = 0.15 \times FOM\ (g\,kg^{-1}\,DM)$$

where 0.15 indicates that 150 g of MCP per kg of FOM is assumed to be synthesized.[6]

The DVE/OEB system also considers MCP synthesized from RDP ($MCP_{RDP}{}^{DVE}$) for the estimation of OEB. The $MCP_{RDP}{}^{DVE}$ value was calculated as:

$$MCP_{RDP}{}^{DVE}\ (g\,kg^{-1}\,DM) = CP\ (g\,kg^{-1}\,DM) \times \{1 - [1.11 \times RUP\ (\%\ CP)/100]\}$$

where the factor 1.11 is the regression coefficient between in situ RUP and in vivo RUP according to the French PDI system.[26]

The truly absorbable MCP synthesized in the rumen ($AMCP^{DVE}$) was calculated as:

$$AMCP^{DVE}\ (g\,kg^{-1}\,DM) = 0.75 \times 0.85 \times MCP_{FOM}{}^{DVE}\ (g\,kg^{-1}\,DM)$$

where 0.75 means that 75% of the microbial N is present in amino acids, while the remainder is present in nucleic acids. The value of 0.85 indicates the true digestibility of microbial protein.[27]

Truly Absorbed Rumen Undegraded Feed Protein in the Small Intestine. The content of truly absorbed RUP in the small intestine ($ARUP^{DVE}$) is based on the content and digestibility of ruminally undegraded feed CP (RUP^{DVE}) and was calculated as follows:

$$RUP^{DVE}\ (g\,kg^{-1}\,DM) = 1.11 \times RUP\ (\%\ CP)/100 \times CP\ (g\,kg^{-1}\,DM)$$

$$ARUP^{DVE}\ (g\,kg^{-1}\,DM) = dRUP\ (\%\ RUP)/100 \times RUP^{DVE}\ (g\,kg^{-1}\,DM)$$

where dRUP = estimated intestinal digestibility of RUP and 1.11 represents the regression coefficient between in situ RUP and in vivo RUP according to the French PDI system.[26]

Endogenous Protein Loss in the Small Intestine. The calculation of DVE requires a correction for endogenous protein loss (ENDP) to account for N lost as a consequence of incomplete digestion. The ENDP is associated with the amount of undigested DM (UDM), which was estimated as:

$$UDM\ (g\,kg^{-1}\,DM) = DM \times [(100 - dDM\ (\%)]/100$$

where dDM is the digestibility of DM after a 48 h rumen incubation.

$$ENDP\ (g\,kg^{-1}\,DM) = 0.075 \times UDM\ (g\,kg^{-1}\,DM)$$

where 0.075 stands for 75 g of absorbed protein per kg of UDM in feces that is required to compensate for the endogenous protein loss.[6]

Total Truly Absorbed Feed Protein in the Small Intestine. Total truly absorbed feed protein in the small intestine (DVE value) was calculated as follows:

$$DVE\ (g\,kg^{-1}\,DM) = AMCP^{DVE}\ (g\,kg^{-1}\,DM) + ARUP^{DVE}\ (g\,kg^{-1}\,DM) - ENDP\ (g\,kg^{-1}\,DM)$$

where $AMCP^{DVE}$ is the truly absorbable microbial protein synthesized in the rumen, $ARUP^{DVE}$ is the truly absorbed undegraded feed protein in the small intestine, and ENCP is the endogenous protein loss in the small intestine.

The OEB value was calculated as:

$$OEB\ (g\,kg^{-1}\,DM) = MCP_{RDP}{}^{DVE}\ (g\,kg^{-1}\,DM) - MCP_{FOM}{}^{DVE}\ (g\,kg^{-1}\,DM)$$

where $MCP_{RDP}{}^{DVE}$ is MCP synthesized from RDP and $MCP_{FOM}{}^{DVE}$ is MCP synthesized from potentially available energy from the fermentation of OM in the rumen. Therefore, a positive OEB indicates a potential N loss from the rumen, while a negative OEB indicates a shortage of N that impairs MCP synthesis. The optimal OEB value of a diet is zero or slightly higher than zero.[6]

Predicted Metabolizable Protein Supply to Dairy Cattle by TDN-Based Model. The detailed concepts and formulas of the NRC-2001 model are given in NRC.[7] The NRC-2001 model considers the amount of true absorbed protein reaching the small intestine to be an important factor in estimating feed quality.

Estimation of Total Digestible Nutrients. The NRC-2001 model requires the TDN_{3X} value to estimate rumen microbial protein synthesis. TDN_{1X} can be calculated according to the NRC:

$$TDN_{1X} = tdNFC + tdCP + (tdFA \times 2.25) + tdNDF - 7$$

where 7 represents estimated metabolic fecal TDN.

When the intake level increases, TDN declines.[7] Therefore, a discount factor is required to determine TDN_{3X}. Assuming the diet TDN at maintenance is 74%, the discount factor at three times maintenance (i.e., production level) is 0.918.[7] Therefore, the TDN_{3X} can be calculated as:

$$TDN_{3X} = 0.918 \times TDN_{1X}$$

Truly Absorbed Rumen Synthesized Microbial Protein. Ruminally synthesized microbial crude protein (MCP^{NRC}) is calculated based on discounted TDN and is dependent on the availability of RDP. Thus, MCP^{NRC} was first calculated as follows:

$$MCP_{TDN}{}^{NRC}\ (g\,kg^{-1}\,DM) = 0.13 \times TDN_{3X}$$

where 0.13 represents 130 g of MCP synthesized per kg TDN (discounted).[7]

Then, RDP^{NRC} was calculated as:

$$RDP^{NRC}\ (g\,kg^{-1}\,DM) = CP\ (g\,kg^{-1}\,DM) \times [100 - RUP\ (\%\ CP)]/100$$

when RDP^{NRC} is higher than $1.18 \times MCP_{TDN}{}^{NRC}$; the $MCP_{TDN}{}^{NRC}$ value is used as MCP^{NRC} for the final $AMCP^{NRC}$ calculation; otherwise, MCP^{NRC} was calculated as:

$$MCP_{TDN}{}^{NRC}\ (g\,kg^{-1}\,DM) = 0.85 \times RDP^{NRC}\ (g\,kg^{-1}\,DM)$$

where 0.85 indicates the amount of RDP converted to microbial protein and 1.18 results from 1.00/0.85.[7] Because both the true protein content of ruminally synthesized microbial CP and the digestibility of ruminally synthesized microbial CP are 0.80,[7] thus, $AMCP^{NRC}$ was estimated as:

$$AMCP^{NRC}\ (g\,kg^{-1}\,DM) = 0.80 \times 0.80 \times MCP^{NRC}\ (g\,kg^{-1}\,DM)$$

Truly Absorbed Rumen Undegraded Feed Protein in the Small Intestine. The prediction of $ARUP^{NRC}$ is based on the content and digestibility of RUP^{NRC} and was calculated as:

$$RUP^{NRC}\ (g\,kg^{-1}\,DM) = CP\ (g\,kg^{-1}\,DM) \times RUP\ (\%\ CP)/100$$

$$ARUP^{NRC}\ (g\,kg^{-1}\,DM) = dRUP\ (\%\ RUP)/100 \times RUP^{NRC}\ (g\,kg^{-1}\,DM)$$

Truly Absorbed Endogenous Protein in the Small Intestine. Endogenous protein (ECP) in the rumen is based on DM content.[7] Thus, ECP is calculated as:

$$ECP\ (g\,kg^{-1}\,DM) = 6.25 \times 1.9 \times DM\ (\%)/100$$

where 6.25 represents the Kjeldahl/N conversion factor and 1.9 indicates that 1.9 g of endogenous N is obtained from a kg of DM.[7]

It is assumed that 50% of ECP passes to the small intestine of which 80% is true protein, which is assumed to be fully digestible.[7] Thus, truly absorbed endogenous protein in the small intestine (NRC-2001) (AECP) was calculated as:

$$AECP\ (g\,kg^{-1}\,DM) = 0.50 \times 0.80 \times ECP\ (g\,kg^{-1}\,DM)$$

Metabolizable Protein and Degraded Protein Balance. In the NRC-2001 model, total metabolizable protein (MP) is calculated as:

Table 1. Comparison of Cereal Grains with Their DDGS in Terms of Protein Molecular Structure Spectral Profiles Using DRIFT Spectroscopy[a]

	grains			DDGS						
	wheat (n = 3)	corn (n = 3)	triticale (n = 3)	wheat DDGS (n = 5)	corn DDGS (n = 3)	triticale DDGS (n = 3)	blend[b] DDGS (n = 3)	SEM	overall P value	grains vs DDGS P value
Protein Molecular Structure Spectral Profiles (Unit: Absorbance)										
amide I area	19.21 b	13.56 c	21.17 a	11.30 d	8.48 e	6.18 f	9.04 e	0.399	<0.01	<0.01
amide II area	3.94 c	2.74 d	3.74 c	5.53 b	6.54 a	3.57 c	5.14 b	0.139	<0.01	<0.01
amide I to amide II ratio	4.91 b	4.95 b	5.70 a	2.03 c	1.29 e	1.73 d	1.75 cd	0.075	<0.01	<0.01
α-helix height	0.26 a	0.21 b	0.28 a	0.14 c	0.12 d	0.08 e	0.11 d	0.005	<0.01	<0.01
β-sheet height	0.21 b	0.15 c	0.23 a	0.10 d	0.10 de	0.07 f	0.09 ef	0.004	<0.01	<0.01
α-helix to β-sheet ratio	1.26 bc	1.38 a	1.21 c	1.31 b	1.21 c	1.20 c	1.26 bc	0.018	<0.01	<0.01

[a]SEM, standard error of mean. For letters a–f, means with different letters in the same row are significantly different ($P < 0.05$). Multitreatment comparison method, Tukey–Kramer. [b]Blend DDGS produced from a blend of 70% wheat and 30% corn.

$$\text{MP (g kg}^{-1}\text{ DM)} = \text{AMCP}^{\text{NRC}}\text{ (g kg}^{-1}\text{ DM)} + \text{ARUP}^{\text{NRC}}\text{ (g kg}^{-1}\text{ DM)} + \text{AECP (g kg}^{-1}\text{ DM)}$$

where AMCP^{NRC} = absorbable microbial crude protein synthesized in the rumen, ARUP^{NRC} = truly absorbed bypass feed protein in the small intestine, and AECP = truly absorbed endogenous crude protein in the small intestine.

In contrast to the DVE/OEB system, endogenous protein losses are added rather than subtracted from supply. Although the estimation of degraded protein balance (DPB^{NRC}) is not provided by the NRC-2001 model, it can be calculated based on predicted data and according to the principles of the DVE/OEB system. However, in the NRC-2001 model, DPB^{NRC} is considered as the difference between the potential MCP synthesis based on RDP and that based on TDN at a production level rather than on FOM as in the DVE/OEB system. Therefore, DPB^{NRC} is calculated as:

$$\text{DPB}^{\text{NRC}}\text{ (g kg}^{-1}\text{ DM)} = \text{RDP}^{\text{NRC}}\text{ (g kg}^{-1}\text{ DM)} - 1.18 \times \text{MCP}_{\text{TDN}}{}^{\text{NRC}}\text{ (g kg}^{-1}\text{ DM)}$$

where RDP^{NRC} = rumen degraded protein, factor 1.18 = 1/0.85, and $\text{MCP}_{\text{TDN}}{}^{\text{NRC}}$ = microbial crude protein synthesis from energy that is provided by total digestible nutrients (discounted at three times maintenance).

Statistical Analysis. *Molecular Spectral Profile, Chemical Profile, Protein and Carbohydrate Fractions, and Energy Content.* Statistical analyses were performed using the MIXED procedure of SAS[23] (version 9.1.3). The model used for the analysis was $Y_{ij} = \mu + F_i + e_{ij}$, where Y_{ij} was an observation of the dependent variable ij; μ was the population mean for the variable; F_i was the effect of feed source as a fixed effect (triticale n = 3, wheat n = 3, corn n = 3, triticale DDGS n = 3, wheat DDGS n = 5, and corn DDGS n = 3), and e_{ij} was the random error associated with the observation ij.

In Situ Rumen Degradation Kinetics, In Vitro Digestion of RUP, and Predicted Nutrient Supply. Statistical analyses were performed using the MIXED procedure of SAS[23] (Version 9.1.3). The model used for the analysis was $Y_{ijk} = \mu + F_i + S_j + e_{ijk}$, where Y_{ijk} was an observation of the dependent variable ijk; μ was the population mean for the variable; F_i was the effect of feed source as a fixed effect; S_j was the run effect as a random effect; and e_{ijk} was the random error associated with the observation ijk.

For all statistical analyses, multitreatment comparison was used. Tukey–Kramar method significance was declared at $P < 0.05$ and trends at $P \leq 0.10$.

RESULTS AND DISCUSSION

Using DRIFT Spectroscopy To Compare Protein Structure Profiles between Grains and Their DDGS. *Quantifying Protein Molecular Structure Using Amide I to Amide II Ratio.* Table 1 gives the protein molecular structure parameters of the protein in triticale grain and triticale DDGS in comparison with two other common cereal grains—wheat and corn and their DDGS as references. As compared with cereal grains, DDGS exhibited differences in their amide profiles ($P < 0.01$). These differences most likely result from changes during bioethanol processing. The decrease ($P < 0.01$) in intensity of the amide I to amide II ratio from the grains to their DDGS agrees with the results of Yu et al.[39] (wheat and corn grain vs DDGS = 4.58 vs 2.84, $P < 0.05$). By comparing each grain with its corresponding DDGS, it was found that all three grains were higher in their spectral intensity of amide I as well as the amide I to amide II ratio than their DDGS. Amide II did not change in a similar pattern, as it was increased in intensity in the DDGS except for triticale DDGS. The reason is still not clear but could be due to different response of functional bonding in amide II to bioethanol processing as compared with amide I. In terms of the amide I to amide II ratio, there were differences among wheat DDGS, corn DDGS, and triticale DDGS ($P < 0.01$). However, the blend DDGS had a similar ratio to the wheat DDGS and triticale DDGS. A comparison among the different grains showed that triticale is higher in the amide I to amide II ratio than wheat and corn ($P < 0.01$).

Quantifying Protein Molecular Structure Using α-Helix to β-Sheet Ratio. Table 1 shows the protein molecular structure characteristics in terms of α-helix to β-sheet ratio. Grains showed significantly different results in the α-helix, β-sheet, and their ratio as compared with their DDGS. The intensity of the α-helix and β-sheet height was higher in all three grains (wheat, corn, and triticale) than their DDGS. However, in terms of the α-helix to β-sheet ratio, only corn and corn DDGS showed a decrease ($P < 0.01$). This result disagrees with the results of Yu et al.[39] who showed increases from the original corn and wheat blend grains to their DDGS in the α-helix and β-sheet heights. The discrepancy might arise from differences in heating conditions because bioethanol processing requires a series of heating procedures such as cooking (nonpressurized or pressurized) and drying under different temperatures.

Effect of Bioethanol Processing on Chemical Profile of Triticale Grain and DDGS. Triticale grain and DDGS chemical profiles are presented in Table 2. As expected, the chemical profiles were dramatically different between triticale grain and DDGS. Significant differences between triticale grain and DDGS were found for all of the nutrients. Triticale had

Table 2. Chemical Profile of Triticale Grain and DDGS

	triticale (n = 3)	triticale DDGS (n = 3)	SEM[a]	P value
Basic Chemical Profile				
DM (%)	90.3	90.3	1.00	0.99
ash (% DM)	1.7	4.2	0.03	<0.01
organic matter (OM, % DM)	98.3	95.8	0.03	<0.01
EE (% DM)	1.5	6.5	0.73	<0.01
Carbohydrate Profile				
starch (% DM)	63.6	5.2	1.21	<0.01
NDF (% DM)	13.5	40.3	1.56	<0.01
ADF (% DM)	3.6	14.0	0.49	<0.01
ADL (% DM)	0.8	4.7	0.04	<0.01
ADL (% NDF)	5.9	11.8	0.40	<0.01
CP Profile				
CP (% DM)	13.3	31.5	1.61	<0.01
SCP (% CP)	33.0	21.9	2.81	<0.05
NPN (% CP)	8.5	21.9	2.97	<0.05
NPN (% SCP)	26.2	100.0	3.97	<0.01
NDICP (% DM)	1.6	12.6	0.76	<0.01
NDICP (% CP)	11.9	39.9	0.39	<0.01
ADICP (% DM)	0.1	3.7	0.23	<0.01
ADICP (% CP)	1.0	12.0	1.20	<0.01

[a]SEM, standard error of mean.

lower ash, EE, all of the fiber and protein fractions than triticale DDGS (e.g., for EE 1.5 vs 6.5%, $P < 0.01$), except for SCP. In contrast, triticale grain was higher in starch content than triticale DDGS ($P < 0.01$). The residual starch content in DDGS indicated that the fermentation of starch is not complete during bioethanol processing. The NPN value (100% SCP) of triticale DDGS is in agreement with that of wheat DDGS as reported by Nuez-Ortín and Yu.[28] The chemical analyses of the present data were in general consistent with previous studies in triticale DDGS.[2,4,5] Some of them reported lower NDF values (29.6 to 39.4% DM) but similar CP (average 31.8%) and EE.[2,4,29] The ADIN content of triticale DDGS in the current study was similar to the 11.4% of total N for triticale DDGS[5] and wheat DDGS[30] (average: 10.5% of total N).

Effect of Bioethanol Processing on Protein and Carbohydrate Subfractions of Triticale Grain and DDGS. Significant differences between triticale grain and DDGS were observed for all protein and carbohydrate subfractions (Tables 3 and 4). Triticale grain was lower in the rapidly degradable PA fraction ($P < 0.05$), the slowly degradable PB3 fraction (PB3, $P < 0.01$), and the unavailable PC fraction ($P < 0.01$) and higher in the rapidly degradable PB1 fraction ($P < 0.01$) and the intermediately degradable PB2 fraction ($P < 0.05$) than triticale DDGS. The true protein content decreased from 90.5% in triticale grain to 66.2% in triticale DDGS (Table 3), indicating overheating during drying and/or changes in protein structure during the bioethanol processing. A decrease for PA (21.9 vs 16.3% CP) and no change for true protein (78.1 vs 78.9% CP) were previously reported for wheat and wheat DDGS.[28] This inconsistency can be attributed to the different chemical composition of wheat and triticale grain (e.g., 21.9 vs 8.5% NPN for wheat and triticale, respectively).

For the carbohydrate fractions (Table 4), triticale was 8.5-fold higher in CB1 fraction ($P < 0.01$) but lower ($P < 0.01$) in CA, CB2, and CC fractions than triticale DDGS. The decrease in the CB1 value from triticale to triticale DDGS confirms that starch was fermented to produce ethanol. However, 5% starch remained in triticale DDGS, which indicated that its

Table 3. Protein Subfractions in Triticale Grain and DDGS Determined with the "Cornell Net Carbohydrate and Protein System"[a]

	triticale (n = 3)	triticale DDGS (n = 3)	SEM[b]	P value
Protein Subfractions (% CP)				
PA	8.5	21.9	2.97	<0.05
PB1	24.4	0.0	1.68	<0.01
PB2	55.1	38.3	3.03	<0.05
PB3	11.0	27.9	1.44	<0.01
PC	1.0	12.0	1.20	<0.01
Protein Subfractions (% TP[c])				
true protein (% CP)	90.5	66.2	1.99	<0.01
PB1 (% TP)	26.9	0.0	1.54	<0.01
PB2 (% TP)	61.0	57.5	3.43	0.52
PB3 (% TP)	12.1	42.5	3.09	<0.01
Protein Subfractions (% DM)				
PA	1.1	7.0	1.17	<0.05
PB1	3.3	0.0	0.24	<0.01
PB2	7.3	11.9	0.48	<0.01
PB3	1.5	8.9	0.90	<0.01
PC	0.1	3.7	0.23	<0.01

[a]Abbreviations: TP, true protein; PA, fraction of CP that is instantaneously solubilized at time zero, calculated as NPN; PB1, rapidly degradable protein fraction that is soluble in borate phosphate buffer and precipitated with trichloroacetic acid, calculated as SCP − NPN; PB2, intermediately degradable protein fraction calculated as total CP − the sum of fractions PA, PB1, PB3, and PC; and PB3, slowly degradable protein fraction, calculated as NDICP − ADICP; PC, fraction of undegradable protein, calculated as ADICP. It contained the proteins associated with lignin and tannins and/or heat-damaged proteins such as Maillard reaction products. [b]SEM, standard error of mean. [c]True protein = PB1 (% CP) + PB2 (% CP) + PB3 (% CP).

Table 4. Carbohydrate (CHO) Subfractions in Triticale Grain and DDGS Determined with the "Cornell Net Carbohydrate and Protein System"[a]

	triticale (n = 3)	triticale DDGS (n = 3)	SEM[b]	P value
CHO Subfractions (% DM)				
CA	8.0	24.8	1.28	<0.01
CB1	63.6	5.2	1.21	<0.01
CB2	10.0	19.4	1.20	<0.05
CC	1.9	11.4	0.10	<0.01
CHO Subfractions (% Total CHO[c])				
CHO (% DM)	83.4	57.8	0.96	<0.01
CA (% CHO)	9.6	43.0	1.81	<0.01
CB1 (% CHO)	76.2	9.0	1.50	<0.01
CB2 (% CHO)	12.0	28.4	1.98	<0.01
CC (% CHO)	2.3	19.7	0.40	<0.01

[a]Abbreviations: CA, fraction of total carbohydrate with a rapidly K_d (300% h^{-1}) and is degradable soluble sugars and organic acids; CB1, fraction of total carbohydrate with an intermediate K_d (20−50% h^{-1}); CB2, fraction of total carbohydrate with a slow K_d (2−10% h^{-1}) and is available cell wall; and CC, fraction of total carbohydrate and is unavailable cell wall and not fermented. CC is calculated as 0.024 × ADL, CB1 is calculated as NDFn − CC, CB1 is starch and pectin, and CA is calculated as NFC − CB1. [b]SEM, standard error of mean. [c]CHO = 100 − CP − EE − ash.

fermentation was not completed. Similar values on CC (unavailable carbohydrate) have been reported in wheat and corn.[28] However, after fermentation, the corn DDGS had lower CC values than triticale DDGS and wheat DDGS. These differences could be due to different DDGS processes and/or due to the different inherent structure between corn and wheat or triticale.

Effect of Bioethanol Processing on Energy Content of Triticale Grain and DDGS. Triticale was lower in tdCP ($P < 0.01$), tdNDF ($P < 0.05$), and tdFA, $P < 0.01$), while it was higher in tdNFC ($P < 0.01$) than triticale DDGS (Table 5). TDN was higher for triticale than triticale DDGS ($P < 0.01$).

Table 5. Truly Digestible (td) Nutrients and Energy Content in Triticale Grain and DDGS[a]

	triticale ($n = 3$)	triticale DDGS ($n = 3$)	SEM[b]	P value
Digestible Nutrient (% DM)				
tdNFC	70.1	29.5	1.49	<0.01
tdCP	13.3	30.0	1.67	<0.01
tdNDF	6.9	12.0	0.80	<0.05
tdFA	0.5	5.5	0.73	<0.01
Total Digestible Nutrients (% DM)				
TDN_{1X}	84.5	76.9	1.12	<0.01
Predicted Energy Values (Mcal/kg DM)				
DE_{3X}	3.42	3.34	0.028	0.10
ME_{3X}	3.01	2.94	0.030	0.20
NEL_{3X}	1.92	1.89	0.024	0.48
NE_m	2.08	2.02	0.020	0.11
NE_g	1.41	1.36	0.020	0.13

[a]Abbreviations: tdNFC, digestible nonfiber carbohydrate (% DM); tdCP, digestible crude protein (% DM); tdNDF, digestible neutral detergent fiber (% DM); tdFA, digestible fatty acid (% DM); TDN_{1X}, total digestible nutrients at maintenance estimated from NRC dairy model 2001 (% DM); DE_{3X}, digestible energy three times maintenance estimated from the NRC dairy model 2001 (Mcal/kg DM); ME_{3X}, metabolizable energy at three times maintenance estimated from the NRC dairy model 2001 (Mcal/kg DM); NE_{L3X}, net energy for lactation at three times maintenance estimated from the NRC dairy model 2001 (Mcal/kg DM); NE_m, net energy for maintenance estimated from the NRC beef model 1996 (Mcal/kg DM); and NE_g, net energy for growth estimated from the NRC beef model 1996 (Mcal/kg DM). [b]SEM, standard error of mean.

The energy content of triticale grain and DDGS is presented in Table 5. The energy content (DE_{3X}, ME_{3X}, NE_{L3X}, NE_m, and NE_g for dairy cattle) was not different ($P > 0.05$) between triticale grain and DDGS. These results indicate that triticale DDGS has a similar energy content to triticale grain and can replace triticale grain in a dairy diet as has been obtained for wheat.[28] In contrast, corn DDGS has been reported higher NE_g (1.87 Mcal/kg DM[31] and 1.67 Mcal/kg DM[28]) than the corn grain (1.48 Mcal/kg DM).[28] These differences may be due to the different chemical profiles (e.g., fat content is much higher in DDGS) and/or inherent structures between corn and wheat or triticale in terms of protein and carbohydrate conformations.[39]

Effect of Bioethanol Processing on in Situ Rumen Degradability of Triticale Grain and DDGS. For DM degradation characteristics (Table 6), triticale was higher ($P < 0.05$) in K_d, degradable DM fraction, and EDDM content, but lower ($P < 0.05$) in S, U, and RUDM than triticale DDGS. K_d for wheat grain was reported as 12.4% h^{-1},[32] which is lower than the triticale in this study. The difference is likely due to the different processing methods (particle sizes) utilized for the cereal grain prior to incubation in the rumen. In the current study, the triticale grain was put through a roller mill (gap size, 0.203 mm), while in the previous study, samples were ground through a 1 mm screen. Finely grinding process[32] resulted in a very high S fraction (61.1%) at 0 h, which is usually eliminated from the K_d calculation. Therefore, K_d was low in the study.[32] In a previous study, which used the same in situ techniques and same roller milling procedure as in the current study, the wheat K_d of DM was 36.7% h^{-1}, which was higher than the current result (27.1% h^{-1}).[33] Given that the chemical profiles of wheat and triticale are similar, the difference in the rate of DM degradation was likely due to the different inherent structures of the different nutrients (e.g., CP, NDF, and starch). Comparing triticale with triticale DDGS, the decreased S value and increased D, K_d and EDDM values demonstrated the same pattern with the previous study on wheat and wheat DDGS.[33]

Table 6. In Situ Rumen Degradation Kinetics of DM, CP, and NDF in Triticale Grain and DDGS

	triticale ($n = 3$)	triticale DDGS ($n = 3$)	SEM[a]	P value
In Situ Rumen Degradation Characteristics of DM				
degradation rate (% h^{-1})	27.1	10.3	1.70	<0.01
lag time (h)	0.1	0.0	0.04	0.17
soluble fraction (S, %)	7.3	30.5	1.25	<0.01
degradable fraction (D, %)	82.7	50.1	1.18	<0.01
undegradable fraction (U, %)	10.0	19.4	0.74	<0.01
rumen undegraded feed DM (g kg^{-1} DM)	255	379	12.4	<0.01
effectively degraded feed DM (g kg^{-1} DM)	745	621	12.4	<0.01
In Situ Rumen Degradation Characteristics of CP				
degradation rate (% h^{-1})	16.9	9.5	1.14	<0.01
lag time (h)	0.2	0.0	0.10	0.32
soluble fraction (S, %)	3.9	19.3	1.69	<0.01
degradable fraction (D, %)	91.1	64.6	1.67	<0.01
undegradable fraction (U, %)	5.0	16.1	1.65	<0.01
rumen undegraded feed protein (% CP)	29.3	41.4	1.79	<0.01
effectively degraded feed protein (% CP)	70.7	58.6	1.79	<0.01
In Situ Rumen Degradation Characteristics of NDF				
degradation rate (% h^{-1})	14.1	5.6	0.84	<0.01
lag time (h)	0.3	0.2	0.21	0.53
washable fraction (S, %)	5.2	18.3	1.82	<0.01
degradable fraction (D, %)	45.9	56.6	2.59	<0.05
undegradable fraction (U, %)	48.9	25.1	2.54	<0.01
rumen undegraded feed NDF (% NDF)	62.9	55.2	1.05	<0.01
effectively degraded feed NDF (% NDF)	37.2	44.8	1.05	<0.01
rumen undegraded feed NDF (g kg^{-1} DM)	85	222	5.4	<0.01
effectively degraded feed NDF (g kg^{-1} DM)	50	181	5.5	<0.01

[a]SEM, standard error of mean.

The removal of starch during bioethanol fermentation led to a 3-fold increase in CP content in the triticale DDGS as compared with triticale grain. Therefore, even if the EDCP (%) is decreased from triticale to triticale DDGS, the EDCP (g kg^{-1}

DM) still increased due to the larger CP content in triticale DDGS. For CP degradation characteristics (Table 6), triticale was higher ($P < 0.05$) in degradation rate (K_d) and degradable fraction (D) but lower ($P < 0.05$) in soluble fraction (S), undegradable fraction (U), RUP (43 vs 144 g kg^{-1} DM), and EDCP (94 vs 185 g kg^{-1} DM) than triticale DDGS. According to the tabular data from NRC (2001), triticale grain has higher K_d (43 vs 16.9% h^{-1}), higher S (51.3 vs 3.9%), lower D (45.9 vs 91.1%), and lower U (2.8 vs 5.0%) for protein than the current results. The difference is likely due to the in situ processing method as samples were finely ground (1 mm) in the NRC[7] vs coarsely rolled at a gap size of 0.203 mm in the current study. However, in practical terms, the coarsely rolled is more commonly used in dairy nutrition.

As compared with the in situ data for wheat,[32] both K_d and S were higher than triticale. It was also reported that the K_d of wheat was 29.0% h^{-1},[34] which is higher than triticale in the current study (16.9% h^{-1}). However, the EDCP of wheat was lower (55.5%)[34] than triticale (70.7%) in the current study. The differences were likely generated not only from the different types and genotypes of cereal grains but also from the processing methods (ground or rolled) used in the experiments. Normally, the smaller the particle size used in the chemical analysis and the in situ procedures, the higher the soluble fraction of CP. This is confirmed by comparing the soluble CP (ground through 1 mm screen) content and the in situ S fraction (roller gap, 0.203 mm) values.

The lower proportion of soluble CP in both wheat and wheat DDGS samples in the study of Nuez-Ortín and Yu[33] as compared with the triticale grain and DDGS in the present study, along with different bioethanol processing procedures (such as fermentation temperatures, and drying period), might contribute to the difference. As compared with triticale DDGS, a similar RUP content for wheat DDGS was observed (41.5 vs 41.4% CP, respectively),[35] but a higher RUP value (wheat DDGS vs triticale DDGS: 222 vs 143 g kg^{-1} DM) was reported by Nuez-Ortín and Yu.[33] It has been concluded that the RUP value was positively correlated to the ADICP in various sources of DDGS.[31,36,37] Boila and Ingalls[36] reported that when ADICP in DDGS was increased from 8.9 to 16.7% of CP, the RUP value was also increased in DDGS. The ADICP of triticale DDGS in the current study (12.0% CP) is in agreement with what was observed by Boila and Ingalls.[36]

For NDF degradation characteristics (Table 6), triticale was higher ($P < 0.05$) in K_d and U but lower ($P < 0.05$) in S, D, RUNDF, and EDNDF than triticale DDGS. Nuez-Ortín and Yu[33] reported in situ NDF degradation characteristics of wheat grain that were similar in terms of K_d (11.6 vs 14.1% h^{-1}), S (5.9 vs 5.2%), D (46.4 vs 45.9%), and EDNDF (50 vs 50 g kg^{-1} DM) to triticale grain. However, wheat DDGS was different from triticale DDGS in terms of higher D (68.5 vs 56.6%) and lower S (0 vs 18.3%) and lower EDNDF (107 vs 181 g kg^{-1} DM).

Effect of Bioethanol Processing on Intestinal Protein Digestion in Triticale Grain and DDGS. The effects of bioethanol processing on the estimated intestinal protein digestibility of triticale grain and DDGS are shown in Table 7. The results show no difference in estimated intestinal digestibility of RUP (IDP) between triticale grain and DDGS. This indicated that bioethanol processing did not change the intestinal digestibility of RUP. The estimated intestinally absorbable feed protein (IADP) and the total digestible feed protein (TDP) were higher in triticale DDGS than in triticale grain (IADP, 94 vs 29 g kg^{-1} DM; TDP, 279 vs 123 g kg^{-1} DM). These results indicate that triticale DDGS is a rich source of RUP as compared with triticale grain, although the intestinal digestibilities of RUP (IDP) in triticale grain and DDGS were similar. Both IADP and TDP of triticale grain agree with those of wheat grain,[33] but the IADP of triticale DDGS was lower than that of wheat DDGS (29.8 vs 44.0% CP), while the TDP values from wheat and triticale DDGS were similar.[33] This indicates that wheat and triticale DDGS have different protein digestive characteristics, although their original grains are similar in their ruminal and intestinal availability of protein. This difference is likely due to different processing conditions (e.g., fermentation duration and temperatures) used by different ethanol plants when producing wheat and triticale DDGS.

Table 7. Estimated Intestinal Digestibility and Availability of CP in Triticale Grain and DDGS[a]

	triticale ($n = 3$)	triticale DDGS ($n = 3$)	SEM[b]	P value
Protein Value				
CP (g kg^{-1} DM)	133	315	16.1	<0.01
Rumen Phase				
RUP^{DVE} (g kg^{-1} DM)	43	144	4.1	<0.01
RUP^{NRC} (g kg^{-1} DM)	39	129	3.7	<0.01
EDCP (g kg^{-1} DM)	94	185	9.5	<0.01
Intestinal Phase				
IDP[c] (% RUP)	75.3	72.3	2.72	0.46
IADP[d] (% CP)	21.9	29.8	0.98	<0.01
IADP (g kg^{-1} DM)	29	94	3.4	<0.01
TDP[e] (% CP)	92.6	88.4	1.30	<0.05
TDP (g kg^{-1} DM)	124	279	11.7	<0.01

[a]Abbreviations: CP, crude protein (% DM or g kg^{-1} DM); RUP, rumen undegraded feed protein (% CP); RUP, rumen undegraded feed protein (g kg^{-1} DM) estimated from the DVE/OEB 1994 model, calculated as 1.11 × CP (g kg^{-1} DM) × RUP (% CP); RUP, rumen undegraded feed protein (g kg^{-1} DM) estimated from the NRC-2001 model, calculated as CP (g kg^{-1} DM) × RUP (% CP); EDCP, effective degradation of feed CP (% CP or g kg^{-1} DM); IDP, estimated intestinal digestibility of RUP (% RUP); IADP, estimated intestinally absorbable feed protein (% CP or g kg^{-1} DM); and TDP, total digestible feed protein (% CP or g kg^{-1} DM). [b]SEM, standard error of mean. [c]Estimated intestinal digestibility using the three-step in vitro procedure (Calsamiglia and Stern[25]). [d]Estimated intestinally absorbable feed protein: IADP = RUP × IDP/100. [e]Total digestible feed protein: TDP = EDCP + IADP.

Nutrient Supply to Dairy Cattle from Triticale Grain and DDGS Using the DVE/OEB System. The prediction of protein supply from triticale grain and DDGS to dairy cattle using the DVE/OEB system is presented in Table 8. Triticale had a higher FOM value ($P < 0.05$); as a result, there was higher $MCP_{FOM}{}^{DVE}$ and higher AMCP^{DVE} values ($P < 0.05$) from triticale grain than DDGS. However, triticale grain had a lower $MCP_{RDP}{}^{DVE}$ value ($P < 0.05$) than triticale DDGS. ARUP^{DVE} was lower in triticale than in triticale DDGS ($P < 0.05$). This is because no difference was found with regards to dRUP (%RUP, $P > 0.05$) between the triticale grain and the DDGS, while a lower RUP^{DVE} ($P < 0.05$) was found in triticale than triticale DDGS.

For endogenous protein losses in the small intestine, triticale was lower than triticale DDGS ($P < 0.05$). The DVE value for triticale was lower than triticale DDGS ($P < 0.05$). The OEB value for triticale was also lower (−24 g kg^{-1} DM) than triticale

Table 8. Prediction of the Potential Nutrient Supply from Triticale Grain and DDGS to Dairy Cattle Determined with the DVE/OEB System[a]

	feed			
	triticale (n = 3)	triticale DDGS (n = 3)	SEM[b]	P value
Truly Absorbed Rumen Synthesized Microbial Protein in the Small Intestine (g kg^{-1} DM)				
FOM	761	613	11.6	<0.01
MCP_{FOM}^{DVE} (based on FOM)	114	92	1.8	<0.01
MCP_{RDP}^{DVE}	90	171	9.5	<0.01
$AMCP^{DVE}$	73	59	1.1	<0.01
Truly Absorbed Rumen Undegraded Feed Protein in the Small Intestine (g kg^{-1} DM)				
RUP^{DVE}	43	144	4.1	<0.01
$ARUP^{DVE}$	33	104	3.8	<0.01
Endogenous Protein Losses in the Digestive Tract (g kg^{-1} DM)				
DOM	925	822	3.7	<0.01
UDM	64	151	3.7	<0.01
ENDP	5	11	0.3	<0.01
Total Truly Absorbed Protein in the Small Intestine (g kg^{-1} DM)				
DVE (=$AMCP^{DVE}$ + $ARUP^{DVE}$ − ENDP)	101	151	4.2	<0.01
Degraded Protein Balance (OEB, g kg^{-1} DM)				
OEB	−24	79	8.6	<0.01

[a]Abbreviations: FOM, organic matter fermented in the rumen (g kg^{-1} DM); MCP_{FOM}^{DVE}, microbial crude protein synthesized in the rumen based on available energy (g kg^{-1} DM); MCP_{RDP}^{DVE}, microbial crude protein synthesized in the rumen based on available nitrogen (g kg^{-1} DM); $AMCP^{DVE}$, truly absorbed microbial protein in the small intestine (g kg^{-1} DM); RUP^{DVE}, rumen undegraded feed protein (g kg^{-1} DM) estimated from the DVE/OEB 1994 model, calculated as 1.11 × CP (g kg^{-1} DM) × RUP (% CP); dRUP, estimated intestinal digestibility of RUP (% RUP); $ARUP^{DVE}$, truly absorbed rumen undegraded protein in the small intestine (g kg^{-1} DM); DOM, digestible organic matter (g kg^{-1} DM); UDM, undigested dry matter (g kg^{-1} DM); ENDP, endogenous protein in the small intestine (g kg^{-1} DM); DVE, truly digested protein in the small intestine (g kg^{-1} DM); and OEB, degraded protein balance (g kg^{-1} DM). [b]SEM, standard error of mean.

Table 9. Prediction of the Potential Nutrient Supply from Triticale Grain and DDGS Determined with the NRC-2001 Model[a]

	feed			
	triticale (n = 3)	triticale DDGS (n = 3)	SEM[b]	P value
Truly Absorbed Rumen Synthesized Microbial Protein in the Small Intestine (g kg^{-1} DM)				
MCP_{RDP}^{NRC} (based on RDP)	80	158	8.1	<0.01
MCP_{TDN}^{NRC} (based on TDN)	101	92	0.9	<0.01
MCP^{NRC}	80	92	1.7	<0.01
$AMCP^{NRC}$	51	59	1.1	<0.01
Truly Absorbed Rumen Undegraded Feed Protein in the Small Intestine (g kg^{-1} DM)				
RUP^{NRC}	39	129	3.7	<0.01
$ARUP^{NRC}$	29	94	3.4	<0.01
Endogenous Protein in the Digestive Tract (g kg^{-1} DM)				
ECP	11	11	0.1	1.00
AECP	4	4	0.0	0.99
Total Truly Absorbed Protein in the Small Intestine (g kg^{-1} DM)				
MP (=$AMCP^{NRC}$ + $ARUP^{NRC}$ + AECP)	85	157	3.5	<0.01
Degraded Protein Balance (DPB^{NRC}, g kg^{-1} DM)				
DPB^{NRC}	−25	77	10.4	<0.01

[a]Abbreviations: MCP_{RDP}^{NRC}, microbial crude protein synthesized in the rumen based on RDP (g kg^{-1} DM); MCP_{TDN}^{NRC}, microbial crude protein synthesized in the rumen based on discounted TDN (g kg^{-1} DM); MCP^{NRC}, microbial crude protein synthesized in the small intestine (g kg^{-1} DM); $AMCP^{NRC}$, truly absorbed microbial protein in the small intestine (g kg^{-1} DM); RUP^{NRC}, rumen undegraded feed protein (g kg^{-1} DM) estimated from the NRC dairy 2001 model, calculated as CP (g kg^{-1} DM) × RUP (% CP); $ARUP^{NRC}$, truly absorbed rumen undegraded protein in the small intestine (g kg^{-1} DM); ECP, endogenous protein (g kg^{-1} DM); AECP, truly absorbed endogenous protein in the small intestine (g kg^{-1} DM); MP, metabolizable protein (g kg^{-1} DM); and DPB^{NRC}, degraded protein balance (g kg^{-1} DM). [b]SEM, standard error of mean.

DDGS (79 g kg^{-1} DM) (P < 0.05). FOM values of several feedstuffs were decreased after heating (pressure toasting).[38] However, the decreased FOM value from triticale to triticale DDGS in the current study likely results from the removal of starch during bioethanol production. Nuez-Ortín and Yu[9] reported a similar trend regarding the DVE and OEB values from wheat to wheat DDGS. As compared with the present study, DVE for wheat DDGS was higher than for triticale DDGS (249 vs 151 g kg^{-1} DM), but the OEB was similar between them (72 vs 79 g kg^{-1} DM). The higher DVE value of wheat DDGS reported by Nuez-Ortín and Yu[9] than for triticale DDGS in the present study was likely caused by the higher $ARUP^{DVE}$ value (200 vs 104 g kg^{-1} DM) since the $AMCP^{DVE}$ and ENDP were similar.

Nutrient Supply to Dairy Cattle from Triticale Grain and DDGS Using the NRC-2001 Model. The prediction of protein supply from triticale grain and DDGS to dairy cattle using the NRC-2001 model is presented in Table 9. Triticale had a lower MCP_{RDP}^{NRC} value (P < 0.01) but a higher MCP_{TDN}^{NRC} value than triticale DDGS. Because the final MCP^{NRC} value is limited by the lower the MCP_{RDP}^{NRC} and MCP_{TDN}^{NRC} values, the MCP^{NRC} value for the triticale grain and DDGS was 80 vs 92 g kg^{-1} of DM. The $AMCP^{NRC}$ value increased from triticale grain (51 g kg^{-1} DM) to triticale DDGS (59 g kg^{-1} DM). This increase is different from the decreased $AMCP^{DVE}$ values (73 vs 59 g kg^{-1} DM) predicted using the DVE/OEB system. One possible reason for this is that the DVE/OEB system estimates AMCP exclusively from FOM content, while in the NRC-2001 model, there is a comparison between energy-based MCP and RDP-based MCP. Therefore, the higher MCP_{RDP}^{DVE} value did not account for the increase of $AMCP^{DVE}$. For $ARUP^{NRC}$, there is a significant increase from triticale grain to DDGS. Considering the similar intestinal digestibility of RUP (dRUP, % RUP), this increase is consistent with $ARUP^{DVE}$ in the DVE/OEB system. Both systems calculate ARUP by multiplying RUP and dRUP (% RUP). Considering the different calculation methods (estimation based on unavailable DM for the DVE/OEB system rather than estimation based on the DM of each sample for the NRC-2001 model) for endogenous protein in the two systems, the NRC-2001 model did not distinguish differences in AECP (endogenous protein) between triticale grain and DDGS. However, the truly absorbed protein in the small intestine (DVE or MP) and degraded protein balance (OEB or DPB^{NRC}) show consistent results for both systems. The truly absorbed protein in the small intestine is lower in triticale than in triticale

DDGS. This indicates that bioethanol processing concentrates the protein and consequently increases the total metabolizable protein. The higher degraded protein balance for triticale DDGS suggests that when formulating diets, other feed ingredients with a lower degraded protein balance should be included in the diet to achieve optimum protein efficiency.

In summary, bioethanol processing changed the protein molecular structure of cereal grain. These differences in protein molecular structure between cereal grains and their DDGS can be detected with DRIFT molecular spectroscopy. Triticale grain and DDGS are significantly different in protein molecular structural conformation, metabolic characteristics of protein, chemical profile, and protein and carbohydrate fractions, but similar in estimated energy content. The results indicate that bioethanol processing increases the concentration of nutrients in triticale DDGS except starch. Triticale DDGS provides a higher truly absorbed protein in the small intestine and degraded protein balance for ruminants than the original triticale grain, which indicated that triticale DDGS is a superior source of metabolizable protein than triticale.

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Funding

We thank FOBI-ABIP, BCRC, ADF, NSERC, and the Ministry of Agriculture Strategic Feed Research Chair Program for funding our feed research programs.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Tim McAllister, Lethbridge Research Center, AAFC, and Dr. Oba Masahito, University of Alberta, for providing the samples, and Zhiyuan Niu at the Department of Animal and Poultry Science, University of Saskatchewan, Canada, for helpful assistance.

ABBREVIATIONS USED

ADICP, acid detergent insoluble crude protein; AECP, truly absorbed endogenous protein in the small intestine (NRC-2001); $AMCP^{DVE}$, truly absorbed microbial protein synthesized in the rumen (DVE/OEB); $AMCP^{NRC}$, truly absorbed microbial protein synthesized in the rumen (NRC-2001); $ARUP^{DVE}$, truly absorbed rumen undegraded feed protein in the small intestine (DVE/OEB); $ARUP^{NRC}$, truly absorbed rumen undegraded feed protein in the small intestine (NRC-2001); CA, rapidly degradable soluble sugar fraction (K_d = 200−350% h^{-1}); CB1, intermediately degradable carbohydrate subfraction (K_d = 20−50% h^{-1}); CB2, slowly degradable carbohydrate subfraction (K_d = 2−10% h^{-1}); CC, unfermentable carbohydrate subfraction; D, potential degradable fraction in the in situ ruminal incubation; DE_{3X} (DE_p), digestible energy at three times maintenance (i.e., production level); DOM, digestible organic matter; DPB^{NRC}, degraded protein balance (NRC-2001); dRUP, estimated intestinal digestibility of RUP; DVE, truly absorbed protein in the small intestine defined by DVE/OEB system; ECP, endogenous crude protein (NRC-2001); EDCP, effectively degraded crude protein; EDDM, effectively degraded dry matter; EDNDF, effectively degraded neutral detergent fiber; ENDP, endogenous protein loss; FOM, fermented organic mater; IADP, estimated intestinally absorbable feed protein; IDP, estimated intestinal digestibility of RUP; K_d, degradation rate; MCP, microbial protein synthesized in the rumen; $MCP_{FOM}{}^{DVE}$, microbial protein synthesized based on available energy (fermentable organic matter in the DVE/OEB system); $MCP_{TDN}{}^{NRC}$, microbial protein synthesized based on available energy (total digestible nutrient in the NRC-2001 model); MCP_{RDP}, microbial protein synthesized based on rumen degraded protein; ME, metabolizable energy; ME_{3X} (ME_p), metabolizable energy at three times maintenance (i.e., production level); MP, metabolizable protein; NDFn, nitrogen free neutral detergent fiber (NDFn = NDF − NDICP); NE_g, net energy for growth; NE_{L3X} (NE_{Lp}), net energy for lactation at three times maintenance (i.e., production level); NE_m, net energy for maintenance; OEB, degraded protein balance (DVE/OEB); PA, nonprotein nitrogen (K_d is assumed to be infinity); PB1, rapidly degradable protein subfraction (K_d = 120−400% h^{-1}); PB2, intermediately degradable protein subfraction (K_d = 3−16% h^{-1}); PB3, slowly degradable protein subfraction (K_d = 0.06−0.55% h^{-1}); PC, undegradable protein subfraction; RCBD, randomized complete block design; RDP, rumen degraded protein; RUDM, rumen undegraded dry matter; RUNDF, rumen undegraded neutral detergent fiber; RUP, rumen undegraded protein; RUST, rumen undegraded starch; S, soluble or washable fraction in the in situ ruminal incubation; tdCP, total digestible crude protein; tdFA, total digestible fatty acid; TDN_{1X}, total digestible nutrients at maintenance; TDN_{3X}, total digestible nutrients at three times maintenance; tdNDF, total digestible neutral detergent fiber; tdNFC, total digestible nonfiber carbohydrate; TDP, total digestible feed protein; U, potential undegradable fraction in the in situ ruminal incubation; UDM, undigested dry matter.

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