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Impact of ethanol bioprocessing on association of protein structures at a molecular level to protein nutrient utilization and availability of different co-products from cereal grains as energy feedstocks

Limei Chen [a,b], *Xuewei Zhang* [a,**], *Peiqiang Yu* [a,b,*]

[a] *Department of Animal Science, Tianjin Agricultural University, Tianjin, 300384, China*
[b] *Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada*

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ABSTRACT

The objective of this study was to study impact of ethanol (CAS # 64-17-5) processing on association of protein structures at a molecular level to protein nutrient utilization and availability of different co-products from cereal grains as energy feedstocks in China and to identify the correlation between protein molecular structures and protein nutritional profiles of co-products from ethanol bioprocessing in China in terms of: 1) protein chemical profiles; 2) protein subfractions; 3) total digestible protein; 4) protein degradation and estimated intestinal CP digestibility. The proteins molecular structure were determined using FT/IR-ATR molecular spectroscopy. The protein 2nd structure alpha-helix and beta-sheet were modeled based on amide I component peaks centered at ca. 1650 and 1630 cm^{-1}, respectively identified by using 2nd derivative function. The protein subfractions were analyzed used CNCPS system. Total digestible protein were estimated according to a summary chemical approach in NRC model. Protein degradation and intestinal CP digestibility were determined using in situ nylon bag technique and three-step in vitro method, respectively. The results indicated that co-products from corn and barley differed in both protein nutritional profiles and protein structure parameters in terms of α-helix, β-sheet spectral intensity and their ratio and amide I, amide II spectral intensity and their ratio. Protein amide II height had a weakly positive correlation with ($p < 0.05$) PB2 fraction with $R = 0.53$, but that other protein amide parameters had no correlation with ($p > 0.05$) PA, PB1, PB3 and PC fractions. Protein amide II height had a positive correlation with ($p < 0.05$) TDN with $R = 0.74$. Protein amide II height has a negative correlation with ($p < 0.05$) protein degradability (**R_DCP**) with $R = -0.67$, and a positive correlation with ($p < 0.05$) intestinal protein digestibility (**I_DCP**) with $R = 0.60$, and a positive correlation with ($p < 0.05$) total tract available protein (**T_ACP**) with $R = 0.58$. For protein secondary structure, the α-helix to β-sheet ratio was negatively correlated with ($p < 0.05$) total protein digestibility (**T_DCP**) with $R = -0.56$ and positively correlated with ($p < 0.05$) total digestible crude protein (**tdCP**) with

* *Corresponding author*. College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada. Tel.: +1 306 966 4132.
** *Corresponding author*. Tel.: +86 138 21461560.
E-mail addresses: zhangxuewei63@163.com (X. Zhang), peiqiang.yu@usask.ca (P. Yu).
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R = 0.55. In conclusion, the relationship between mid-IR spectroscopic data and nutritional profiles and digestibility parameters illustrated that the co-products intrinsic structures are closely related to nutritive quality, nutrient utilization and digestive behavior.

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1. Introduction

As to protein value, most of published studies in the literature often focus on protein composition, not protein molecular structure. Protein quality relies not only on total protein content but also on inherent structures, such as protein secondary structures matrix, protein and carbohydrate combined matrix on the molecular basis [1–3]. Protein secondary structures mainly composed of alpha-helix and beta-sheet as well as a small amount of beta-turn and random coil [4,5]. The secondary structures impacted on protein quality, protein utilization and availability [6,7].

The great deal of co-products such as dried distiller grains with solubles (DDGS) has been produced with the high use of cereal grains (corn, wheat and barley) as energy stocks for ethanol and beer producing. Due to starch removal, the remaining chemical components in the distillers grains products become concentrated approximately threefold compare to the parent grain [8,9]. Since its high protein content, DDGS has become a protein sourced feedstuff and is widely used in animal feed and livestock production industry. A systematical study on dry matter, mineral and other nutrient profiles of these co-products has been conducted using conventional 'wet' chemical analysis. On the other hand, several studies have been reported the information with protein structures. Especially the unique studies on correlation between protein molecular structure spectra and protein chemical and nutrient profiles, protein utilization and availability have been conducted by our research team in recent years. However, the research report how the molecular structure changes were associated with nutrient availability in the rumen and intestinal and total nutrient supply is still limited. Therefore, study on the protein molecular structures of these co-products can help us to have a better understanding to protein nutritional value.

The objectives of this study were: (1) to determine the protein chemical profiles, protein degradation and intestinal digestion of the co-products from cereal grains; (2) to reveal the protein molecular structures (in terms of amide I and amide II intensity and their ratio; α-helix and β-sheet intensity and their ratio); (3) to investigate the relationship between protein molecular structures and chemical profiles, protein subfractions partitioned by Cornell Net Carbohydrate and Protein System (CNCPS), protein degradation and digestion; (4) to determine the most important structural features for the co-products from cereal grains. In this study, the hypothesis is that the protein molecular structure was associated with protein utilization and availability. Different ethanol and beer-making co-products have different protein molecular structures and different protein degradation and digestion behaviors.

2. Materials and methods

2.1. Different types and sources of co-products from cereal grain

In this study, five corn DDGS and two barley DDGS samples were collected from seven different manufactures in the north of China from 2012 to 2013. They were numbered as "1, 2, 3, 4, 5, 6, and 7" samples according to the order of sampling.

This work was performed on substrates of unknown provenance, for which the chain of custody is not known. The species and the cultivars cannot be specified and while the authors BELIEVE that this work exemplifies the difference between DDGS processing - there is a reasonable concern that there may be substrate factors that influence the results obtained.

The detailed chemical compositions of each co-product were analyzed by the standard methods of AOAC [11–14]. Their protein and carbohydrate subfractions were partitioned by CNCPS [15,16], and their true digestible nutrition and energy values of these co-products were estimated using the NRC-2001 model [14,17,18].

2.2. ATR-FT/IR molecular spectroscopy

The protein molecular spectral experiment was carried out at the Department of Animal and Poultry Science, University of Saskatchewan. The DDGS samples were ground to pass through a 0.5 mm screen twice (Retsch ZM-1, Brinkmann Instruments (Canada) limited, ON, Canada). The molecular spectral was performed using a JASCO FT/IR-4200 (JASCO Corporation, Tokyo, Japan), which is equipped with an MI RacleTM attenuated total reflectance (ATR) accessory module, and outfitted with a ZnSe crystal and pressure clamp (PIKE Technologies, Madison, WI, USA). Spectra were collected at the mid-IR infrared region from 4000 to 600 cm^{-1} with a spectral resolution of 4 cm^{-1} with 128 scans co-added with 8 replications for each sample. To minimize infrared absorption by CO_2 and water vapor in the ambient air, a background spectrum is collected with 256 scans per 8 replications. JASCO Spectra manager II software was used as a tool to collect data.

2.3. Molecular spectral analysis

OMNIC 7.3 (Thermo-Nicolet, Madison, Wisconsin) software will be used to analyze molecular spectral data. The following chemical functional groups centered differently which are related to protein structure were focused on (peaks centered at): ca. 1648–1658 cm^{-1} (protein 2nd structure α-helix); ca. 1620–1640 cm^{-1} (protein 2nd structure β-sheet); ca. 1650 cm^{-1}

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