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Relationship of carbohydrates and lignin molecular structure spectral profiles to nutrient profile in newly developed oats cultivars and barley grain

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ABSTRACT

The objectives of this study were to quantify the chemical profile and the magnitude of differences in the oat and barley grain varieties developed by Crop Development Centre (CDC) in terms of Cornell Net Carbohydrate Protein System (CNCPS) carbohydrate sub-fractions: CA4 (sugars), CB1 (starch), CB2 (soluble fibre), CB3 (available neutral detergent fibre – NDF), and CC (unavailable carbohydrate); to estimate the energy values; to detect the lignin and carbohydrate (CHO) molecular structure profiles in CDC Nasser and CDC Seabiscuit oat and CDC Meredith barley grains by using Fourier transform infrared attenuated total reflectance (FTIR-ATR); to develop a model to predict nutrient supply based on CHO molecular profile. Results showed that NDF, ADF and CHO were greater ($P < 0.05$) in oat than in barley. The starch content was greater ($P < 0.05$) in barley than in oat. The CDC Meredith showed greater total rumen degradable carbohydrate (RDC), intestinal digestible fraction carbohydrate (FC) and lower total rumen undegradable carbohydrate (RUC). However, the estimated milk production did not differ for CDC Nasser oat and CDC Meredith barley. Lignin peak area and peak height did not differ ($P > 0.05$) for oat and barley grains as well as non-structural CHO. However, cellulosic compounds peak area and height were greater ($P < 0.05$) in oat than barley grains. Multiple regressions were determined to predict nutrient supply by using lignin and CHO molecular profiles. It was concluded that although there were some differences between oat and barley grains, CDC Nasser and CDC Meredith presented similarities related to chemical and molecular profiles, indicating that CDC Meredith barley could be replaced for CDC Nasser as ruminant feed. The FTIR was able to identify functional groups related to CHO molecular spectral in oat and barley grains and FTIR-ATR results could be used to predict nutrient supply in ruminant livestock systems.

1. Introduction

Barley (*Hordeum vulgare* L.) significantly contributes to the world's food supply. Approximately, 64% of the world's barley is channelled to the feed industry which malt production is its most important destiny [1]. Additionally, barley has been used as livestock feed due to its excellent nutrient composition. Since barley price has increased in the last few years, barley grains have been replaced for oats (*Avena sativa* L.) [2]. Although, oats are considered a protein source in feed rations and also contain good source of fibre, fat and minerals, oat hulls are rich in structural carbohydrates and lignin, which could contribute for low metabolizable energy, and consequently low nutritional value when compared to barley grains [2–4]. In order to improve the grains quality, researchers at the Crop Development Centre (CDC) at the University of Saskatchewan (U of S), Saskatoon, Canada, have developed two new varieties of oats: CDC Nasser and CDC Seabiscuit. The CDC Nasser is a feed oat and has been developed to combine low-lignin hull with high fat content and with more digestible hull; while CDC Seabiscuit is a milling oat variety with low fat, high plump content, low thins content and high-yielding.

The traditional "wet chemistry" is used to determine the total composition of feed. However, the traditional "wet chemistry" analysis is not able to identify the inherent molecular structure of the grains and it is not also able to connect the structural information to chemical information [5]. Fourier transform infrared attenuated total reflectance (FTIR-ATR) molecular spectroscopy is a novel technique that has been employed to reveal molecular structural features within the tissue in several kinds of material [6]. This is a rapid, direct, noninvasive, nondestructive and bioanalytical technique that could provide information related to the chemical profile of feed in a short-time using small amount of sample and demanding little or no previous preparation of the samples [7]. The technique is based on the principle that when the infrared (IR) radiation is applied to organic molecules (functional groups), it breaks

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down the molecule's equilibrium stage and promotes energy transitions in a molecule between rotational and vibrational energy. When transitions between rotational and vibrational energy levels occur, this event causes a net change in the dipole moment and the molecule will absorb IR. This IR absorption profile is unique to a specific molecular vibration frequency. Furthermore, when IR passes through a sample, some IR is absorbed by the sample and some IR is transmitted. The resulting spectrum represents the molecular absorption–transmission, which creates a molecular fingerprint of the sample [8].

The hypothesis for this study was that the newly developed oat varieties and CDC Meredith barley (cereal usually used as livestock feed) have similar chemical profiles, ruminal degradation, intestinal digestion and nutrient supply. We also hypothesized that lignin and carbohydrates (CHO) molecular spectral profiles could be revealed by FTIR-ATR in the newly developed varieties of oats and it might have no difference for lignin and CHO molecular spectral profiles between the newly developed oat varieties and barley. In addition, it was hypothesized that there are correlations between FTIR-ATR results and nutrient supply for ruminants. The objectives of this study were (1) to quantify the magnitude of differences in the newly developed oat grains in terms of chemical profile, Cornell Net Carbohydrate Protein System (CNCPS) carbohydrate fractions and nutrient supply (a modeling approach), (2) to detect the lignin and carbohydrates molecular structure features of CDC Nasser and CDC Seabiscuit oat grains (new varieties) in comparison to CDC Meredith barley grain using FTIR-ATR, and (3) to develop model to predict carbohydrate degradation, digestion, and nutrient supply based on CHO molecular profile from oat and barley grains by using FTIR-ATR.

2. Material and Methods

2.1. Samples Collection and Processing

This study was performed at the Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada. The grains, CDC Nasser and CDC Seabiscuit oats and CDC Meredith barley, were supplied by Crop Development Centre (U of S, Saskatoon, Canada). Grains were sampled from harvested plots grown in 2013, 2014, and 2015. The samples were crashed using Sven roller mill with gap to 1.78 mm (Apollo Machine and Products Ltd., Saskatoon, SK, Canada) at the Department of Agricultural Engineering (U of S). An amount of 100 g of each grain from each year was ground using Retsch SM 2000 (Retsch, Inc., Newtown, PA) fitted with a 1.0 mm screen and stored individually in plastic containers with lid at room temperature (20–22 °C) for further chemical analyses, while 50 g from each original grain was ground using Retsch SM 2000 (Retsch, Inc., Newtown, PA), fitted with a 0.5 mm screen and stored individually in plastic containers with lid at room temperature (20–22 °C) for synchrotron-based microspectroscopic analysis.

2.2. Chemical Profile

The samples ground at 1.0 mm were analyzed for quantification of dry matter (DM), organic matter (OM) and ether extract (EE) according to AOAC [9]. The neutral detergent fibre (NDF) was analyzed according to Van Soest et al. [10] and heat-stable enzyme alpha-amylase (Ankon Tech. Corp., Fairport, NY) was added according Mertens [11]. The acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined according to the procedures described by Van Soest et al. [10]. Starch content was performed using the Megazyme Total Starch Assay Kit (Megazyme International Ltd., Wicklow, Ireland, [12]). Sugar content was analyzed according to Dubois et al. [13] method. The contents of non-fibre carbohydrates (NFC) were calculated according to the equation NFC = 100 − (% CP + % NDFp + % EE + % ash), where % NDFp is NDF corrected for protein [10]. The equation: CHO (% DM) = 100 − (% CP + % EE + % Ash) were used to calculate the contents of total CHO, while non-structural carbohydrates (NSC) were calculated considering the starch (% DM) and sugar (% DM) contents [3,10].

2.3. Carbohydrates Fractions Partitioning

The CHO fraction is based on rumen fermentation and microbial action on carbohydrate availability [14]. Carbohydrate profile was partitioned according to CNCPS version 6.5 in the following sub-fractions: CA4 is rapidly degradable (water-soluble carbohydrates) that have a degradation rate of 40–60% h^{-1}; CB1 is the fraction intermediately degradable (starch) with an intermediate degradation rate of 20–40% h^{-1}; CB2 is the available cell wall (soluble fibre) with an intermediate degradation rate of 20–40% h^{-1}; CB3 is the fraction that contains available NDF with a slow degradation rate of 1–18% $h^{-1}$and, CC is the fraction with unavailable CHO (indigestible fibre) [15,16]. In the CNCPS v6.5, the determination of CC fraction has changed and the unavailable NDF value is determined based on 240-h of in vitro digestibility [17]. The energy values of total digestible nutrients at maintenance (TDN_{1X}), digestible energy for lactation (DE_{3x}), and net energy for lactation (NE_{L3x}) were estimated according to the NRC [3] model. The net energy for maintenance (NE_m) and net energy for growth (NE_g) were estimated according to the NRC [18] model. Estimated milk was calculated by dividing NEL for milk protein by NEL concentration in milk, assuming 3.3% protein and 3.5% fat in the milk.

2.4. Molecular Spectroscopy Data Collection and Analyses

The molecular spectral data from CDC Nasser, CDC Seabiscuit oat grains and CDC Meredith barley grains were collected using JASCO FTIR-ATR-4200 spectrometer (JASCO Corp., Tokyo, Japan). Five replicate samples were randomly determined for each treatment. FTIR-ATR-4200 was equipped with a ceramic infrared light source and a deuterated l-alanine-doped triglycine sulfate detector that contains an MIRacle attenuated total reflectance (ATR) accessory module and was outfitted with a ZnSe crystal and pressure clamp (Pike Technologies, Madison, WI). Spectra were collected at the mid-infrared region from approximately 4000 to 700 cm^{-1} with a spectral resolution of 4 cm^{-1} with 128 scans co-added. Background spectra were collected with 256 scans to minimize infrared absorption by CO_2 and water vapour in the environment air.

2.5. Univariate Analysis on Lignin and Carbohydrates Molecular Structure of Oats and Barley

The spectrum data and the classification of molecular features spectral data were analyzed by using the OMNIC 7.3 (Spectra Tech, Madison, WI, USA) software program. The normalization was performed in each obtained spectra which allows the comparison amongst spectra in the same intensity scale, as well as it removes the effects of varying path lengths on the data. Each one of the biological components has unique molecular chemical-structural features, and consequently, they have their own unique IR spectrum. For CHO, depending on bond linkage and type of sugar, the major absorption bands are found in the region at ~1180–950 cm^{-1} and are related to C—O stretching vibrations [8]. However, the bands in this spectra region are very complicated due to the great number of OH and CO bonds and it is customary to look for structural CHO (STCHO), such as cellulose, and non-structural CHO (NSCHO) such as starch in studies with plant material [8,19,20]. In the whole spectra, the main difference between STCHO and NSCHO is the presence of bands of moderate intensity at approximately 1420, 1370 and 1335 cm^{-1}, which indicates the characteristics of STCHO and a peak around 1025 cm^{-1} indicates NSCHO such as endosperm of cereal grain [8,21]. As particularly in the 1100–1025 cm^{-1} region, strong CHO bands are present for STCHO and NSCHO, the 1188–947 cm^{-1} region has been denominated total CHO (ttCHO) [22]. In this study, the assessed items of CDC Nasser and CDC Seabiscuit oats and CDC Meredith barley