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Relationship between protein molecular structural makeup and metabolizable protein supply to dairy cattle from new cool-season forage corn cultivars

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ABSTRACT

Protein solubility, ruminal degradation and intestinal digestibility are strongly related to their inherent molecular makeup. This study was designed to quantitatively evaluate protein digestion in the rumen and intestine of dairy cattle, and estimate the content of truly metabolizable protein (MP) in newly developed cool-season forage corn cultivars. The second objective was to quantify protein inherent molecular structural characteristics using advance molecular spectroscopic technique (FT/IR-ATR) and correlate it to protein metabolic characteristics. Six new cool-season corn cultivars, including 3 Pioneer (PNR) and 3 Hyland (HL), coded as PNR-7443R, PNR-P7213R, PNR-7535R, HL-SR06, HL-SR22, HL-BAXXOS-RR, were evaluated in the present study. The metabolic characteristics, MP supply to dairy cattle, and energy synchronization properties were modeled by two protein evaluation models, namely, the Dutch DVE/OEB system and the NRC-2001 model. Both models estimated significant ($P < 0.05$) differences in contents of microbial protein (MCP) synthesis and truly absorbable rumen undegraded protein (ARUP) among the cultivars. The NRC-2001 model estimated significant ($P < 0.05$) differences in MP content and degraded protein balance (DPB) among the cultivars. The contents MCP, ARUP and MP were higher ($P < 0.05$) for cultivar HL-SR06, resulting in the lowest ($P < 0.05$) DPB. However, none of the cultivars reached the optimal target hourly effective degradability ratio [25 g N g/kg organic matter (OM)], demonstrating N deficiency in the rumen. There were non-significant differences among the cultivars in molecular-spectral intensities of protein. The amide I/II ratio had a significant correlation with ARUP ($r = -0.469$; $P < 0.001$) and absorbable endogenous protein ($AECP^{NRC}$) ($P < 0.001$; $r = 0.612$). Similarly, amide-II area had a weak but significant correlation ($r = 0.299$; $P < 0.001$) with RUP and ARUP, and with $AECP^{NRC}$ ($P < 0.001$; $r = 0.411$). Except total digestible nutrients and $AECP^{NRC}$, the amide-I area did not show significant correlations with DVE/OEB and NRC predicted protein fractions. This study shows that molecular spectroscopy can be potentially used as a rapid tool to quantify protein molecular makeup and screen the protein nutritive value of forage corn.

1. Introduction

After grass, ensiled corn (*Zea mays L.*) is the major forage component in the ration of dairy cows. The crop has a comparatively stable and high yield under different agronomic and climatic conditions, high metabolizable energy content and good ensiling characteristics. Moreover, the inclusion of corn silage in grass based diets improves the performance of dairy cows [1–3] and finishing beef cattle [4,5]. Traditionally corn was grown in warmer climates, however, recent progress in plant breeding and improvements in agronomic practices have markedly increased the potential yield and metabolizable energy content of corn at more northern latitudes [4]. As a result, the area under corn production has been increased rapidly in temperate environment zones. However, the nutritive value of corn grown in cooler climates such as in Canadian prairies (Saskatchewan) is different from conventional corn grown in warm climates [6]. These differences are attributed to the shorter growing season and lower growing temperatures in Canadian prairies as compared to warm season corn, such as in the United States [7]. Moreover, there are known differences in nutrient composition of corn due to genetic differences in warm and cool-season cultivars [8].

Corn cultivars requires 2000 or more crop heat units (CHU) to reach harvest maturity, with 45% kernel dry matter, in Western Canada [9].The CHU is the summation of day-time temperatures above 10 °C and night-time temperature above 4 °C from seeding to harvest. For such environmental conditions, recently, 6 cool-season corn cultivars have been developed: Hyland SR22, Hyland Baxxos RR and Hyland

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SR06, and Pioneer P7213R, Pioneer P7443R, and Pioneer P7535R. However, to the author's knowledge, no systematic research has been conducted on the metabolic characteristics of protein in the gastrointestinal tract, and total truly metabolizable protein (MP) supply to dairy cattle from the new cool-season corn cultivars.

The nutritive value of feed protein in ruminants not only depends on the amino acid content and composition but also on the rate and extent of protein degradation in the rumen, microbial protein (MCP) synthesis from the degraded protein, and intestinal digestibility of rumen undegraded feed protein (RUP), which markedly influence the supply of metabolizable amino acids to the animal [10]. With the recent progress in feed protein metabolism in ruminants, various mechanistic protein evaluation models such as the NRC-2001 dairy model [11], and the DVE/OEB system [12], have been developed to quantitatively estimate MP supply to dairy cows. The present study was therefore designed to evaluate the metabolic characteristics of the protein in the rumen and intestine of dairy cattle, and estimate the potential MP supply to dairy cow from new cool-season silage corn cultivars. We hypothesized that corn cultivar influences digestive characteristics of protein, MCP synthesis in rumen, and truly absorbable MP availability in the small-intestine.

Recent research has demonstrated that the intrinsic molecular structures of protein strongly influence protein degradation in the rumen and digestibility of RUP in post-ruminal tract [10,13,14].Moreover, Fourier transform infrared attenuated total reflectance (FT/IR-ATR) molecular spectroscopy can be used to quantify molecular structural features of feeds biopolymers, such as protein [14,15]. The amide-1: amide-II ratio and α-helix:B-sheet ratios are important indices for protein molecular structural characteristics [14], which may be used as a marker for estimating protein digestion in ruminants. However, studies on protein internal structures of forages are scarce, because the wet chemical analyses do not reveal information regarding molecular structures and the advanced molecular spectroscopic techniques are seldom used in feed research [14].Therefore, the second objective was to investigate the correlation between protein molecular structural characteristics, and their digestion and utilization in digestive tract of dairy cows.

2. Materials and Methods

2.1. Corn Cultivation, Experimental Design and Sampling

Six newly developed cool-season corn cultivars were sown in 24 plots on 20 May 2011 in the research fields of Saskatchewan Irrigation Diversification Centre (Outlook, SK, Canada). The cultivars included three Pioneer (PNR; Pioneer Hi-Bred International Inc., Johnston, IA, USA) and three Hyland (HL; Hyland Seeds, Blenheim, ON, Canada), which were coded as PNR-7443R, PNR—P7213R, PNR-7535R, and HL-SR06, HL-SR22, HL-BAXXOSRR. Plots were blocked within 4 fields, and all cultivars were sown in each field. Whole crop samples were collected on September 29, 2011 after a target of 2160 CHU was achieved. The detailed sampling procedure is reported previously [16].

2.2. Chemical Analyses and in Situ Incubations

Green feed samples were chopped to 1 cm size, dried in air draft-oven (55 °C for 72 h), and then ground through a 1 mm screen (Retsch ZM-1, Brinkmann Instruments Canada Ltd., Mississauga, Ontario, Canada). Dry matter (DM, method 930.15), ash (method 942.05) and crude protein (CP; method 984.13) contents were analyzed according to the procedure of the AOAC [17].

Three dry Holstein dairy cattle, fitted with rumen cannula with an internal diameter of 10 cm (Bar Diamond Inc., Parma, ID, USA) were used for the in situ incubations. The cows were cared and handled according to the standard guidelines of the Canadian Council on Animal Care [18].The cows were individually fed with a balanced total mixed ration, containing 570 g/kg of barley silage, 100 g/kg of alfalfa hay, 50 g/kg of dehydrated alfalfa pellets, and 280 g/kg of concentrates on DM basis. The rumen in situ incubations were performed using the standard nylon bag technique as described earlier by Khan et al. [19].

2.3. Nutrient Supply With the DVE/OEB Model

Using mechanistic models to estimate MP supply to dairy cattle from different feeds is an integral part of the modern feed evaluation systems [20]. The Dutch protein evaluation system (the DVE/OEB_{1991} system), as described by Tamminga et al. [12] was used to estimate the truly absorbed MP supply to dairy cattle from the different cultivars of forage corn. In the DVE/OEB system, two characteristics are calculated for each feed: true protein digested in the intestine (DVE) and the rumen degradable protein balance (OEB/DPB). Of these, DVE represents the protein value of a feed, while OEB is the difference between the potential MCP synthesized on the basis of available rumen degradable protein (RDP) and that on the basis of available rumen degradable energy [20]. The DVE is obtained from three components: (i) feed crude protein undegraded in the rumen but digested and absorbed in the small intestine ($ARUP^{DVE}$) (ii) microbial true protein synthesized in the rumen and digested and absorbed in the small intestine ($AMCP^{DVE}$), and (iii) a correction for endogenous protein lost in the digestive processes (ENDP). The DVE was calculated as follows,

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

The detail models and concepts for calculations of $ARUP^{DVE}$, $AMCP^{DVE}$ and ENDP have been reported earlier [21].

The DPB value (OEB) of a feed is the balance (difference) between the potential microbial protein (MCP) synthesized based on RDP (MCP_{RDP}, in the DVE/OEB system) and the potential MCP synthesized based on energy extracted from anaerobic fermentation of organic matter (MCP_{FOM}) in the rumen. Therefore, DPB^{DVE} was calculated as follow

$$\mathrm{DPB^{DVE}} = \mathrm{MCP_{RDP}} - \mathrm{MCP_{FOM}}$$

where MCP_{RDP} was calculated as,

$$\mathrm{MCP_{RDP}} = \mathrm{CP\ (g/kg\ \ DM)} \times \left[1 - \left(1.11 \times \frac{\mathrm{RUP\ (\%CP)}}{100}\right)\right]$$

The factor 1.11 in the formula was taken from the French PDI system, and represents the regression coefficient between in situ RUP and in vivo RUP [22].

2.4. The NRC 2001 Model

The detailed concepts and formulas of the NRC-2001 dairy model are provided by NRC [11]. Briefly, the total truly MP supply from feed to dairy cattle is estimated from (i) truly absorbable ruminally synthesized MCP ($AMCP^{NRC}$) (ii) truly absorbable RUP ($ARUP^{NRC}$), and (iii) truly absorbable rumen endogenous protein ($AECP^{NRC}$). The total truly MP was calculated as follows,

$$\mathrm{MP\ (g/kg\ DM)} = \mathrm{AMCP^{NRC}(g/kg)} + \mathrm{ARUP^{NRC}(g/kg)} + \mathrm{AECP^{NRC}(g/kg)}$$

The detail models and concepts for calculations of $AMCP^{NRC}$, $ARUP^{NRC}$, and AECP have been reported earlier [21].

2.5. Rumen Degradation Model

The first-order kinetic degradation model as described by Ørskov and McDonald [23] was used to describe the rumen degradation