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Technical note: A modified method to quantify prolamin proteins in dry and high-moisture corn

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ABSTRACT

Numerous studies have evaluated laboratory methods to quantify prolamin proteins in dry corn; however, the utility of methods to quantify functional prolamins, which impede starch digestibility, in high-moisture corn (HMC) is less defined. As a result, a common rapid turbidimetric (rTM) laboratory procedure was modified (rapid Bradford method, rBM), extracting buffer-soluble proteins before prolamin solubilization in an effort to better quantify functional prolamins in HMC. Twenty samples of dry and HMC were evaluated by rTM and rBM procedures. Prolamin concentration in dry corn, as estimated by rTM or rBM methods, ranged from 6.12 to 2.20 g/100 g of DM or 5.46 to 2.41 g/100 g of DM, respectively. Dry corn mean prolamin concentrations, as estimated by rTM or rBM methods, were similar at 3.65 or 3.66 g/100 g of DM. Prolamin concentration in HMC, as estimated by rTM, ranged from 4.99 to 3.24 g/100 g of DM, with a mean prolamin concentration of 4.19 g/100 g of DM, but estimation of prolamins in HMC by the rBM method resulted in lower mean (4.19 vs. 3.24 g/100 g of DM) prolamin concentration. Prolamin concentration in dry and HMC measured by rTM was negatively related to peak absolute rates (PAR; mL/0.1 g of DM) of in vitro gas production. However, relationships between rTM prolamin concentration and PAR were not homogeneous and were different between dry and HMC. Prolamin proteins as determined by rBM were likewise negatively related to PAR, but corn type did not influence rBM prolamin concentration by PAR relationships. Data suggest that the rBM method defined more similar functional prolamin proteins, which impede starch degradability, in dry and HMC.
Key words: prolamin, high-moisture corn, starch degradability

Technical Note

Corns with greater percentages of prolamin proteins have been shown to reduce starch degradability or digestibility, both in vitro (Hoffman et al., 2012) and in vivo (Lopes et al., 2009). Recently, Larson and Hoffman (2008) defined a low-cost, rapid turbidity method (**rTM**) to quantify prolamin proteins (zein) in dry corns of varying endosperm type. Since publication, the utility of the rTM has been evaluated in numerous studies. Giuberti et al. (2011) found the rTM to yield similar prolamin values for dried corn compared with the intensive methods of Landry et al. (2000) but yielded lower prolamin values compared with the method of Hamaker et al. (1995). Based on the work of Landry et al. (2002), Giuberti et al. (2012) modified the rTM, replacing isopropanol with *tert*-butanol to extract greater percentages of prolamins from dry and high-moisture corn (**HMC**).

The utility of the rTM in ruminant nutrition has also been evaluated in numerous studies. Lopes et al. (2009) quantified prolamin proteins using the rTM in near-isogenic variants of an Oh43x W64A normal dent endosperm hybrid carrying *floury-2* or *opaque-2* alleles. Hybrids carrying *floury-2* or *opaque-2* alleles contained significantly less prolamin protein, resulting in improved in vivo starch digestibility in lactating dairy cows. Hoffman et al. (2012) and Masoero et al. (2011) demonstrated negative relationships between rTM prolamin contents of dry corn and in vitro starch degradability.

The biological utility of the rTM in evaluating prolamins in HMC or corn silage is less defined because relationships between rTM prolamins in HMC or corn silage and starch degradability are not well established. Using HPLC methods, Hoffman et al. (2011) demonstrated that fermentation of HMC reduced all α, β, γ, and δ prolamin-zein subunits of the starch-protein matrix from 10 to 40%, but rTM did not detect changes in prolamin concentration induced by fermentation. Likewise, Hoffman et al. (2012) could not illicit relationships between prolamin protein in HMC and peak absolute rate (**PAR**) of in vitro gas production when prolamin was determined by rTM. These data suggest

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that the rTM as defined by Larson and Hoffman, (2008) may improperly define the biological functionality of prolamin proteins, as related to starch digestibility, in HMC. It was the objective of the current study to explore whether the rTM could be modified to quantify prolamin proteins in HMC, which are biologically more relevant to starch degradability in ruminants.

The rTM of Larson and Hoffman, (2008) can be generalized as follows: dried ground corn is defatted using acetone (100%), filtered, and dried, and acetone-insoluble DM is retained. Prolamins in acetone-insoluble DM are solubilized with 55.0% aqueous isopropyl alcohol containing 0.6% 2-mercaptoethanol. Turbidity of prolamin protein is achieved by incorporation of aqueous alcohol-solubilized prolamins with 0.15 *M* TCA. The degree of turbidity is measured by the logarithm of absorbance of the sample at 440 nm on a spectrophotometer and prolamin concentration is quantified using a standard absorbance curve developed from purified zein.

Intuitive shortcomings of the rTM exist with regard to measuring prolamins in HMC. First, the acetone rinse is designed to remove fat, with minimal loss of total proteins in the sample, because only prolamin proteins are assumed to become turbid in 0.15 *M* TCA (Larson and Hoffman, 2008). This assumption may be invalid for HMC, as proteolytic activity during fermentation may hydrolyze prolamin proteins into dysfunctional peptides, which may express turbidity in 0.15 *M* TCA. Second, turbidity in chloroacetic acid is rapid but not purely quantitative because turbidity is affected differently by differences in the tertiary structure of proteins (Ebina and Nagai, 1979).

Due to potential shortcomings and new information regarding efficiency of *tert*-butanol to extract prolamins (Giuberti et al., 2012), the following modifications were made to the rTM in an effort to improve its biological utility in determining functional prolamins, as related to starch digestibility, in HMC: (1) to facilitate removal of nonfunctional endosperm proteins (albumins, globulins, and hydrolyzed zein) in corn, defatting the sample with acetone in the rTM was replaced with a borate phosphate buffer extraction to remove buffer-soluble proteins as defined by Krishnamoorthy et al. (1983); (2) use of isopropanol to extract prolamins was abandoned and replaced with *tert*-butanol to more efficiently extract prolamins (Giuberti et al., 2012); and (3) because extraction of prolamins with *tert*-butanol without prior defatting of the sample with acetone would influence turbidity measurements, turbidity in 0.15 *M* TCA was abandoned and replaced with a Bradford protein assay (Bradford, 1976) to quantify prolamin proteins solubilized in *tert*-butanol. The changes were coalesced and defined as a rapid Bradford method (**rBM**) to quantify prolamin proteins in dry and HMC.

Buffer-soluble proteins in dried ground corn are extracted using a borate phosphate buffer as defined by Krishnamoorthy et. al. (1983), and buffer-insoluble DM (**biDM**) is retained. Prolamins in biDM are solubilized with 60.0% aqueous *tert*-butyl alcohol containing 0.6% 2-mercaptoethanol. Prolamin proteins are quantified using Coomassie Brilliant Blue dye (BP100-25 Brilliant Blue G-250; Fisher Scientific, Fair Lawn, NJ) in an acidic media, with the reddish dye turning blue when it binds to protein (Bradford, 1976). The colorimetric shift is measured on a spectrophotometer at a 595-nm wavelength and compared with a prolamin standard. The apparatus include a spectrophotometer set to 595 nm, forced-air ovens set to 55 and 105°C, balance accurate to 1 mg, centrifuge capable of 2,250 × *g*; vortex mixer, a cyclone mill fitted with a 1-mm screen, orbital shaker capable of 240 rpm, funnels capable of holding 125-mm Whatman 541 filter paper, 40-mL centrifuge tubes, 5-mL plastic disposable cuvettes, pipettes capable of dispensing 50 μL or 1 to 10 mL, and glassware including test tubes, 100- and 1,000-mL volumetric flasks, and 125-mL Erlenmeyer flasks with stoppers.

Reagents included borate phosphate buffer [12.2 g of sodium phosphate ($NaH_2PO_4 \cdot H_2O$) and 8.91 g of sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) per liter of distilled water (**dH_2O**), stirred at medium speed until completely dissolved]; aqueous alcohol solution [600 mL of *tert*-butanol (CAS 75-65-0 Fisher Scientific), 6 mL of 2-mercaptoethanol (Fisher Scientific O34461-100), brought to volume in a 1,000-mL volumetric flask with dH_2O]; Bradford reagent [100 mg of Coomassie Brilliant Blue powder dissolved in 5 mL of reagent alcohol (Fisher scientific A962P-4) and 100 mL of 85% (wt/vol) phosphoric acid (Fisher Scientific UN1805) and brought to volume in a 1,000-mL volumetric flask with dH_2O]; and finally, a prolamin standard prepared in a 100-mL volumetric flask: 94 mg of purified zein (CAS 9010-66-6; Acros Organics, Fair Lawn, NJ), 6 mg of albumin standard (23209; Thermo Scientific, Waltham, MA), brought to volume with aqueous alcohol solution and stirred for 1 h. The 94:6-mg ratio of purified zein to albumin was used as the standard to increase the absorbance per unit of CP (N) upon Bradford reagent addition because a prestudy evaluation (data not shown) of absorbance at 595 nm per unit of true N concentration (Lachat Instruments, 1995) in *tert*-butanol, 2- mercaptoethanol extracts of unknown corns was greater per unit of N compared with absorbance per unit of N of a pure zein standard.

Corn samples were dried in a 55°C forced-air oven for 24 to 48 h. Samples were ground in a cyclone mill

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