

## Technical Note: A Method to Quantify Prolamin Proteins in Corn That Are Negatively Related to Starch Digestibility in Ruminants

J. Larson and P. C. Hoffman<sup>1</sup>

Department of Dairy Science, University of Wisconsin, Madison 53706

### ABSTRACT

Compared with flourey or high-moisture corns, dry corn with a greater percentage of vitreous endosperm has been demonstrated to be negatively related to starch digestibility and milk yield of lactating dairy cows. Starch granules in corn are encapsulated by hydrophobic prolamin proteins that are innately insoluble in the rumen environment. Corn prolamin proteins are named zein, and laboratory methods to quantify zein exist but are seldom employed in ruminant nutrition because of their arduous nature. In this study, advances in cereal chemistry were combined with rapid turbidimetric methods yielding a modified turbidimetric zein method (mTZM) to quantify zein in whole corn. Ten dry corns containing unique endosperms were evaluated using the mTZM. Corns with flint, dent, flourey, or opaque endosperms were found to contain 19.3, 11.3, 5.8, and 4.9 g of zein/100 g of starch, respectively. The ability of mTZM to differentiate corn endosperm types as defined by least significant difference was 2.6 g of zein/100 g of starch. Ten high-moisture corns of varying moisture content were also evaluated using the mTZM. Zein content of high-moisture corns as defined by mTZM ranged from 8.3 to 2.8 g of zein/100 g of starch with a least significant difference of 1.2 g of zein/100 g of starch. The mTZM determined that zein contents of high-moisture, flourey, and opaque corns were markedly less than those of flint and dent dry corns, indicating that mTZM has the ability to quantify starch granule encapsulation by hydrophobic prolamin proteins in whole corn.

**Key words:** zein, turbidity, corn endosperm

Corn containing a greater percentage of vitreous endosperm correspondingly contains greater levels of starch-encapsulating prolamin proteins compared with flourey or opaque corns (Hamaker et al., 1995; Philippeau et al., 2000). Corn with greater percentages of vitreous endosperm have reduced in vitro or in situ starch

degradability (Philippeau et al., 2000; Correa et al., 2002; Ngonyamo-Majee et al., 2008), and in vivo starch digestion (Allen et al., 2008) and milk yield (Taylor and Allen, 2005) of lactating dairy cows are reduced when cows are fed vitreous corn compared with flourey corn.

Numerous methods (Landry and Moureaux, 1970; Wallace et al., 1990; Hamaker et al., 1995; Landry et al., 2000) are available to quantify zein in isolated corn endosperm. These methods (Landry and Moureaux, 1970; Wallace et al., 1990; Hamaker et al., 1995; Landry et al., 2000) divide corn endosperm proteins into multiple protein fractions (albumins, globulins, prolamins, and glutelins), which may be too extensive for ruminant nutrition because only the hydrophobic prolamins have been recognized to be negatively associated with starch degradability (Philippeau et al., 2000) in ruminants.

Alternatively, turbidimetric methods (Paulis et al., 1974; Aboubacar et al., 2003; Olakojo et al., 2007) have been used to quantify zein or kafarin in ground whole corn or sorghum. Turbidimetric methods solubilize prolamin proteins in aqueous alcohol and, after solubilization, quantify prolamin content by degree of turbidity upon addition to TCA.

It was the objective of this study to explore whether turbidimetric procedures could be combined with advances in cereal chemistry to quantify prolamin proteins in whole dry or high-moisture corn (**HMC**) as an aid to advance the understanding of factors that influence starch digestion in ruminants.

Turbidimetric prolamin methods (Paulis et al., 1974; Drochioiu et al., 2002; Aboubacar et al., 2003; Olakojo et al., 2007) use different turbidity solvents (Paulis et al., 1974; Olakojo et al., 2007), tend to yield low zein values (Olakojo et al., 2007), or do not incorporate advances in cereal chemistry (Landry et al., 2002). Specific issues relating to advances in cereal chemistry include the following. First, turbidimetric zein methods (Drochioiu et al., 2002; Olakojo et al., 2007) have used 70.0% aqueous ethanol with 0.5% sodium acetate to solubilize zein. Landry et al. (2002) referenced a more-complete extraction of all zein subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) using 55.0% isopropyl alcohol as the solvent and 0.6% 2-mercaptothanol as the reducing agent. Second, standard curves for turbidimetric zein procedures (Drochioiu et al., 2002;

Received May 20, 2008.

Accepted August 19, 2008.

<sup>1</sup>Corresponding author: pchhoffma@wisc.edu

Olakojo et al., 2007) have been developed by determining the aqueous alcohol-soluble CP content of selected corn samples and relating the turbidity (absorbance) of the same samples to their alcohol-soluble CP content. Developing standard curves from a universally available zein source (purified zein) would be advantageous to facilitate procedural uniformity among laboratories wishing to conduct such assays. As a result the following modified turbidimetric zein method (**mTZM**) was developed and is presented as follows.

### Concept

Dried ground corn is defatted using acetone (100%), filtered, and dried; acetone-insoluble DM (**aiDM**) is retained. Zein(s) in aiDM are solubilized with 55.0% aqueous isopropyl alcohol containing 0.6% 2-mercaptoethanol. The turbidity of zein is achieved by incorporation of aqueous alcohol-solubilized zein with 0.15 *M* TCA. Degree of turbidity is measured by log absorbance of the sample at 440 nm on a spectrophotometer, and zein is quantified using a standard absorbance curve developed from purified zein.

### Apparatus

Apparatus included a spectrophotometer with a double beam set at 440 nm; convection ovens capable of drying samples at 55 and 105°C; balance capable of weighing to 0.001 g; centrifuge fit to hold 50-mL tubes and capable of operating at  $4,500 \times g$ ; grinding mill capable of grinding samples to 1 mm; vortex mixer; magnetic stir plates and bars; tubes and glassware including 50-mL plastic centrifuge tubes, 100-mL Erlenmeyer flask, 10-mL spectrophotometer tubes or cuvettes; Buchner funnels capable of holding 125-mm 541 Whatman filter paper; and apparatus to apply light vacuum (optional).

### Reagents

Reagents included acetone (100%); aqueous alcohol solution (275 mL of 100% isopropyl alcohol, 3 mL of 2-mercaptoethanol, brought to 500 mL with distilled H<sub>2</sub>O); and TCA solution (0.15 *M*; 12.5 g of TCA brought to 500 mL with distilled H<sub>2</sub>O).

Purified zein was obtained from Acros Organics (17931100, Thermo Fisher Scientific, Waltham, MA). Zein standard solutions were prepared as follows. One hundred milligrams of zein body CP equivalents from purified zein were added to a 100-mL volumetric flask, brought to volume with aqueous alcohol solution, and mixed thoroughly for 1 h (1,000 µg/mL zein solution). The actual amount (mg) of purified zein DM required

to make the 1,000 µg/mL zein standard solution is dependent on zein body, CP and DM contents of the purified zein. Zein bodies in purified zein are estimated to be 90% of total CP (Kale et al., 2007). The CP ( $\pm 80.0\%$ ) and DM ( $\pm 95\%$ ) contents of purified zein should be ascertained by Kjeldahl and DM analysis (AOAC, 1990) before preparing the 1,000 µg/mL zein standard solution using a factor of 5.71 instead of 6.25 to convert N to CP (Hamaker et al., 1995). The actual amount (mg) of purified zein required ( $\pm 146$  mg) for the 1,000 µg/mL zein solution =  $[(100/\text{purified zein CP, \% of DM} \times 0.9)]/\text{purified zein DM percentage}$ .

Zein standard solutions containing 750, 500, 250, and 0 µg/mL zein were subsequently developed by mixing 15, 10, 5, and 0 mL of the 1,000 µg/mL zein solution with 5, 10, 15, and 20 mL of the aqueous alcohol solution, respectively.

### Sample Preparation

Corn samples were dried in a 55°C forced air oven for 24 to 48 h. Samples were ground in a Udy (Udy Corp., Boulder, CO) or equivalent mill with a 1-mm screen. The DM of the sample was determined by drying at 105°C for 3 h.

### Defatting Procedure

One gram of dry, ground (1-mm grind) corn was placed into a 100-mL Erlenmeyer flask. Twenty milliliters of acetone (100%) was added, the flask was placed on magnetic stir plate and the contents mixed for 1 h. The hot weight of 125-mm 541 Whatman filter paper was recorded to the nearest 0.001 g, folded, and fit to a Buchner funnel. The acetone and corn mixture (after rinsing the flask with acetone to remove remaining corn particles) was then filtered through the funnel. The filter paper containing the aiDM was dried at 55°C for 24 h. After drying, the filter was weighed, the weight recorded to the nearest 0.001 g, and the defatting DM recovery (**dfDMr**: see equation below) was calculated. Typically, defatting DM recoveries are near 90.0%. The DM content of the aiDM was determined by drying in a 105°C oven for 3 h with the DM of aiDM retained for inclusion in final zein determination calculations.

### Zein Solubilization

Two hundred milligrams of aiDM from the defatting procedure was weighed in duplicate into 50-mL polystyrene centrifuge tubes. Then, 20 mL of the aqueous alcohol solution containing 2-mercaptoethanol was added and the mixture placed on a magnetic stir plate and mixed for 4 h. After mixing, the solution was cen-

trifuged at  $4,500 \times g$  for 20 min; 0.5 mL of the supernatant was pipetted into 10-mL spectrophotometer tubes or cuvettes containing 5.5 mL of 0.15 M TCA solution and vortexed. The turbidity was allowed to equilibrate for 45 min.

### Standard Curve Preparation

To prepare the standard curve, 0.5 mL of each of the 1,000, 750, 500, 250, and 0  $\mu\text{g/mL}$  zein standard solutions was pipetted into spectrophotometer tubes or cuvettes containing 5.5 mL of 0.15 M TCA solution and vortexed; the turbidity was allowed to equilibrate for 45 min. The log absorbance was read at 440 nm on the spectrophotometer. By specific spectrophotometer standard curve functions or by external equation, a standard curve was fit where the known zein content of the standard curve samples ( $\mu\text{g/mL}$ ) were the dependent variables and log absorbance at 440 nm of the standard curve samples were the independent variables.

### Zein Quantification and Calculations

The log absorbance at 440 nm of samples with unknown zein concentration from the zein solubilization step was determined, and the content of zein ( $\mu\text{g/mL}$ ) was predicted from the standard curve.

Defatting DM recovery was calculated as

$$\text{dfDMr, \% of DM} = (\text{filter paper} + \text{aiDM residue, g} - \text{filter paper weight, g}) / (\text{dry corn sample weight, g} \times \text{DM});$$

and zein content (g/100 g of DM) was calculated as

$$\text{Zein content (g/100 g of DM)} = \{[(\text{zein, } \mu\text{g/mL}) / (\text{aiDM sample weight, mg/DM} \times 50)] \times \text{dfDMr}\} \times 100,$$

where DM is the DM content of the sample material defined within the equation(s).

To evaluate repeatability and ability of mTZM to discern corn endosperm type, 10 corns of varying endosperm type were procured from a commercial corn breeding company (Brown Seed Genetics, Bay City, WI) and corn breeding seed stock at the University of Wisconsin. Corn endosperms types included 5 dent (BSG 07AB05, BSG 214686, BSG 07ABO5X1736, W64aX-Oh43, Reid Yellow Dent), 2 flourey (BSG 1736f2/f2, W64aXOh43f2/f2), 1 opaque (W64aXOh43o2/o2), 1 waxy (BSG A635wx), and 1 flint (Peace River Flint) varieties. Germplasm sources were from both hybrid

and inbred lines providing robust physical and chemical endosperm characteristics (Ngonyamo-Majee et al., 2008).

Whole corn kernels were ground through a Udy mill fit with a 1-mm screen and analyzed for DM, CP, fat (AOAC, 1990), and starch (Ehrman, 1996). Corns were determined for zein by mTZM with each sample run in duplicate in 2 separate runs to provide inference of method precision. An mTZM zein value was determined as the mean of duplicate samples for the individual run. Estimates of mTZM precision were obtained by calculating the between-run repeatability standard deviation ( $S_r$ ) and the corresponding relative repeatability standard deviation [ $RS_r = (S_r/\text{mean value}) \times 100$ ], which expresses laboratory error as a percentage of the mean (Theander et al., 1995). Finally, least significant difference (LSD) was calculated using the ANOVA procedures of SAS (SAS Institute, 2001) to estimate the potential of mTZM to discriminate corn endosperm types.

A second set of 10 of HMC were randomly selected from commercial samples of HMC sent to the Marshfield Soil and Forage Testing Laboratory (Marshfield, WI) for routine analysis. Samples of HMC were prepared and analyzed by the same procedures as for the dry corn. Estimates of mTZM precision ( $S_r$ ,  $RS_r$ , and LSD) for HMC were also calculated or derived as described previously.

Before evaluation of mTZM to determine zein in corns, 2 ancillary evaluations of mTZM were made. First, the DM, CP, fat, and starch contents of the 10 corns of varying endosperm characteristics were evaluated by the aforementioned procedures before and after defatting (aiDM). The defatting step of mTZM resulted in an  $80.1 \pm 5.1\%$  reduction in the fat content of corn. Starch and DM losses associated with the defatting step of mTZM were  $10.8 \pm 1.0$  and  $10.9 \pm 1.0\%$ , respectively, but the loss of protein was negligible ( $3.0 \pm 2.7\%$ ).

Second, comparison of zein as determined by mTZM and zein determined by another laboratory method would have been desired but no predominant method to determine zein in whole corn has been established. To evaluate continuity with previously published literature, alcohol-soluble protein of aiDM (Landry et al., 2000) for the 10 corns of varying endosperm type was evaluated using a Bradford assay (Pierce, Rockford, IL; Bradford, 1976) and compared with zein concentration as estimated by mTZM. There was close agreement ( $R^2 = 0.88$ , bias = 0.13 g/100 g of DM) between alcohol-soluble protein of aiDM (Landry et al., 2000) and zein contents of aiDM estimated by mTZM for the 10 corns of varying endosperm type. Data suggest the solubilization step and quantification of zein by turbidity (mTZP procedure) yielded similar zein estimates as zein solu-

**Table 1.** Determination of zein in corn varieties by a turbidimetric method<sup>1</sup>

Corn variety	Nutrient, % of DM			Zein, <sup>2</sup> g/100 g of DM			Zein	
	CP	Fat	Starch	1	2	Mean	g/100 g of CP	g/100 g of starch
Flint								
Peace River Flint	17.1	7.4	47.5	8.7	9.6	9.2	53.5	19.3
Dent								
Reid Yellow Dent	9.6	3.9	64.0	3.2	4.2	3.7	38.6	5.8
BSG 07AB05	10.5	3.1	65.8	7.0	7.8	7.4	70.8	11.3
BSG 214686	12.3	3.4	64.2	8.5	7.6	8.1	65.8	12.6
BSG O7ABO5X1736	11.0	3.3	65.7	8.6	9.1	8.8	80.2	13.4
W64aXOh43	12.9	3.7	71.5	8.6	10.5	9.5	74.0	13.3
Waxy								
BSG A635wx	11.7	2.9	60.6	7.6	9.0	8.3	71.0	13.6
Floury								
BSG 1736fl2/fl2	10.6	4.2	68.4	4.9	4.5	4.7	44.5	6.9
W64aXOh43fl2/fl2	10.3	4.3	68.2	2.9	3.5	3.2	30.8	4.7
Opaque								
W64aXOh43o2/o2	11.8	4.3	64.3	3.1	3.3	3.2	26.8	4.9
Mean						6.6	55.6	10.6
SD						2.6	19.4	4.8
S <sub>r</sub> <sup>3</sup>						0.8	7.2	1.3
RS <sub>r</sub> <sup>3</sup> % of mean						12.8	13.0	12.7
LSD						1.6	13.5	2.6

<sup>1</sup>BSG = Brown Seed Genetics (Bay City, WI); other varieties were from corn breeding seed stock at the University of Wisconsin (Madison).

<sup>2</sup>Values 1 and 2 indicate individual zein determinations by the turbidimetric method.

<sup>3</sup>S<sub>r</sub> = repeatability standard deviation; RS<sub>r</sub> = relative repeatability standard deviation.

bilization by Landry et al. (2000) and quantification of zein as protein by a Bradford assay.

Zein content, measures of mTZM precision, and various units of expressing zein for the 10 corns of differing endosperm type are presented in Table 1. Zein, as estimated by mTZM, ranged from 9.5 to 3.2 g/100 g of DM with a mean zein content of 6.6 g/100 g of DM. The S<sub>r</sub> of mTZM was 0.8 g/100 g of DM and the RS<sub>r</sub> was

12.8% of the mean. The RS<sub>r</sub> is the between-run assay error expressed as a percentage of the mean, which is an index of method precision (Theander et al., 1995). Mentink and Hoffman (2006) observed the RS<sub>r</sub> of CP, starch, and fat analyses to be 1.2, 3.5, and 8.6% of the mean, respectively. Theander et al. (1995) observed the RS<sub>r</sub> of Klason lignin analysis to be >20.0% of the mean. Based on literature comparisons (Theander et al., 1995;

**Table 2.** Determination of zein in high-moisture corn by a turbidimetric method<sup>1</sup>

Corn moisture, %	Nutrient, % of DM			Zein, <sup>2</sup> g/100 g of DM			Zein	
	CP	Fat	Starch	1	2	Mean	g/100 g of CP	g/100 g of starch
22.6	9.9	3.4	69.7	5.5	6.1	5.8	58.6	8.3
23.5	9.3	4.2	67.8	2.1	2.0	2.1	22.3	3.0
25.3	8.4	3.0	69.1	2.5	1.9	2.2	26.1	3.2
26.0	10.1	4.3	67.6	2.2	1.6	1.9	18.6	2.8
28.0	9.0	3.4	69.1	4.1	4.6	4.3	47.9	6.2
29.2	9.9	3.8	66.4	3.9	4.6	4.2	42.8	6.4
32.7	9.4	4.8	67.3	5.3	4.5	4.9	52.1	7.3
33.7	9.9	3.6	68.3	4.2	3.7	4.0	40.2	5.8
37.5	10.1	4.7	64.5	2.4	2.1	2.2	21.8	3.4
37.6	10.3	4.2	63.5	2.5	2.6	2.6	24.8	4.0
Mean						3.4	35.5	5.0
SD						1.4	14.5	2.0
S <sub>r</sub> <sup>2</sup>						0.5	5.0	0.7
RS <sub>r</sub> <sup>2</sup> % of mean						14.0	14.1	13.9
LSD						0.8	8.4	1.2

<sup>1</sup>Values 1 and 2 indicate individual zein determinations by the turbidimetric method.

<sup>2</sup>S<sub>r</sub> = repeatability standard deviation; RS<sub>r</sub> = relative repeatability standard deviation.

Mentink and Hoffman, 2006), mTZM was less precise than CP or starch analysis, more precise than lignin analysis, and comparably precise to determination of fat by acid hydrolysis.

The LSD of zein as determined by mTZM was 1.6 g/100 g of DM suggesting that mTZM had the proficiency to distinguish corn endosperm types. Corn varieties containing floury or opaque genes (BSG 1736fl2/fl2, W64aXOh43fl2/fl2, W64aXOh43o2/o2) contained 72 and 56% less zein, respectively, compared with flint (Peace River Flint) or dent varieties (BSG 07AB05, BSG 214686, BSG O7ABO5X1736, W64aXOh43), with the exception of Reid Yellow Dent (Table 1).

Zein is commonly expressed on a DM or CP basis (Wallace et al., 1990; Hamaker et al., 1995; Landry et al., 2002). It is questionable if expressing zein on a DM or CP basis is facilitative for ruminant nutrition because zein is negatively associated with starch degradability (Philippeau et al., 2000) and zein exist solely and co-dependently with starch to form the starch matrix in the endosperm (Buchanan et al., 2000). When zein was expressed on a DM or a CP basis, Peace River Flint was not readily distinguishable from dent varieties BSG 07AB05, BSG 214686, BSG O7ABO5X1736, or W64aXOh43. However, Peace River Flint is low in starch and when zein was expressed on a starch basis (g of zein/100 g of starch), differences between Peace River Flint and other corn endosperm types became distinguishable. Peace River Flint contained 19.3 g of zein/100 g of starch, whereas dent varieties BSG 07AB05, BSG 214686, BSG O7ABO5X1736, and W64aXOh43 contained 11.3, 12.6, 13.4, and 13.3 g of zein/100 g of starch respectively. Floury and opaque varieties BSG 1736fl2/fl2, W64aXOh43fl2/fl2, and W64aXOh43o2/o2 contained less zein at 6.9, 4.7, and 4.9 g of zein/100 g of starch. The LSD of zein (2.6 g of zein/100 g of starch) indicated that mTZM could differentiate corn endosperm types when expressed on a starch basis.

Zein content, measures of mTZM precision, and various units of expressing zein for the 10 HMC are presented in Table 2. Zein in HMC as estimated by mTZM ranged from 5.8 to 1.9 g/100 g of DM with a mean zein content of 3.4 g/100 g of DM. The  $S_r$  of mTZM for HMC was 0.5 g/100 g of DM with an  $RS_r$  of 14.0% of the mean. The  $RS_r$  of mTZM was similar (14.0 vs. 12.8% of the mean) to that observed for dry corns. The LSD of mTZM as g/100 g of DM, g/100 g of CP, or g/100 g of starch were 0.8, 8.4, and 1.2 respectively. Measures of laboratory precision indicate that mTZM had similar precision when used to evaluate the zein content of HMC compared with dry corn varieties.

Zein contents of HMC were comparable to or, in some cases, less than zein contents of floury or opaque corns

(BSG 1736fl2/fl2, W64aXOh43fl2/fl2, W64aXOh43o2/o2). Two possible explanations for these observations exist. First, HMC is harvested at an earlier physiological stage than dry corn and, because zein increases with advancing maturity (Murphy and Dalby, 1971), lower zein contents in HMC compared with mature dry corns could be expected. Second, bacterial proteolysis (Baron et al., 1986) or solubilization of zein by lactic or acetic acids, which are primary solvents of zein (Lawton, 2002), could have reduced the zein content during the ensiling process. Controlled studies evaluating the effects of maturity and ensiling on zein content of HMC are warranted.

In conclusion, mTZM was found to be feasible in differentiating and quantifying prolamin proteins (zein) in dry corns of varying endosperm type and HMC. The mTZM was moderately precise, and improvements in the assay to increase precision should be pursued. Quantifying prolamin proteins could be used in concert with growth, lactation, in vitro, in situ, or in vivo digestion trials to increase understanding of factors that influence starch digestibility and subsequent ruminant animal performance.

## ACKNOWLEDGMENTS

This project was financially supported by gifts from NuTech Seed (Leland, IA), Bailey Consulting (DeForest, WI), and Agri-Nutrition Consulting (DeForest, WI). The authors thank Magdalena Kurtz, James Bailey, Rob Bailey (Agri-Nutrition Consulting, DeForest, WI), and Corey Catt (NuTech Seed, Leland, IA) for their contributions and support of unrestricted academic research. Special appreciation is also extended to Randy Shaver and Bruce Hamaker for their support and academic input in regard to this project.

## REFERENCES

- Aoubacar, A., J. D. Axtell, L. Nduulu, and B. R. Hamaker. 2003. Turbidity assay for rapid and efficient identification of high protein digestibility sorghum lines. *Cereal Chem.* 80:40–44.
- Allen, M. S., R. A. Longuski, and Y. Ying. 2008. Endosperm type of dry ground corn grain affects ruminal and total tract digestion of starch in lactating dairy cows. *J. Dairy Sci.* 91(Suppl.1):529. (Abstr.)
- AOAC. 1990. *Official Methods of Analysis*. 15th ed. AOAC, Arlington, VA.
- Baron, V. S., K. R. Stevenson, and J. G. Buchanan-Smith. 1986. Proteolysis and fermentation of corn-grain ensiled at several moisture levels and under several simulated storage methods. *Can. J. Anim. Sci.* 66:451–461.
- Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.* 72:248–254.
- Buchanan, B. B., W. Gruissem, and R. L. Jones. 2000. *Biochemistry and Molecular Biology of Plants*. Am. Soc. Plant Physiol., Rockville, MD.

- Correa, C. E. S., R. D. Shaver, M. N. Pereira, J. G. Lauer, and K. Kohn. 2002. Relationship between corn vitreousness and ruminal in situ starch degradability. *J. Dairy Sci.* 85:3008–3012.
- Drochioiu, G., I. Druta, M. N. Petrovanu, and S. Strajeru. 2002. A rapid method used at the Suceava Genebank to evaluate protein quality of some cereal grains. *Plant Genet. Resour. Newsl.* 129:47–51.
- Ehrman, T. 1996. Determination of starch in biomass samples by chemical solubilization and enzymatic digestion. LAP-016. US Dept. Energy, National Bioenergy Center, Washington, DC.
- Hamaker, B. R., A. A. Mohamed, J. E. Habben, C. P. Huang, and B. A. Larkins. 1995. Efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamins contents than the conventional method. *Cereal Chem.* 72:583–588.
- Kale, A., F. Zhu, and M. Cheryan. 2007. Separation of high-value products from ethanol extracts of corn by chromatography. *Ind. Crops Prod.* 26:44–53.
- Landry, J., S. Delhaye, and C. Damerval. 2000. Improved method for isolating and quantitating  $\alpha$ -amino nitrogen as nonprotein, true protein, salt-soluble proteins, zeins, and true glutelins in maize endosperm. *Cereal Chem.* 77:620–626.
- Landry, J., S. Delhaye, and C. Damerval. 2002. Comparative efficiencies of isopropyl and tert-butyl alcohols for extracting zeins from maize endosperm. *J. Agric. Food Chem.* 50:4131–4134.
- Landry, J., and T. Moureaux. 1970. Heterogeneity of the glutelins of the grain core. Selective extraction and composition in amino acids of the three isolated fractions. *Bull. Soc. Chem. Biol.* 52:1021–1037.
- Lawton, J. W. 2002. Zein: A history of processing and use. *Cereal Chem.* 79:1–18.
- Mentink, R. L., and P. C. Hoffman. 2006. Utility of near infrared reflectance spectroscopy to predict nutritional components in total mixed rations. *J. Dairy Sci.* 89:2320–2326.
- Murphy, J. J., and A. Dalby. 1971. Changes in the protein fractions of developing normal and opaque-2 maize endosperm. *Cereal Chem.* 48:336–349.
- Ngonyamo-Majee, D., R. D. Shaver, J. G. Coors, D. Sapienza, and J. G. Lauer. 2008. Relationship between kernel vitreousness and dry matter degradability for diverse corn germplasm. II. Ruminal and post-ruminal degradabilities. *Anim. Feed Sci. Technol.* 142:259–274.
- Olakojo, S. A., O. Omueti, K. Ajomale, and B. A. Odunbodede. 2007. Development of quality protein maize: Biochemical and agronomic evaluation. *Trop. Subtrop. Agroecosys.* 7:97–104.
- Paulis, J. W., J. S. Wall, and W. F. Kwolek. 1974. A rapid turbidimetric analysis for zein in corn and its correlation with lysine content. *J. Agric. Food Chem.* 22:313–315.
- Philippeau, C., J. Landry, and B. Michalet-Doreau. 2000. Influence of the protein distribution of maize endosperm on ruminal starch degradability. *J. Sci. Food Agric.* 80:404–408.
- SAS Institute. 2001. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, NC.
- Taylor, C. C., and M. S. Allen. 2005. Corn grain endosperm type and brown midrib 3 corn silage: Feeding behavior and milk yield of lactating cows. *J. Dairy Sci.* 88:1425–1433.
- Theander, O., P. Aman, E. Westerlund, R. Anderson, and D. Petterson. 1995. Total dietary fiber determined as neutral sugar residues, uronic acid residues, and Klason lignin (The Uppsala Method): Collaborative study. *J. AOAC Int.* 78:1030–1044.
- Wallace, J. C., M. A. Lopes, E. Paiva, and B. A. Larkins. 1990. New methods for extraction and quantitation of zeins reveal a high content of g-zein in modified opaque-2 maize. *Plant Physiol.* 92:191–196.