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Evaluation of a modified method to measure total starch in animal feeds

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ABSTRACT

The AOAC method 996.11 (AOAC, 2005) has been recognized as an accurate, repeatable, and efficient method to measure total starch in animal feeds. However, analyzing starch using the AOAC method can be expensive and associated with technical challenges. The objectives of this study were to determine if an alternative modified starch method (MAOAC) could be more economical and minimize technical challenges associated with the AOAC method. Modification of the AOAC method, 996.11 (AOAC, 2005) was done by combining the AOAC method with the acetate buffer method of Hall (2009) and introducing alpha-amylase (1162 liquefon units/assay) and amyloglucosidase (400 units/assay) from different sources (ANKOM Technology Inc., Macedon, NY and Sigma–Aldrich Inc., St. Louis, MO). Dried rumen and fecal samples, alfalfa hay, dried distillers grains with solubles, corn silage, total mixed ration (TMR), concentrate mixture, ground corn, and pure corn starch were analyzed using the AOAC and MAOAC methods. Two technicians performed two runs of each method and all samples were analyzed in duplicate within each run. The average starch concentration for 9 samples was not affected by method (AOAC method = 298.7 ± 1.84; MAOAC = 298.0 ± 1.39 g/kg; $P = 0.49$), technician (technician I = 297.8 ± 12.8 g/kg; technician II = 299.0 ± 19.6 g/kg; $P = 0.24$), or run (run I = 298.4 ± 15.3 g/kg; run II = 298.1 ± 17.0 g/kg; $P = 0.59$). The average time spent to analyze 18 assays was approximately 3 h for both methods. Average chemical cost per assay with the MAOAC method was $0.88 compared with $3.41 for the AOAC method. There was a 79% decrease in water consumption for the samples containing >100 g/kg starch with the MAOAC method compared with the AOAC method. The MAOAC starch assay could be considered a cost-effective, more environmentally friendly, and less technically difficult method compared with the AOAC starch method.

Abbreviations: AOAC method, Association of Official Analytical Chemists method; MAOAC method, modified Association of Official Analytical Chemists method; TMR, total mixed ration; DMSO, dimethyl sulfoxide; GOPOD, glucose oxidase–peroxidase–aminoantipyrine buffer mixture.

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1. Introduction

Starch is a major source of energy in livestock diets. However, elevated dietary starch may cause undesirable effects on glycemic response and animal health measures, such as ruminal acidosis (Hall, 2009). Accordingly, accurate measurement of starch is important for diet formulations and digestibility evaluation of animal feeds.

Over the last few decades, various chemical and enzymatic methods have been introduced to measure total starch in animal feeds (Moreels and Amylum, 1987). However, the lack of specificity and the use of corrosive and dangerous chemicals have caused chemical methods to become less popular compared with enzymatic methods. Furthermore, the availability of amylases from various sources has resulted in a proliferation of enzymatic methods to analyze starch (Karkalas, 1985). These methods vary in ease of use, time spent on analysis, and cost per assay.

The AOAC Official starch method 996.11 (AOAC, 2005) was developed by McCleary et al. (1997). This method is a quantitative and reliable total starch assay using thermostable α-amylase and amyloglucosidase which allows for measurement of total starch concentration in a wide range of food, feed, plant, and cereal products. In this method, samples that contain minimal concentrations of resistant starch are incubated at 100 °C with thermostable alpha-amylase followed by amyloglucosidase which allows for hydrolysis to glucose. Samples that contain large concentrations of resistant starch are completely solubilized by pretreatment with dimethyl sulfoxide (DMSO) at 100 °C, followed by thermostable alpha-amylase treatment. The resulting maltodextrins are hydrolyzed to glucose with amyloglucosidase, and the glucose is measured using glucose oxidase/peroxidase reagent. This method has been recognized as an accurate, repeatable, and efficient method to measure total starch; however, the method can be expensive and associated with technical challenges.

An acetate buffer method was introduced to determine dietary starch in animal feeds by Hall (2009). This method is a modification of the assay developed by Bach Knudsen (1997) and introduced techniques to avoid known technical defects that decrease the accuracy of other starch assays (Hall, 2009). However, this method requires approximately 5 h to complete and is time consuming. Therefore, the objectives of this study were to determine if an alternative starch method would be more economical and could minimize technical challenges associated with AOAC method 996.11 (AOAC, 2005).

2. Materials and methods

2.1. Materials

Total Starch Assay Kits were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). Thermostable α-amylase from *Bacillus licheniformis* was purchased from ANKOM Technology Inc. (Macedon, NY). All other chemicals and reagents were purchased from Sigma–Aldrich Inc. (St. Louis, MO).

Samples of (1) rumen contents; (2) fecal samples; (3) alfalfa hay; (4) dried distillers grains with solubles; (5) corn silage; (6) total mixed ration (TMR) for lactating dairy cows; (7) concentrate mixture for lactating dairy cows; (8) dry ground corn; and (9) pure corn starch were analyzed for total starch concentrations. All feed, rumen, and fecal samples were collected at the South Dakota State University Dairy Research and Teaching Facility. Pure corn starch was purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). All samples with the exception of pure corn starch were dried at 55 °C for 48 h in a Despatch oven (style V-23, Despatch Oven Co., Minneapolis, MN). The dried samples were ground to a 4-mm particle size (Wiley mill, model 3; Arthur H. Thomas Co., Philadelphia, PA) and then further ground to 1-mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). Ground samples were analyzed for DM which was determined at 105 °C for 3 h (Shreve et al., 2006). All samples, except the pure corn starch sample, were further ground to pass 0.5-mm screen using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY) before the starch analysis.

2.2. Experimental design

The modification of the AOAC method was done by combining the AOAC method 996.11 (AOAC, 2005) with the acetate buffer method by Hall (2009) and using α-amylase (EC 3.2.1.1; ANKOM Technology Inc., Macedon, NY), amyloglucosidase (EC 3.2.1.3), glucose oxidase (EC 1.1.3.4), and peroxidase (EC 1.11.1.7) from different sources (Sigma–Aldrich Inc., St. Louis, MO). The experiment was conducted in 3 steps.

2.2.1. Step 1: determination of optimal α-amylase (ANKOM Technology Inc., Macedon, NY) concentration

Alpha-amylase from ANKOM Technology Inc. (Macedon, NY) was introduced as a new enzyme source to replace α-amylase from Megazyme International Ireland Ltd. (Wicklow, Ireland). The optimal α-amylase concentration for the assay was tested at increasing α-amylase concentrations of 465, 697, 930, 1162, 1394, and 1627 liquefon units (LU)/assay to measure total starch concentration of pure corn starch. The range of α-amylase concentrations (465–1627 LU/assay) for step 1 was based on the total number of α-amylase units/assay use in the AOAC method 996.11 (AOAC, 2005; 300 U/assay) and acetate buffer method (~1500 LU/assay; Hall, 2009). The starch analysis in step 1 was completed according to AOAC method 996.11 (AOAC, 2005). Reagents and enzymes from a Total Starch Assay Kit (Megazyme International Ireland Ltd., Wicklow, Ireland) were used except for α-amylase which was purchased from ANKOM Technology Inc. (Macedon, NY). The optimal α-amylase concentration was determined based on the percentage of starch recovery of the pure corn starch (100%).