Concentration of ruminal ammonia nitrogen (NH3-N) was analyzed after centrifuging ruminal fluid samples at 10,000 × *g* for 15 min at 4°C (Avanti J-E, Beckman Coulter Inc., Palo Alto, CA) following the phenol-hypochlorite technique described by Broderick and Kang (1980) with the following modification: absorbance was read on 200 µL samples at OD620 in flat-bottom 96-well plates (Corning Costar 3361, Thermo Fisher Scientific Inc., Waltham, MA) using a plate reader (Fisherbrand UV/VIS AccuSkan GO Spectrophotometer, Thermo Fisher Scientific Inc., Hampton, NH). All assays were conducted in duplicate determinations with subsequent analyses performed when CVs were above 5%. Intra- and inter-assay CVs were x.x% and x.x%, respectively, for concentrations of NH3-N.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64-75.