Letter to the Editor
Possible Errors in the Analysis of Lactic Acid and Volatile Fatty Acids in the Gastrointestinal Tracts of Pigs and Chickens

Recent articles by van Wissen et al. (2001) (16) and van der Wielen et al. (2000) (14) have described the measurements of lactate and volatile fatty acids (VFA) in the hind guts (ceca and colon) of pigs and broiler chickens, respectively, in an attempt to link these concentrations with the growth of certain pathogenic bacteria. In broiler chickens, the concentrations of lactate and acetate in the ceca were shown to be approximately 20 and 65 μmol/g (or mmol/kg), respectively, when broilers were around 9 days of age. These concentrations appear to be representative of concentrations that could be expected according to previously published results (13).

The concentrations of lactate and VFA reported in the study with pigs, however, appear to be erroneous and particularly low (for example, 0 for lactate and 0.3 to 3.7 mmol/liter for acetate). Lactic acid concentrations in the pig ceca have previously been reported to be between 0.6 and 2.0 mmol/liter (see references for VFA). Total VFA concentrations in the ceca of pigs have been reported to be between 80 and 200 mmol/kg in several studies using either steam distillation (1–3) or gas-liquid chromatography (4, 10, 12). Likewise, the concentration of VFA in the ceca using gas chromatography analysis is reported to be between 70 and 120 mmol/kg in humans (8) and between 80 and 250 mmol/kg in rats (5, 9), which both have fermentation characteristics similar to those of pigs (15).

There are several possible reasons for the apparently erroneous values in the pig study. Firstly, only 1 g of cecal material was diluted 1:4 (wt/vol) with water prior to mixing, centrifugation, and analysis. Sampling such a small weight of gut contents compared to that expected to be available could give rise to sampling errors, as the entire cecum would not be expected to contain a homogenous sample. Perhaps more importantly, the samples were not stabilized by acidification prior to freezing or analysis. VFA are, by their nature, volatile at pH values above physiological pH values (11). Previously reported methods have used concentrated H2SO4 to stabilize the pH in the gastrointestinal tract (GIT) contents collected from cattle (7) and sheep (6) and, in methods reported more recently by our research group for poultry (13) and rats (9), a mixture of perchloric acid and formic acid is used to stabilize pH and to precipitate protein.

Lastly, xylitol and crotonic acid are solid at room temperature (mp = 94 to 96°C and 71 to 73°C, respectively) and have characteristics quite different from those of VFA, and so the use of these internal standards (IS) will not give an indication of the volatilization of the VFA themselves during the handling and analysis process. It would be expected that the recovery of xylitol and crotonic acid through processing would be greater than that of the more volatile fatty acids being recovered, so high recovery of IS versus low recovery of the analyte could lead to apparent low concentrations. 4-Methyl valeric is our preferred IS, as it has physical properties similar to those of the VFA of interest. It is added to the acidic stabilizing solution to reflect losses of VFA throughout the entire sample preparation and subsequent storage prior to analysis. A second sample with sufficient gut contents (which should be available from the pig cecum) can be prepared with water for the assessment of pH.

These errors, which are obvious from the reported values in the hind guts of pigs, leads to questions as to the accuracy of measures in other GIT sections and as to whether the correlation between Enterobacteriaceae sp. numbers in the stomach and organic acid concentration is a real phenomenon.

REFERENCES
E. H. Clayton*
Nexus Unit
John Hunter Hospital
Locked Bag 1
Hunter Regional Mail Centre, NSW 2310, Australia

R. J. Blake
Disciplines of Nutrition & Dietetics and Human Physiology
Level 3, Medical Sciences Building
University of Newcastle
University Drive
Callaghan NSW 2308, Australia

*Phone: 61 249 855 800
Fax: 61 249 855 815
E-mail: Edward.Clayton@hnehealth.nsw.gov.au

Editor’s Note: The authors of the published article declined to respond.