

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 Winsen 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 colons) 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 $\mu\text{mol/g}$ (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 site of 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 approximately 3.0, and the loss of VFA can occur rapidly at physiological pH values (11). Previously reported methods have used concentrated H_2SO_4 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 ($\text{mp} = 94$ 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

1. Alexander, F., and M. E. Davies. 1963. Production and fermentation of lactate by bacteria in the alimentary canal of the horse and pig. *J. Comp. Pathol.* **73**:1–8.
2. Argenzio, R. A., and M. Southworth. 1975. Sites of organic acid production and absorption in gastrointestinal tract of the pig. *Am. J. Physiol.* **228**:454–460.
3. Bach Knudsen, K. E., B. Borg Jensen, J. O. Anderson, and I. Hansen. 1991. Gastrointestinal implications in pigs of wheat and oat fractions. 2. Microbial activity in the gastrointestinal tract. *Br. J. Nutr.* **65**:233–248.
4. Bach Knudsen, K. E., H. Jørgensen, and N. Canibe. 2000. Quantification of the absorption of nutrients derived from carbohydrate assimilation: model experiment with catheterised pigs fed on wheat- or oat-based rolls. *Br. J. Nutr.* **84**:449–458.
5. Berggren, A. M., I. M. E. Björck, E. Margareta, and G. L. Nyman. 1993. Short-chain fatty acid content and pH in the caecum of rats given various sources of carbohydrates. *J. Sci. Food Agric.* **63**:397–406.
6. Clayton, E. H., and G. P. D. Jones. 2001. Preliminary observations of tumour necrosis factor-alpha in the faeces of sheep following acute lactic acidosis. *Aust. J. Agric. Res.* **52**:869–873.
7. Clayton, E. H., I. J. Lean, J. B. Rowe, and W. J. Cox. 1999. Effects of feeding virginiamycin and sodium bicarbonate to grazing lactating dairy cows. *J. Dairy Sci.* **82**:1545–1554.
8. Cummings, J. H., E. W. Pomare, W. J. Branch, C. P. E. Naylor, and G. T. MacFarlane. 1987. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **28**:1221–1227.
9. Hanstock, T. L., E. H. Clayton, K. M. Li, and P. E. Mallet. 2004. Anxiety and aggression associated with the fermentation of carbohydrates in the hind-gut of rats. *Physiol. Behav.* **82**:357–368.
10. Imoto, S., and S. Namioka. 1978. VFA production in the pig large intestine. *J. Anim. Sci.* **47**:467–478.
11. Leng, R. A. 1970. Formation and production of volatile fatty acids in the rumen, p. 406–421. *In* A. T. Phillipson (ed.), *Physiology of digestion and metabolism in the ruminant*. Oriel Press, Newcastle upon Tyne, England.
12. Marsono, Y., R. J. Illman, J. M. Clarke, R. P. Trindle, and D. L. Topping. 1993. Plasma lipids and large bowel volatile fatty acids in pigs fed on white rice, brown rice and rice bran. *Br. J. Nutr.* **70**:503–513.
13. Taylor, R. D., and G. P. D. Jones. 2004. The incorporation of whole grain into pelleted broiler diets. II. Gastrointestinal and digesta characteristics. *Br. Poultry Sci.* **45**:237–246.
14. van der Wielen, P. W., S. Biesterveld, S. Notermans, H. Hofstra, B. A. Urlings, and F. van Knapen. 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Appl. Environ. Microbiol.* **66**:2536–2540.
15. Van Soest, P. J., J. Jeraci, T. Foose, K. Wrick, and F. Ehle. 1983. Comparative fermentation of fibre in man and other animals, p. 75–80. *In* G. Wallace and L. Bell (ed.), *Fibre in human and animal nutrition: Proceedings of*

Dietary Fibre in Human and Animal Nutrition Symposium. Massey University, Palmerston North, New Zealand.

16. **van Winsen, R. L., B. A. Urlings, L. J. Lipman, J. M. Sniijders, D. Keuzen-**

kamp, J. H. Verheijden, and F. van Knapen. 2001. Effect of fermented feed on the microbial population of the gastrointestinal tracts of pigs. *Appl. Environ. Microbiol.* 67:3071–3076.

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

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