HYDROGEN AND METHANE BREATH TESTS FOR ASSESSING METABOLIC ACTIVITY OF GUT MICROFLORA IN BROILER CHICKENS

R.J. HUGHES¹, D.R. TIVEY² and R.N. BUTLER³

Summary

Breath hydrogen measurement is used as an indicator of carbohydrate malabsorption in humans and for estimating the rate of passage of digesta through the small intestine. Both tests rely on bacterial fermentation of undigested carbohydrate in the large bowel. The development of similar breath tests for chickens for non-invasive measurement of gastro-intestinal functions will provide more insight than the snap-shot view obtained in conventional nutrient balance studies involving the slaughter of birds to obtain digesta. Using breath tests, it should be possible to pre-select individual chickens with known physiological characteristics, subject them to dietary or other treatments, and follow the resulting changes in gastro-intestinal functions such as digesta transit time and microbial proliferation in the small intestine. Preliminary breath testing studies with broiler chickens indicated that gut microflora competed for energy and other nutrients thus slowing the rate of growth and reducing feed efficiency.

I. INTRODUCTION

Analysis of expired breath is a non-invasive method for diagnosing gastro-intestinal function in humans (Butler, 1996). Hughes et al. (2000b) demonstrated that $\text{^{13}CO}_2$ breath tests could be developed as non-invasive tools for studying gut physiology in broiler chickens. Other breath tests used routinely in medical practice are based on release of hydrogen and methane following microbial fermentation of carbohydrates such as lactulose which is a disaccharide not absorbed in the small intestine (Wutzke et al., 1997). Studies on humans and other species indicate that samples of breath can be taken with simple, inexpensive equipment and remain stable for long periods, enabling these tests to be used in the field. Tivey and Butler (1999) concluded that breath tests should prove to be powerful analytical tools for nutrition research and veterinary diagnostics. The breath tests likely to be of most benefit for broiler nutrition studies include those which examine digesta transit time and microbial growth in the small intestine in order to address problems such as variation in energy metabolism in broilers (Hughes et al., 2000a).

This paper describes (1) an initial experiment to determine whether gut microflora of broiler chickens produce hydrogen and methane, and (2) application of a hydrogen breath test to assess metabolic activity of gut microflora in chickens given a wheat-based diet.

II. MATERIALS AND METHODS

Helmets of different dimensions (40 or 50 mm internal diameter and 95 or 100 mm length, respectively) were constructed from capped PVC pipe to suit chickens of different ages and hence size. The helmet was placed over the head and neck of the chicken and held firmly against the shoulders and breast to minimise loss of expired $\text{H}_2$ and $\text{CH}_4$. After 30 sec, a sample of breath was drawn through the cap via Luer lock fittings into a 10 mL evacuated tube. Preliminary work indicated that 30 sec provided a suitable breath sample without distressing the

¹ SARDI, Pig and Poultry Production Institute, University of Adelaide, Roseworthy, South Australia 5371.
² Department of Animal Science, University of Adelaide, Roseworthy, South Australia 5371.
³ Women's and Children's Hospital, North Adelaide, South Australia 5006.
chicken. Hydrogen and methane concentrations were analysed by gas chromatography.

In Experiment 1, sixteen 23-d-old chickens fed a commercial diet *ad libitum* were breath tested. Two days later, after an overnight fast, chickens were weighed and breath tested commencing at 0830 h to establish a base-line. Each chicken was given 5 mL of diluted lactulose solution via a disposable syringe fitted with a plastic tube which was inserted 4 cm into the oesophagus. A total of 12 chickens were given approximately 130 mg lactulose, two other chickens were given double the dose (260 mg lactulose) and a further two were given quadruple the dose (520 mg lactulose). The lower dose rate (1 g carbohydrate per 10 kg body weight) was equivalent to that given to human subjects to assess carbohydrate malabsorption. The higher dose rates (2 g per 10 kg and 4 g per 10 kg) were given to obtain an indication of whether carbohydrate loading needed to be greater to achieve measurable levels of hydrogen or methane in expired breath for subsequent experiments with chickens. Each chicken was breath tested 3 h post-feeding of lactulose. Chickens were denied access to feed in this 3 h period.

In Experiment 2, a total of 48 chickens of 21-22 d of age were housed individually in metabolism cages and given a practical diet comprising (per kg): wheat 700 g, meat and bone meal 76 g, soybean meal 170 g, sunflower oil 40 g, salt 2.5 g, lysine HCl 2.5 g, methionine 3 g, and vitamins and minerals 6 g. Cold-pressed pellets were fed for seven d. The first 3 d enabled the chickens to adapt to the cages and feeds. During the following 4 d, all excreta were collected and dried. Feed intake was measured during the adaptation and collection phases of the study. Birds were weighed at the start and end of the 7 d period. Dry matter contents of samples of pelleted and milled feed were measured. Gross energy values of dried excreta and milled feed were measured with a Parr isoperibol bomb calorimeter for calculation of apparent metabolizable energy (AME) of the diet. Chickens had free access to feed and water throughout the 7 d experimental period. On days 0 and 6 each chicken was breath tested commencing at 0830 h.

III. RESULTS AND DISCUSSION

There was large variability in hydrogen concentration (in ppm) in breath samples from non-fasted chickens given a commercial diet, and in fasted chickens both before and after dosing with lactulose in Experiment 1 (Figure 1). In 9 out of 12 chickens studied, there was an increase in hydrogen concentration in the 3 h period following dosing with lactulose. There was no change in one chicken and the other two chickens showed a small decline.

![Graph](image)

Figure 1. Breath hydrogen concentration (in ppm) in chickens fed a commercial diet *ad libitum*, and then two days later from the same chickens (fasted overnight) immediately before and 3 h after dosing with lactulose (130 mg in 5 mL water).
All chickens hosted microflora (presumably in the caeca) which were capable of fermenting carbohydrate to hydrogen. In addition, methane (up to 13 ppm) was detected in the breath of all 16 chickens at some stage during this experiment, either before or after dosing with lactulose (data not shown). These results tend to suggest that other factors such as the rate of passage of digesta, proliferation of facultative anaerobes (Choc et al., 1996) in the small intestine, and combinations of these contributed significantly to the large variation in breath hydrogen and methane. It is also possible that the dose rate of non-absorbable carbohydrate was too low.

In Experiment 2, change in hydrogen concentration in breath during the 7 d study was highly variable, as were the concentrations at the start and end of the study (Figure 2).

![Diagram](image)

**Figure 2.** Variation in expired concentrations of breath hydrogen (in ppm) from chickens given a wheat-based diet (30 g crude fibre/kg). Data from individual chickens are sorted by increasing change from start to end of the 7 d metabolism experiment.

There were no associations (P>0.05) between AME and breath hydrogen or between feed intake and breath hydrogen. However, growth rate of chickens was negatively correlated (P<0.01) with breath hydrogen samples taken at the end of the study and feed conversion ratio was positively correlated (P<0.001) with breath hydrogen (Figure 3). These preliminary results demonstrate the potential usefulness of non-invasive breath tests for detecting malabsorption of carbohydrate in commercial broiler flocks and for measurement of digesta transit time in nutrient balance experiments.

In addition, it was clearly evident that gut microflora competed for energy and other nutrients thus slowing the rate of growth and reducing feed efficiency. The possibility that undigested carbohydrate leaving the small intestine was fermented to volatile products such as short-chain fatty acids also in a variable manner, like hydrogen and methane production, needs further study. Bacterial overgrowth of the gut is likely to have detrimental effects in addition to significant losses of nutrients. Microbial proliferation could ultimately lead to health problems through general inflammation of the gut and invasion of tissue by organisms pathogenic to the bird or to humans consuming contaminated carcasses.
IV. CONCLUSIONS

Changes in hydrogen and methane concentrations in breath during two metabolism studies were highly variable. However, observed variation between individual birds in AME was not directly associated with breath hydrogen concentration. Elevated levels of hydrogen in breath were indicative of significant reductions in growth rate and feed efficiency brought about by malabsorption, coupled with losses of energy and other nutrients through proliferation of gut bacteria.

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REFERENCES