BREATH TESTS FOR ESTIMATING DIGESTA TRANSIT TIME IN CHICKENS

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Summary

The lactulose breath test is a non-invasive diagnostic procedure for estimation of digesta transit time in human subjects. A similar procedure applied to ten broiler chickens yielded a mean and standard error for oro-caecal transit time of 166 ± 9 mins. In comparison, whole tract transit time for ferric oxide administered in a gelatine capsule was 162 ± 9 mins. The sensitivity of the breath hydrogen test was improved by fasting chickens for 3 h prior to dosing with lactulose. The gut microflora of chickens produced large increases in breath hydrogen from fermentation of lactulose without the need for prior dosing. Finally, it may be possible to devise a breath test for chickens based on fermentation of naturally-occurring complex carbohydrates in commercial chicken feed.

I. INTRODUCTION

Transit time, or inversely the period of residence of digesta in the gastrointestinal tract, influences the rates of digestion and absorption of nutrients in chickens (van der Klis and van Voorst 1993; Uni et al., 1995). Transit time in human subjects is measured by a non-invasive diagnostic procedure involving rise in hydrogen concentration in breath following dosing with the synthetic disaccharide lactulose. The test relies on the production of hydrogen by hindgut fermentation of lactulose which is not absorbed in the small intestine (Wutzke et al., 1997). Hydrogen not otherwise utilised as a food source by other bacteria in the gut diffuses through the gut wall to the bloodstream and is then expired via the lungs. Recent studies by Hughes et al. (2001) point to the usefulness of breath tests as non-invasive methods for studying gastrointestinal function in chickens.

This paper describes two experiments with chickens to develop a hydrogen breath test for non-invasive measurement of transit time of digesta. The first experiment examined whether prior dosing with lactulose was necessary to prime gut microflora and whether fasting prior to test dosing with lactulose improved the sensitivity of the test. The second experiment compared oro-caecal transit time (OCTT) determined by rise in breath hydrogen with whole tract transit time (WTTT) determined by appearance of ferric oxide in excreta.

II. MATERIALS AND METHODS

(a) Experiment 1 - Prior dosing with lactulose and fasting

Male broiler chickens (Ross breed) were reared from hatch under electric brooders in a floor pen in a controlled temperature room. The chickens had free access to commercial starter crumbles and water. At 20 days of age chickens were transferred to single-bird metabolism cages in a room kept at 25-27°C and given commercial finisher pellets. Commencing at 26 days of age, four chickens entered the testing cycle which ran over two days. The cycle was repeated with separate sets of four chickens on the following two days. On day 1 of each cycle, two chickens were given a priming dose of lactulose at 1100 h. Each chicken was treated with approximately 130 mg lactulose in 5 mL of water. The solution was administered via a disposable syringe fitted with a soft plastic tube which was inserted 4 cm into the oesophagus. On day 2, two chickens (one prime-dosed and one not dosed) were fasted for 3 h from 0800 h. The other two chickens (one prime-dosed and one not dosed) had free access to feed. Commencing at 1100 h, all four chickens were test-dosed with
approximately 130 mg lactulose in 5 mL of water. Following the test dosing with lactulose chickens had free access to feed and water. Serial breath testing of each chicken commenced immediately before test-dosing at 1100 h then at 60, 90, 105, 120, 135, 150, 165, 180, 210, 240 and 300 minutes thereafter. A 50 mL gas sample from the head space was taken 15 sec after a prototype helmet was placed over the head of the chicken and held firmly against the shoulders to minimise loss of expired gas, as described previously by Hughes et al. (2000 and 2001).

(b) Experiment 2 – Measurement of oro-caecal and whole tract transit time

Male and female broiler chickens (Ross) were reared from hatch under electric brooders in separate floor pens in a controlled temperature room. The chickens had free access to commercial broiler starter crumbles and water. At 14 days of age, chickens were transferred to single-bird metabolism cages in a room kept at 25-27°C and given commercial starter crumbles. Commencing at 18 days of age, 14 chickens in total were fasted for 3 h starting at 0800 h. At 1100 h, all chickens were administered with a gelatine capsule containing ferric oxide (Fe3O4 200 mg/kg live weight) as described by Iskander and Pym (1987). Then ten chickens (six male and four female) were dosed with approximately 130 mg lactulose in 5 mL of water, as described in (a) above. The remaining four chickens (two of each sex) not dosed with lactulose provided a measure of base-line variation in hydrogen production from undigested carbohydrate by gut microflora. Serial breath testing of each chicken commenced immediately before test-dosing at 1100 h then at 120, 150, 165, 180, 195, 210 and 240 minutes thereafter. Breath samples were collected as described in (a) above. Excreta trays were examined frequently for signs of ferric oxide in voided droppings.

III. RESULTS AND DISCUSSION

(a) Experiment 1 - Prior dosing with lactulose and fasting

All chickens showed an increase in breath hydrogen after dosing with lactulose whether or not they received a priming dose on the previous day. Fasting prior to dosing appeared to reduce variation in hydrogen concentration between chickens at the same time after test dosing, and to also reduce the within-chicken variation. Results of serial breath sampling of fasted chickens are shown in Figure 1. Estimates of oro-caecal transit time for lactulose following fasting ranged from 165 mins for chicken E, 180 mins for chickens B and F, to 210 mins for chickens A, C and D. The mean and standard error for transit time were 193 ± 8 mins. The above estimate is likely to be biased upwards because the interval between serial breath samples was 15 minutes up to 180 minutes post dosing, then 30 minutes thereafter. That is, mean transit time occurred after the period of most frequent sampling.

The hydrogen profile for chicken D with two peaks in hydrogen concentration at 180 and 240 minutes (Figure 1) is very similar in appearance to breath profiles observed in rats and humans with small bowel bacterial overgrowth (proliferation of facultative anaerobes in the small intestine). Choc et al. (1996) observed microbial proliferation in the small intestine of chickens associated with an increase in digesta transit time as a result of the gelling properties of soluble non-starch polysaccharides in wheat.
Figure 1. Breath hydrogen concentration (in ppm) in male chickens fasted for 3 h prior to test dosing with lactulose (130 mg in 5mL water) in Experiment 1.

(b) Experiment 2 – Measurement of oro-caecal and whole tract transit time

Oro-caecal transit time and standard error were 165 ± 12 mins and 164 ± 13 mins for whole tract transit time in male chickens (Figure 2). For female chickens (Figure 3), the corresponding values were 158 ± 13 mins for OCTT and 166 ± 112 mins for WTTT. That is, there was no difference due to sex of the chicken for either measurement. OCTT and WTTT were significantly correlated ($r=0.76$, $P<0.05$) with WTTT being generally shorter than OCTT. Possible explanations for this anomalous observation are (a) lactulose and ferric oxide move in a different manner through different sections of the alimentary tract, or (b) there is a pause when undigested carbohydrate reaches the caeca in fasted animals while bacterial fermentation gets under way.

Figure 2. Breath hydrogen concentration (in ppm) in male chickens fasted for 3 h prior to test dosing with lactulose (130 mg in 5mL water) in Experiment 2. The vertical arrows indicate whole tract transit time for ferric oxide marker.
Figure 3. Breath hydrogen concentration (in ppm) in female chickens fasted for 3 h prior to test dosing with lactulose (130 mg in 5mL water) In Experiment 2. The vertical arrows indicate whole tract transit time for ferric oxide marker.

Finally, it is possible that a non-invasive breath test for estimation of oro-caecal transit time can be devised without the need to dose chickens with lactulose. OCTT and WTTT were 184 ± 8 mins and 172 ± 5 mins, respectively, in four chickens fasted for 3 h but not dosed with lactulose. That is, caecal microflora may produce enough hydrogen from normal levels of complex carbohydrates in commercial broiler feed.

IV. CONCLUSIONS

Serial breath testing of fasted chickens dosed with lactulose can be used to measure oro-caecal transit time in chickens. There may be a sufficient level of dietary fibre in normal broiler diets to avoid the need for lactulose in field testing of commercial flocks.

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REFERENCES