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PASSAGE RATES OF DIGESTA MARKERS IN THE GUT OF THE HOATZIN, A FOLIVOROUS BIRD WITH FOREGUT FERMENTATION[1]

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Abstract. Foregut fermentation in the Hoatzin (*Opisthocomus hoazin*) appears to be a unique adaptation among birds. Passage rates of liquid and solid digesta were measured in five Hoatzins. Four markers were given to captive birds: Cr-EDTA (a liquid marker), ytterbium (Yb) oxide mordanted on plant fiber particles of 1 mm² (solid marker), and 1 mm² and 4 mm² plastic particles (solid markers). The markers were given orally as a single pulse dose. Hoatzins were fed ad libitum and housed in metabolic cages with removable floor trays. Excreta were sequentially collected for 4–5 days. Plastic markers were visually counted and Cr and Yb were measured by atomic absorption spectrometry. Transit times were significantly shorter for the liquid marker (2.6 hr ± 0.5) than for solid particles. Transit lines of solid particles were significantly longer for the largest particles: Yb (4.5 hr ± 3.0), 1 mm² (7.5 hr ± 1.0) or 4 mm² (10.7 hr ±4.6). Mean retention times were significantly shorter for the liquid marker (18.3 hr ± 3.3) than for Yb (25.1 hr ± 2.8), 1 mm² (33.6 hr ± 11.3) or 4 mm² (45.7 hr ± 7.8). Mean retention times of the Yb particles were significantly shorter than for the 4 mm² plastic marker. Hoatzin passage rates are among the longest recorded for a bird; they are similar to mean retention times found in some large foregut fermenting mammals. Long retention times, efficient separation of solid and liquid digesta and selective particle size retention probably maximize energy and nutrient utilization of both plant cell contents and cell walls. This digestive strategy contrasts with that of other herbivorous birds, in which fast passage rates maximize the rate of assimilation from cell content at the expense of little cell wall digestion.

Key words: Herbivory; passage rate; digesta markers; foregut fermentation; selective digesta retention; cell wall.

Resúmen. La fermentación pregástrica en la Chenchena es una adaptación única entre las aves. Tiempos medios de retención y tiempos de tránsito de la digesta sólida y líquida fueron medidos en cinco Chenchenas. Dosis puntuales de cuatro marcadores se les administraron a las aves en cautiverio: Cr-EDTA (un marcador líquido), óxido de ytterbio (Yb) mordante en partículas de fibra vegetal de 1 mm² (marcador sólido), y marcadores plásticos de 1 mm² y 4 mm² (marcadores solidos). Las Chenchenas fueron alimentadas ad libitum en jaulas metabólicas con bandejas removibles en el piso. Las excretas fueron colectadas secuencialmente por 4–5 días. Los marcadores plásticos fueron contados visualmente y Cr Yb fueron medidos mediante espectrometría de absorción atómica. Los tiempos de tránsito fueron significativamente más cortos para el marcador líquido (2.6 h ± 0.5) que para los marcadores sólidos: Yb (4.5 h ± 3.0), 1 mm² (7.5 h ± 1.0) y 4 mm² (10.7 h ± 4.6). Los tiempos medios de retención fueron significativamente más cortos para el marcador líquido (18.3 h ± 3.3) que para Yb (25.1 h ± 2.8), 1 mm² (33.6 h ± 11.3) o 4 mm² (45.7 h ± 7.8). Los tiempos medios de retención fueron significativamente más cortos para Yb que para marcadores plásticos de 4 mm². Estos son los tiempos de retención más largos registrados para cualquier ave pero similares a los tiempos de retención de mamíferos grandes con fermentación pregástrica. Tiempos de retención largos, separación eficiente de digesta sólida y líquida y retención selectiva de partículas probablemente maximizan la utilización de energía y nutrientes tanto de los contenidos celulares como de las paredes celulares. Esta estrategia digestiva contrasta con la de otras aves herbivoras, en la que las rápidas tasas de paso maximizan la tasa de asimilación de los contenidos celulares a expensas de poca digestión de las paredes celulares.

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INTRODUCTION

Food passage rates of birds have been poorly studied (Warner 1981b). Although retention times in birds seem to be generally short, a few common trends can be seen (Warner 1981b, Karasov 1990). In particular, herbivorous birds have slower passage rates than other birds of similar size (Warner 1981b). Within this group, birds with active caecal fermentation (e.g., Tetraonidae) have longer retention times than herbivorous birds with little or no fermentation, like geese, or Emus (Warner 1981b, Karasov 1990).

The few studies that have analyzed particle retention in birds seem to indicate that some birds are able to pass refractory solids faster than the more digestible liquids (Björnhag and Sperber 1977, Warner 1981b). Furthermore, herbivorous birds with caecal fermentation seem to retain the liquid phase almost twice as long in the caeca than the solid phase (Gasaway et al. 1975). However, foregut fermenting mammals generally show the opposite trend: solids are retained longer than liquids (Warner 1981a, Warner 1981b). The particular gastrointestinal morphology of foregut fermenting mammals also results in selective particle retention, with smaller particles and liquids passing faster along the gut than larger particles. This is an important trait for foregut fermenting mammals because the larger particles are retained in the fermentation chambers for further digestion, while the digestible liquids and small particles are passed to the small intestine where assimilation takes place. This selective particle retention seems to occur at the foregut chambers and not at other gut sites (Grovum and Williams 1973, Warner 1981b).

The Hoatzin (*Opisthocomus hoazin*) is the only known obligate avian folivore with a well-developed foregut fermentation system (Grajal et al. 1989). Most of the fermentation takes place in the anterior portion of the gut (i.e., crop and posterior esophagus). The capacity of these foregut sections is about 10% of the adult Hoatzin's body mass (Grajal et al. 1989; Grajal 1995b). The morphology of these organs is unique. The crop is divided by a fold into two connected chambers, while the posterior esophagus is heavily sacculated, with multiple semilunar folds and constrictions. Particle size is significantly reduced at these fermentation sites (Grajal et al. 1989; Grajal 1995b), suggesting a combined abrasive action by the internal lining of the muscular crop and intense microbial attack on the fiber components of the diet. Some additional fermentation takes place in the small paired caeca (Grajal et al. 1989).

In the Hoatzin, digesta dynamics would be more similar to the trends in foregut fermenting mammals than trends in other herbivorous birds. Differential passage of solid and liquid digesta or small and large particles are important attributes of foregut fermentation digestive systems, because these traits can increase nutrient and energy extraction from a herbivorous diet. Therefore, this study was undertaken to examine differential particle passage rates and retention times of digesta in the Hoatzin.

MATERIALS AND METHODS

The experiments were performed with one Hoatzin in 1988 and four Hoatzins in 1989. All birds had ad libitum access to a diet consisting of romaine lettuce with a powdered mix of ground alfalfa pellets, ground Timothy grass hay and ground roasted soybeans (Grajal 1995a). Additionally, fresh young shoots of plants in their natural diet (e.g., *Enterolobium cyclocarpum, Pithecellobium saman, Guazuma ulmifolia* and *Pthyrhusa* cf. *venezuelensis*) were offered ad libitum twice daily. The birds were housed indoors in adjacent 1 × 1 × 2 m individual custom-made metabolic cages with removable floor trays for quantitative collection of excreta. Mean body mass for all birds was 613.7 g (66.1 SD, $n = 5$). At the end of the experiments, the birds were on average 100.9% of the original body mass (1.8 SD, $n = 5$). All trials started in the morning (7:30–10:00 hr) just before the morning feeding.

Hoatzins were force-fed a gel capsule with markers as a single pulse dose (Warner 1981b). Plastic markers consisted of two sizes (1 mm^2 and 4 mm^2) of squares of brightly-colored pink and orange commercial flagging tape respectively. The specific gravity of this flagging tape was 1.01, very similar to the specific gravity of wet fiber plant fractions (Warner 1981b). The liquid phase was marked with Cr-EDTA (chromium ethylene-diamine tetra acetic acid) prepared following the procedure of Binnerts et al. (1968). The Cr concentration in excreta was measured using atomic absorption spectrometry (Williams et al. 1962). Fiber from a mature grass hay was mordanted with ytterbium (Yb) oxide, using the procedure of Ellis et al. (1982). Before mordanting, the hay was ground and sieved to 1 mm^2

particles, with an upper size limit of 2 mm^2. The cell contents were extracted using neutral detergent (Goering and VanSoest 1970), and the residue (neutral detergent fiber NDF) was dried over absorbent filter paper at ambient temperature for 24 hr and then stored in a dessicator until needed for marking. The ytterbium concentration in excreta was measured by atomic absorption spectrometry using a nitrous oxide-acetylene flame.

The individual doses for the trials were estimated at approximately 13.6 mg Cr, 15 mg Yb (mordanted to 2 g fiber), 400 plastic markers of 1 mm^2 and 200 plastic markers of 4 mm^2 per bird. However, the exact dose of markers for each animal was not known, because the sizes of the gel capsules were slightly different, and some material remained in the beak and mouth of the birds. Therefore, the total amount of marker was estimated as the amount of marker at the asymptotic portion of the cumulative marker excretion curve. When the cumulative curve did not achieve a clear asymptote, additional values were predicted from an exponential regression of marker excretion points after the time of maximum marker excretion (see below for regression model).

Excreta were quantitatively collected on black plastic film liners on the cage's floor tray. The black liners provided a color contrast that enhanced the recovery of the tiny 1 mm^2 plastic markers, although nearly total recovery was achieved only in two samples.

Excreta were collected at regular intervals of 2–3 hr the first day, 4–5 hr the second day, 8 hr the third day and 10 hr the fourth day. For each batch of excreta, the total number and size-class of plastic markers were visually counted and removed. All excreta from each collection were removed from the plastic liners, dried in a forced-air oven at 65°C to constant weight and kept in labeled individual plastic bags for later analysis.

For Cr and Yb analysis, the excreta from each sample were ashed at 600°C. Some samples were too small (e.g., from the early excreta), and were pooled with a larger sample corresponding to the next sample time. Extraction of Cr and Yb from the ash was based on the procedure of Christian and Coup (1954) and modified for simultaneous extraction of Cr and Yb by Siddons et al. (1985). The Yb extraction method of Siddons et al. (1985) was modified so that instead of centrifugation after ash extraction, the extract was filtered through Whatman® GPA filter paper and stored until analyzed. Standards for both Cr and Yb were prepared using excreta from previous experiments that contained no Cr or Yb (Christian and Coup 1954, Siddons et al. 1985).

Transit time was the time of first appearance of a marker in the excreta. Mean retention times were calculated as $MRT = \Sigma m_i t_i / \Sigma m_i$ where m_i is the amount of marker excreted per unit dry matter excreta at the ith defecation at time t_i after dosing (Blaxter et al. 1956). This method makes no assumptions about the frequency distribution of marker excretion (Warner 1981b), an important advantage because excretion curves are generally not uniform in shape. Additionally, the times needed for the excretion of 5, 10, 50, 90, and 99% of the marker were expressed as t_5, t_{10}, t_{50}, t_{90}, and t_{99}. The time of maximum marker concentration in the excreta was defined as t_{max}. Most of the decline in marker concentration over time after t_{max} was approximately exponential. Therefore, a linear regression was calculated from the natural logarithm of the marker concentration against time. The inverse of the regression slope was the exponential retention time, t_{exp}, which in turn is an estimate of the rate of turnover in the main fermentation chamber (crop) (Grovum and Williams 1977).

Trials in 1988 measured retention times using plastic markers. In 1989, one trial consisted of a pulse dose of both plastic markers and Cr-EDTA to four birds (18 Sept.) and another trial consisted of Cr-EDTA and Yb markers (2 Oct.) to four birds.

All statistical analyses were two-tailed with an alpha level of 0.05. Each individual combination of bird-marker was considered as the experimental unit for each test. Mean retention time and transit time were transformed to the inverse of the square root to reduce heteroscedasticity (heterogeneous variances) in the ANOVA tests (Sokal and Rohlf 1981). Post hoc multiple comparisons were performed using the Games-Howell test (Games and Howell 1976). This test is a relatively conservative and robust procedure for unequal sample sizes, heterogeneous variances and even violations of normality (Jaccard et al. 1984). Standard deviations are shown in parentheses.

RESULTS

EXCRETION RATES

Excretion rates of Hoatzins during the trials were relatively constant. No daily fluctuations were

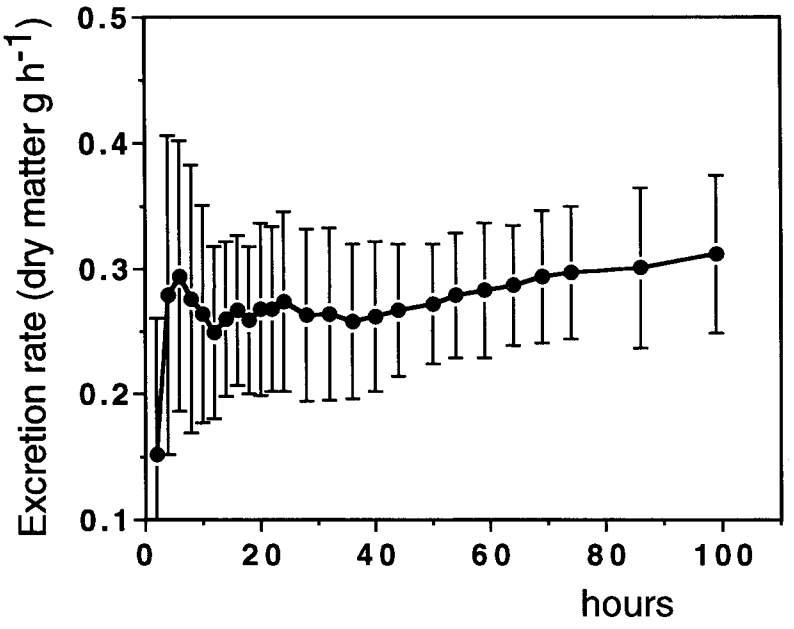

FIGURE 1. Average excretion rate (in dry matter grams/hour), bars represent standard deviations (n = 4). The initial handling of the animals depressed the initial excretion rates, and then remained relatively constant throughout the experiments.

evident at the measuring scale used. However, the handling of birds during the administration of the marker pulse lowered the excretion rate for the fist 4–6 hr of the trials. This initial depression of the excretion rate was present in most birds, but its length was different for each individual. Excretion rate curves are shown in Figure 1. Except for the initial excretion of liquid marker, solid markers appeared for the first time well after the excretion rates stabilized. The effect of initial low excretion rates on liquid transit time remains unknown.

MARKER EXCRETION PATTERNS

Transit times were short for the liquid marker Cr-EDTA (Table 1). This marker appeared in the first collection after the single pulse dose, so the smallest detectable transit time was 2.5 hr (±0.54 SD). Transit times were similar for Yb and 1 mm^2 plastic particles. The longest (and most variable) transit time was recorded for 4 mm^2 plastic particles (Table 1). Overall, transit times were significantly different among markers (One factor ANOVA, F = 16.51, df = 3, P = 0.0001). Multiple comparisons (Games-Howell test) show significant differences between Cr-EDTA and both 1 mm^2 and 4 mm^2 plastic particles.

Mean retention times for each marker are shown in Table 1. No significant difference in mean retention time was found between individual Hoatzins (One factor ANOVA, F = 0.55, df = 4, ns), but the difference was highly significant between markers (One factor ANOVA, F = 13.66, df = 3, P = 0.0002). The liquid marker (Cr-EDTA) had significantly shorter mean retention time than Yb and 4 mm^2 plastic particles, while all other comparisons between markers were not significant (Games-Howell test).

All other indicators of marker behavior were significantly different among markers, with most multiple comparisons showing significant differences between liquid and solid markers. One interesting exception was t_{exp}, where no significant differences were found between markers. This suggests that marker retention in the main fermentation chamber (crop) was independent of particle size.

MARKER EXCRETION CURVES

All but one of the excretion curves were explained by regression of the descending phase of

TABLE 1. Mean retention time (MRT), transit time (TT), and time of appearance of 5, 10, 50, 90, and 99% of the marker expressed as t_5, t_{10}, t_{50}, t_{90}, and t_{99}. The time of maximum concentration of marker was expressed as t_{max}. The inverse of the regression slope was the exponential retention time, t_{exp}. Sample sizes are represented by (*n*). Liquid marker was Cr-EDTA. Solid markers were ytterbium (Yb) mordanted to 1 mm² particles of hay fiber and two sizes (1 mm² and 4 mm²) of cuts of commercial plastic flagging tape. Standard deviations are shown in parentheses.

	Liquid	Solid		
	Cr-EDTA	Yb	1 mm²	4 mm²
MRT (hours)	18.33 (3.32)	25.08 (2.8)	33.56 (11.33)	45.66 (7.83)
TT (hours)	2.57 (0.54)	4.50 (3.0)	7.50 (1.0)	10.67 (4.62)
t_5	3.57 (1.27)	7.00 (2.95)	14.25 (3.75)	18.33 (1.16)
t_{10}	5.43 (1.13)	8.75 (3.5)	15.75 (4.03)	22.00 (2.65)
t_{50}	13.57 (3.26)	18.75 (2.22)	27.25 (9.91)	41.00 (13.08)
t_{90}	31.57 (7.48)	40.75 (11.15)	61.25 (22.74)	78.00 (20.79)
t_{99}	67.57 (13.73)	90.75 (22.41)	97.00 (43.15)	109.67 (17.62)
t_{max}	11.85 (5.2)	16.50 (5.2)	18.00 (4.9)	30.00 (7.2)
t_{exp}	21.78 (9.92)	28.98 (13.65)	17.14 (7.87)	19.85 (7.28)
(*n*)	7	4	4	3

marker concentration (Fig. 2). Nearly half of the curves, however, showed some deviation in curve portions where expected, and observed marker concentration was different from preceding and succeeding portions of the curve. These "waves" were similar to those found in folivorous marsupials by Warner (1981a) and Wallis (1994).

DISCUSSION

All markers seemed satisfactory to measure mean retention times as well as selective particle size retention. Although the exact recovery rate was not accurately estimated, extrapolation from the regression of the descending portion of the marker excretion curve allowed a good estimation of overall marker behavior. The fact that mean retention times of 1 mm² plastic and 1 mm² Yb particles were not significantly different, demonstrates that small plastic markers can provide a simple method to estimate passage rates. Some of the drawbacks of plastic markers include the intensive labor required to obtain a satisfactory recovery. Additionally, the variability of mean retention times measured with plastic markers seems to be inherently higher than measured with chemical markers. This variability is probably related to the fact that plastic markers remain completely inert and do not change in composition or size, while food particles can be physically affected by the grinding action at the crop, esophagus and gizzard.

Although the parameters that describe the passage rates were not significantly different between animals, the patterns of marker defecation curves were quite different both within and among individual Hoatzins. The "waves" found in some marker excretion curves (Figs. 2B, C, F, H, G) suggest a sequestration and subsequent release of marker at the main fermentation chamber (the crop). However, the fact that the t_{exp} were not significantly different between markers, suggests that the observed selective retention of solid and liquid markers may occur outside the crop. The separation may occur in the long sacculated posterior esophagus, which probably behaves as a series of mixing pools for digesta (Grajal 1995b). A similar gut structure is found in some macropodid and potoroine marsupials with a high degree of separation of liquid and solid phases of the digesta (Dellow et al. 1983, Hume 1989, Wallis 1994, Warner 1981a). Therefore, the Hoatzin foregut fermentation system may function as a complex series of mixing reactors, with a large mixing compartment (crop), followed by a series of smaller mixing compartments in the posterior esophagus. This also suggests that a kinetic analysis of marker concentration curves using chemical reactor theory may be too simplistic for this complex system.

The measured mean retention times are among the longest recorded for a herbivorous bird and are more similar to those of ruminants or arboreal folivores (Warner 1981b, Karasov 1990). The short transit time of the Cr-EDTA suggests a fast movement of liquids through the Hoatzin's gut. Fast liquid transit times have been reported for other herbivorous birds (Clemens et al. 1975,

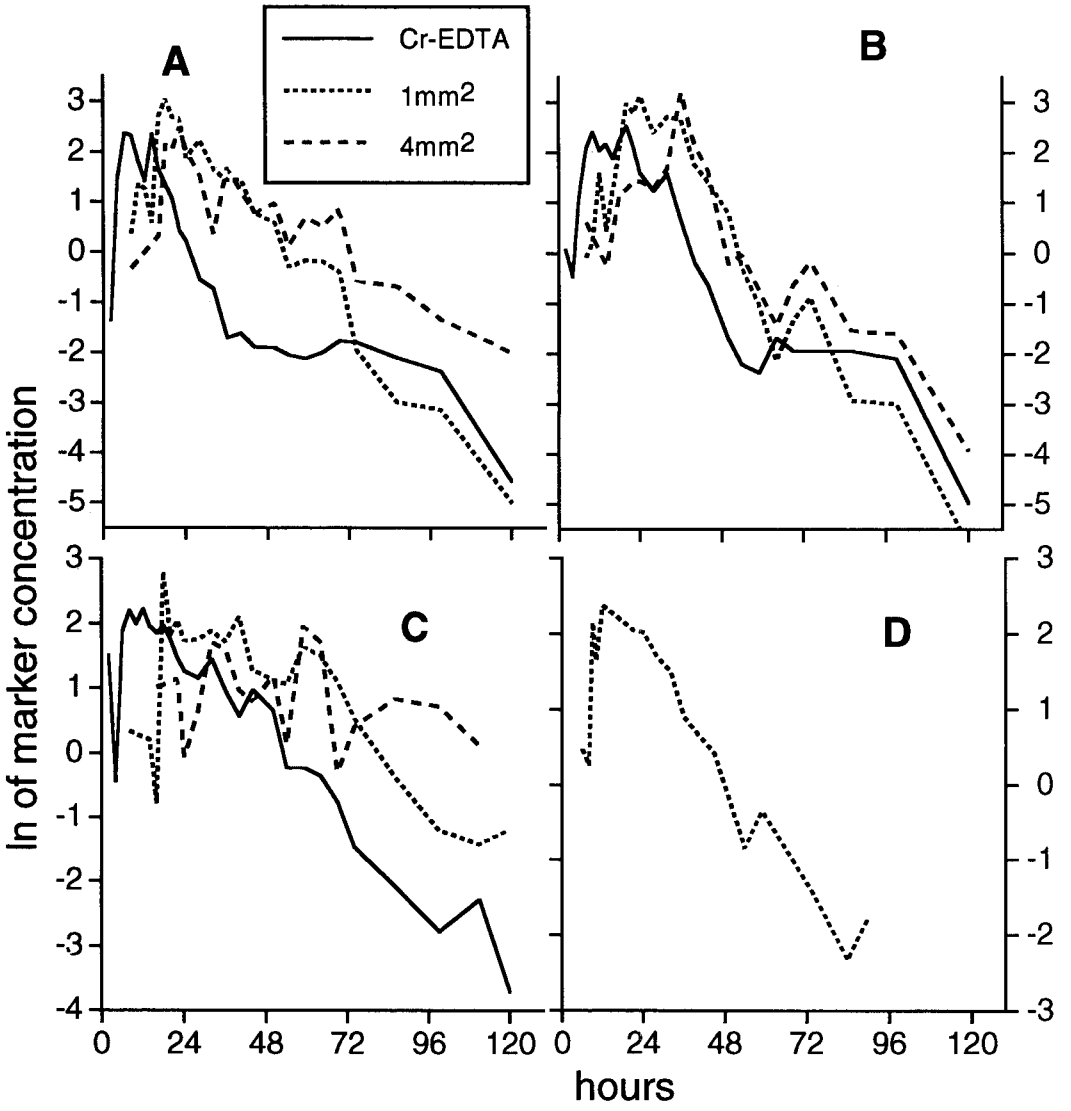

FIGURE 2. Changes in marker concentration through time. Regression equations were fitted to points after t_{max}. The curve for 4 mm² in experiment C) was the only curve that did not significantly fit to a linear regression. Each graph represents one individual Hoatzin given a single pulse dose of a combination of markers. Experiments A), B), and C) consisted of a single pulse dose of Cr-EDTA and both 1 mm² and 4 mm² plastic markers. Experiment D) only consisted of 1 mm² plastic markers. Experiments E), F), G), and H) consisted of a single pulse dose of Cr-EDTA and Yb.

Duke 1989, Björnhag 1989) and herbivorous mammals (Clemens et al. 1975, Warner 1981b).

Selective retention of solid particles compared with the liquid phase has been reported in mammals with foregut fermentation (Grovum and Williams 1973, Hume and Dellow 1980, Warner 1981a, 1981b). Within this group, a higher degree of separation of the two digest phases has been reported for macropodid marsupials than for ruminants (Hume and Dellow 1980, Warner 1981a). However, in spite of the gross morphological and digesta passage similarities, the separation of liquid and solid markers in the Hoatzin does not reach the extent seen in macropod marsupials, and it is more similar to the ratio seen in ruminants. Faster passage of the liquid

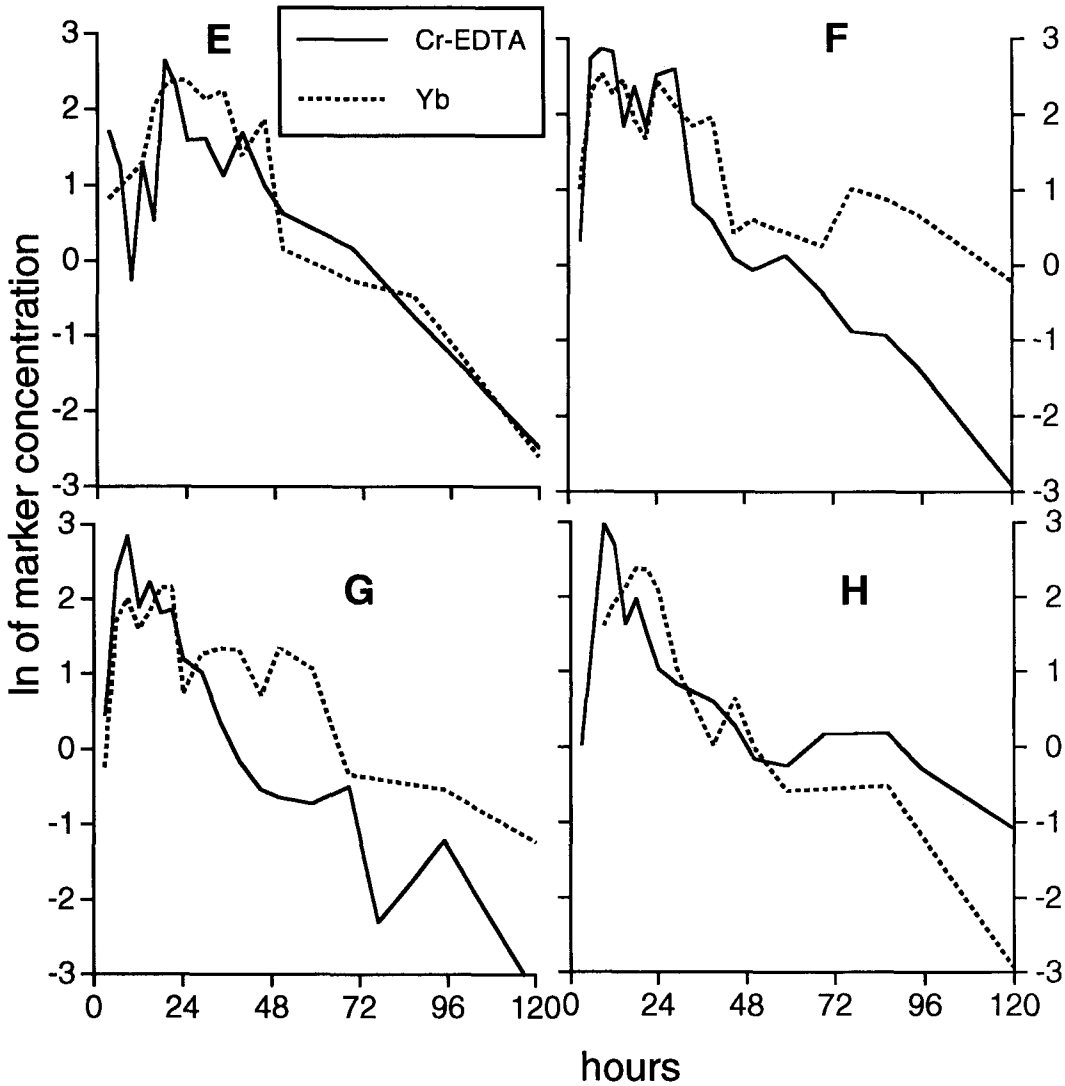

FIGURE 2. Continued.

fraction can be important for a small vertebrate with foregut fermentation, such as the Hoatzin, because it allows the passage of the more fermentable substrates to the small intestine where enzymatic digestion and absorption take place. Meanwhile, increased residence of larger particles in the foregut allows more time for microbial attack of the cell wall and enough time for microbial population turnover. One of the possible drawbacks of a fast liquid passage is that fermentative microbes in solution would be rapidly washed and digested in the small intestine. This may result in a detrimental reduction of the time allowed for microbial population turnover. However, microscopic observations of the crop contents of Hoatzins indicate that most bacteria are firmly attached to the cell walls (Dominguez-Bello et al. 1993) and thus avoid being washed down the gut with the liquid phase.

In Hoatzins, larger particles are retained longer than smaller ones. Although this is common in foregut fermenting mammals (Blaxter et al. 1956, Warner 1981b), it is rarely seen in birds. For example, some herbivorous birds show no selective particle retention (Herd and Dawson 1984), but most herbivorous birds show the op-

posite trend—larger particles are excreted significantly faster than the smaller particles and the liquid fraction (Gasaway et al. 1975, Björnhag and Sperber 1977, Warner 1981b). This pattern is especially prevalent in birds with caecal fermentation (e.g., Tetraonidae), in which the caeca seem to selectively retain very small particles and liquids and reject the less digestible larger food particles (Björnhag and Sperber 1977, Björnhag 1989). This strategy probably maximizes the rate of energy intake in birds with caecal fermentation (Gasaway et al. 1975, Remington 1989). Although a similar mechanism may occur in the caeca of the Hoatzin, its effect on particle retention through the gut is probably negligible, because the Hoatzin's caeca are relatively small (Grajal 1995b).

Long digesta retention times in the Hoatzin seem to maximize the nutritional use of both cell contents and cell walls. In contrast, most herbivorous birds (geese, ducks, Emu) maximize the nutritional use of plant cell contents at the expense of the utilization of cell walls. In the Hoatzin, the relatively faster liquid passage decreases microbial fermentation of the readily digestible cell contents and makes these substrates available to the small intestine, where absorption and digestion take place. Reduced microbial use of cell contents may be an important digestive strategy for Hoatzins. Microbial fermentation of readily digestible cell contents can insert an additional trophic level between the food and the host, increasing microbial metabolic losses (e.g., methane, CO_2 and heat) and decreasing the overall energy available to the Hoatzin. These losses can be significant for a small vertebrate with high mass-specific rates of metabolism (Kleiber 1961, McNab 1988).

Another benefit of differential passage rates is that the more refractory cell wall fraction remains longer in the crop and esophagus, where microbial fermentation takes place. Indeed, Hoatzins digest fiber components to an extent rarely seen in birds. For example, on an experimental diet with 39% neutral detergent fiber (NDF) and 3% nitrogen, Hoatzins digested 63% cellulose, 78% hemicellulose and 71% NDF (Grajal 1995a). These high fiber digestibilities probably result from the combined effect of long retention times, selective retention of liquid and partide sizes, microbial fermentation, and selection of a highly fermentable diet. Indeed, Hoatzins in their natural habitat select plant parts that are lower in cell wall, lignin and higher in protein and water content than non-selected parts (Grajal et al. 1989). Selection of high quality plant parts is possible because Hoatzins are able to fly and therefore can track patchy resources in space and time. Foregut fermentation and the long digesta retention times in the Hoatzin result in a unique evolutionary adaptation that provides for efficient use of a herbivorous diet by a flying bird.

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