NUTRITIONAL ECOLOGY AND DIGESTIVE PHYSIOLOGY OF THE HOATZIN, OPISTHOCOMUS HOAZIN, A FOLIVOROUS BIRD WITH FOREGUT FERMENTATION

BY

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to my parents, Alejandro and Carmina.

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KEY TO SYMBOLS OR ABBREVIATIONS

ADF	Acid Detergent Fiber
ANOVA	Analysis of Variance
BRM	Basal Rate of Metabolism
C_m	Thermal Conductance
Cr	chromium
CS	Concentrate Selectors
CW	Cell Wall
d.f.	degrees of freedom
DM	Dry Matter
EDTA	Chromium Ethylene-Diamine Tetra Acetic acid
g	grams
gDM	grams Dry Matter
GR	Grass and Roughage eaters
h	hour
IF	Intermediate Feeders
IVOMD	In Vitro Organic Matter Digestibility
KJ	Kilojoule
kg	kilogram
l	liter
MEC	Metabolizable Energy Coefficient
mg	milligram
min	minute
ml	milliliter
mm^2	Square millimeter
mmol	millimol
MPS	Mean Particle Size
NDF	Neutral Detergent Fiber
OM	Organic Matter
pers. obs.	personal observation
pers. comm.	personal communication
Ta	Ambient Temperature
Tb	Body Temperature
TT	Transit Time
VFA	Volatile Fatty Acids
VO_2	Rate of Oxygen Consumption
Yb	ytterbium
°C	degrees centigrade

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The hoatzin is the only known obligate folivorous bird with a well-developed foregut fermentation system. Most fermentation takes place at the crop and caudal esophagus, where pH and volatile fatty acid (VFA) levels are similar to those of foregut fermenting mammals. Contents from fermentation organs represent 77% of the total gut capacity and 10% of the adult body mass (average 650g). Large particles are retained longer than small particles at the anterior fermentation sites. Food particle size is reduced by microbial fermentation and grinding by the keratinous interior lining of the muscular crop. Dry matter digestibilities by captive hoatzins were high (70-80%). Fiber digestibilities were higher than values previously reported for other avian herbivores (35-71% neutral detergent fiber, 47-63% cellulose). Passage rates of liquid and solid digesta were measured by giving a single pulse dose of a liquid marker (Cr-EDTA) and three solid markers (ytterbium mordanted to

fiber and cuts of plastic tape of 1 and 4mm^2). Mean retention times in the hoatzin are among the longest ever recorded for a bird (18h for liquid, 24h for ytterbium, 34h for 1mm^2 and 44h for 4mm^2 markers). Production rates of VFA are the highest recorded for a bird, and provide energy for more than 60% of the hoatzin's basal rate of metabolism. In vitro fiber digestibility was similar between hoatzin and cow inoculum. The basal rate of metabolism in hoatzins is low (70% of the expected value for endotherms and 43% of the expected for nonpasserine birds). Body temperature is maintained at 38.5°C at environmental temperatures between 12°C and 36°C.

Some of the extreme adaptations in the hoatzin are more similar to those of mammals with foregut fermentation than to those of a bird. Microbial fermentation breaks down fiber and makes a significant contribution to the energy balance of hoatzins. Additionally, long retention times and selective particle retention enhance digestion of cell wall and cell contents. Other nutritional benefits, such as detoxification of plant secondary compounds and microbial synthesis of essential amino acids, may have been important in the evolution of foregut fermentation in this unique bird.

CHAPTER 1
GENERAL INTRODUCTION

Foregut fermentation as a method to digest plant fiber has been reported for mammals such as ruminants (Parra 1978, Van Soest 1982), colobid monkeys (Bauchop and Martucci 1968), sloths (Bauchop 1978, Montgomery and Sunquist 1978) and macropod marsupials (Dellow et al. 1983, Hume 1982, Hume and Dellow 1980, Moir et al. 1956). Hoatzins are a unique among birds because they are the only known bird with a foregut fermentation system.

Hoatzins are one of the few avian obligate folivores (leaf-eaters) (Morton 1978). Less than 3% of extant bird species feed extensively on green leaves or buds (Morton 1978). Although some birds can digest plant fiber, it is generally of little nutritional value. The reasons for the rarity of avian folivores are that leaves are difficult to digest, bulky, and usually have defensive chemical compounds. These dietary characteristics can impose serious constraints on the high energy demands of powered flight, by increasing the weight to be carried and decreasing the rate of energy extraction from leaves. Consequently, most birds that eat large amounts of plant material maximize the rate of energy or nutrient uptake and minimize the weight of the digesta by extracting the readily digestible cell contents and quickly excreting the undigested bulk of the fiber or cell wall. Examples are geese and ducks (Buchsbaum et al. 1986, Burton et al. 1979, Dawson et al. 1989, Kingsford 1989, Marriot and Forbes 1970, Muztar et al. 1977), some birds within the Galliformes (Inman 1973), Takahe (Notornis mantelli), and kakapo or owl parrot (Strigops habroptilus) (Morton 1978). Some ratites, such as the ostrich

(Struthio camelus) (Mackie 1987, Withers 1983), and the emu (Dromaius novaholliandeae) (Herd and Dawson 1984) can digest plant fiber. Several species of the family Tetraonidae can ferment fiber in enlarged paired caeca (Gasaway 1976a, Gasaway et al. 1976, Hill et al. 1968, Inman 1973, Moss 1973, Moss 1977, Moss and Parkinson 1972, Moss and Trenholm 1987, Pulliainen et al. 1968, Suomalainen and Arhimo 1945).

Hoatzins are obligate folivores (Grajal et al. 1989). In the Venezuelan Llanos, 78.3% of all observed foraging time (>17,000 minutes) was spent on new leaves and shoots, 8.5% on mature leaves, 6.1% on flowers and 7.2% on fruits. Green leaves comprise 86.7% of the hoatzin diet (as % observed foraging time) (Grajal et al. 1989, Strahl and Parra 1985). Among all species included in the diet, samples of portions of the plant that are eaten are significantly higher in water content, nitrogen, and hemicellulose, and lower in total cell wall, cellulose, and lignin than non-eaten portions of the same plants (Grajal et al. 1989). Although hoatzins forage on the leaves of more than 45 species of plants, only a few plants make up the bulk of the diet. For example, two species, Zanthoxylum culantrillo and Acacia articulata, comprise nearly 50% of the observed overall diet in the Central Venezuelan Llanos (Strahl and Parra 1985). In contrast, the remainder of the diet is made up of over 40 plant species, most of which account individually for less than 1% of the observed diet. These include several legumes (Fabaceae) and many additional species in over 20 other families.

Foregut fermentation in the hoatzin is achieved by some unique morphological and physiological adaptations. Their digestive system is unusual both in structure and function among birds. In fact, the hoatzin is the only flying vertebrate with foregut fermentation. The voluminous crop and caudal esophagus have become functional fermentation chambers,

analogous to those of mammalian foregut fermenters. The crop and esophagus are situated in front of a greatly reduced sternal carina that leaves little area for flight muscle attachment. Consequently, hoatzins are not powerful fliers, preferring to hop from branch to branch (Grajal et al. 1989).

Foregut fermentation in hoatzins is a theoretical anomaly. Present models predict a limit of 6-10 kg of body mass, below which foregut fermentation cannot fulfill the nutritional needs of an endotherm (Demment and Van Soest 1985, Parra 1978). At this mass, the predicted total metabolic requirements surpass the rate of energy made available from plant fiber fermentation. These models suggest that herbivores below this threshold should be omnivores with hindgut fermentation. Thus, the small mass of the hoatzin (650g) is an order of magnitude lower than the predicted minimum body mass for a foregut fermenter.

Some of the advantages of foregut fermentation in mammals include an extensive use of plant fiber and an efficient use of microbial byproducts and nitrogen. Microbial byproducts such as volatile fatty acids (VFA) are directly absorbed at the fermentation sites and readily used by the host as an energy source (Blaxter 1962). Microbes also produce essential nutrients such as amino acids and vitamins that are absorbed in the lower gut (Hungate 1966). Furthermore, foregut fermentation can be an important way to synthesize bacterial protein from non-protein nitrogen (e.g., ammonia and urea) (Nolan and Leng 1972, Prins 1977, Van Soest 1982). Finally, foregut fermentation can be an effective method to detoxify some plant secondary compounds before they reach the absorptive tissues of the lower gut (Freeland and Janzen 1974, Mackie 1987).

Foregut fermentation also has some important drawbacks. For example, foregut fermentation can decrease the digestive efficiency of a small

herbivore, because gut flora establishes one or more trophic levels between the host and the readily digestive fractions in the diet. These additional trophic levels can significantly decrease the rate of energy extraction by the host. Fermentation products from microbial metabolism, mainly heat, CO_2 and methane, can cause a substantial loss of nutrients and energy for the host. Thus, some of these nutritional costs can be especially detrimental in a small endotherm with high energy and nutrient turnover rates (Demment and Van Soest 1985, Parra 1978).

The study of the digestive system of the hoatzin can provide numerous insights into the evolution of foregut fermentation in vertebrates and about the evolutionary constraints of herbivory in birds. Therefore, the objectives of this study were to

1) Analyze the structure and function of the gastrointestinal tract of hoatzins,

2) Characterize the dynamics of their digestive process

3) Determine their digestive efficiency

4) Analyze the energetic balance of the hoatzin as a folivorous bird, and

5) Explore the possible selective forces of this unique digestive system in a bird.

This study has been divided in individual chapters that explore these objectives. A final general discussion provides some evolutionary and ecological implications of foregut fermentation in hoatzins, digestive strategies of avian herbivores and the general implications of the evolution of foregut fermentation in vertebrates.

Hoatzins live in gallery forests, forest swamps and oxbow lakes of the Orinoco and Amazon drainages, ranging locally from the Guianas and Venezuela throughout Amazonian Brazil, Colombia, Ecuador, Peru and Bolivia.

Although their range is quite extensive, hoatzins have strict habitat requirements, with narrow local distributions.

The peculiarities of the hoatzin's anatomy were the subject of many descriptive studies in an attempt to establish the evolutionary affinities between the hoatzin and other birds. As a consequence, the taxonomic position of the hoatzin has been one of the most debated topics of avian systematics (Banzhaf 1929, Beebe 1909, Böker 1929, Brigham 1919, Cherrie 1909, Gadow 1891, Garrod 1879, Huxley 1898, L'Hermenier 1837, Parker 1891, Perrin 1877, Pycraft 1895, Verheyden 1956). The presence of functional wing claws in hoatzin chicks, the reduced sternal carina and poor flying abilities of the hoatzin were regarded as the primitive characteristics of a "missing link" between the first fossil birds such as Archaeopteryx and modern birds (Brigham 1919, Huxley 1898, Parker 1891, Young 1888). Later, it was classified within the Galliformes, or chicken-like birds (Huxley 1898). Modern electrophoretic (Sibley and Ahlquist 1973), morphological (de Queiroz and Good 1988, Verheyden 1956) and DNA-DNA hybridization (Sibley et al. 1988) studies have consistently classified the hoatzin as the only member of the family Opisthocomidae, included in the order Cuculiformes and closely related to anis (Crothophaga) and guira cuckoos (Guira).

Surprisingly, the gastrointestinal tract and nutritional ecology received little attention until recently (Grajal et al. 1989). Some early authors attempted to relate the large gut capacity to a folivorous diet (Beebe 1909, Böker 1929, Gadow 1891, L'Hermenier 1837). Also, some authors described the smell of the gut contents as that of fresh cow manure (Beebe 1909, Goeldi 1886, Young 1888). None of these authors, however, suggested foregut fermentation as the primary function of the large gut capacity in the hoatzin.

Young hoatzins are fed regurgitated leaves. Their growth is comparatively slow; they require up to 70-80 days after hatching to fly. During this long growth period, the young can be vulnerable to predators. Thus, young hoatzins display some unique predator-escape mechanisms. Wing claws are actively used to climb branches and vines. Young birds can dive into water and can readily swim underwater in case of imminent danger after the first few days from hatching (Strahl 1988).

During the breeding season (May-Oct. in Venezuela), hoatzins live in social groups that consist of a breeding pair and sometimes up to four helpers at the nest. The social units defend small (200-1000 m^2) multi-use territories. The nests usually are on vegetation overhanging watercourses. The non-breeding season coincides with the dry season in Venezuela, and during this period hoatzins become gregarious, when flocks of up to 200 individuals move to permanent water bodies with green vegetation (Ramo and Busto 1984, Strahl 1988, Strahl and Schmitz 1990).

CHAPTER 2
STRUCTURE AND FUNCTION OF THE DIGESTIVE TRACT OF THE HOATZIN

Introduction

The hoatzin, Opisthocomus hoazin, is a Neotropical folivorous bird that inhabits oxbow lakes, flooded forests and swamps of the Guianas, Orinoco and Amazon basins. Up to 87% of its diet consists of leaves (Grajal et al. 1989). Obligate folivory is unusual in birds because leaves are bulky, have low nutritional value and can have noxious chemicals. These properties can be in direct conflict with the flying ability and energy demands typical of most birds. Much organic matter of plant tissues is structural carbohydrate in cell walls. Cellulose is one of the main components of cell walls and is the most common organic compound in nature. No vertebrate produces the enzymes necessary to digest cellulose. Therefore, many herbivores have enlarged chambers in their gut where anaerobic microbes secrete these enzymes and digest cellulose.

The hoatzin is the only known bird with a well-developed foregut fermentation system (Grajal et al. 1989). The voluminous crop and caudal esophagus have become functional fermentation chambers, analogous to those of mammalian foregut fermenters. The crop and esophagus are situated in front of a greatly reduced sternal carina, leaving little area for flight muscle attachment (Fig. 2.2). Indeed, hoatzins are not powerful fliers, preferring to hop from branch to branch. The peculiarities of the hoatzin's anatomy were the subject of many early descriptive studies in an attempt to establish the evolutionary affinities between the hoatzin and other birds (Brigham 1919,

Goeldi 1886, Huxley 1898, L'Hermenier 1837, Parker 1891, Perrin 1877, Pycraft 1895, Verheyden 1956). The presence of functional wing claws in hoatzin chicks, the reduced sternal carina and poor flying abilities of the hoatzin were regarded as the primitive characteristics of a "missing link" between the first fossil birds such as Archaeopteryx and modern birds (Brigham 1919, Parker 1891). Present systematic studies place the hoatzin within the Cuculiformes (de Queiroz and Good 1988, Sibley and Ahlquist 1973, Sibley et al. 1988). Some of the early authors made an attempt to relate the large gut capacity to a folivorous diet in the hoatzin (Böker 1929, Gadow 1891, L'Hermenier 1837). Moreover, some authors described the smell of the gut contents as that of fresh cow manure (Goeldi 1886, Young 1888). None of these authors suggested foregut fermentation as the primary function of the large gut capacity in the hoatzin.

Foregut fermentation in a 680 g flying endotherm is unexpected on theoretical grounds. In most vertebrate herbivores, gut capacity scales directly with body mass, while metabolism scales with body mass at a power of 0.75 (Demment and Van Soest 1983, Parra 1978). Accordingly, an endotherm below 3-5 kg should not be able to support its normal metabolic requirements on foregut fermentation alone. Moreover, large fermentation chambers place an additional constraint on flying ability, because power requirements scale directly with body mass (Pennycuick 1969). Foregut fermentation is also unexpected in birds, because they do not have the dental adaptations to reduce food particle size as do mammals. While birds can grind their food in the muscular stomach or gizzard before it reaches the main digestion sites of the hindgut, a bird with foregut fermentation requires significant particle size reduction before or during fermentation to increase plant matter digestibility. In fact, particle size is an important factor affecting plant matter digestibility

(Bjorndal et al. 1990), as demonstrated by the independent evolutionary origins of rumination in the typical ruminants (Tragulids and Pecorans), camels (Tylopodidae) and kangaroos (Macropodidae) (Hume and Dellow 1980, Hume and Warner 1980, Langer 1974, 1980, 1984). Finally, selective particle retention is another important gut function that enhances the nutritional use of plant matter by foregut fermenters (Warner 1981b).

This study describes the gross anatomy and function of the gastrointestinal tract of the hoatzin, and then compares the hoatzin's gastrointestinal tract to other herbivorous birds and foregut fermenting mammals. I measured the gut capacity of hoatzins and explored relevant functions, such as particle dynamics and the nutritional and physical characteristics of gut contents. If foregut fermentation is nutritionally important for hoatzins, then it can be expected that gut capacity would be similar to that of mammalian foregut fermenters. Additionally, the hoatzin's digestive tract should be able to reduce particle size and show selective particle retention to optimize the nutritional use of plant cell wall and cell contents. The understanding of the anatomy and function of the hoatzin digestive tract can provide insights into the evolutionary limits of foregut fermentation in vertebrates and in birds in particular.

Materials and Methods

Birds were captured at several sites in the Llanos of Venezuela (see Table 2.1). The total body mass of each bird was recorded immediately after capture with a portable spring scale ($\pm$1 g). Then the gastrointestinal tract was removed and weighed. The gut was divided with string knots into anterior esophagus, crop, posterior esophagus, proventriculus, gizzard, small intestine, caeca, and large intestine. The wet mass of the contents of each section was

determined by subtraction of the mass of each section with and without its contents. The pH of the contents from each segment was measured in situ with a portable pH-meter, usually within 20 min of the bird's death. Samples from each segment were fixed with concentrated sulfuric acid and frozen in dry ice for later measurement of volatile fatty acid (VFA) concentration. Other fresh samples were weighed and dried at 100°C to constant mass for determination of dry matter. Samples from some segments were fixed in buffered formalin for particle size analysis and the remaining contents were frozen and later dried at 60°C to constant mass for nutritional analysis. Tissue samples from the gut were fixed in 10% buffered formalin for histological analysis.

Gut contents were analyzed for dry matter, cell wall, nitrogen, and ash. Fiber content was determined following the neutral detergent (NDF) method of Goering and Van Soest (1970). Nitrogen content was determined by the Kjeldahl method. The concentration of VFA was determined using gas chromatography (Wilkie et al. 1986). Mean particle size in some gut sections was measured using a computerized particle analysis video system with a camera mounted on a microscope. The small sample sizes at specific hindgut sites did not allow an accurate measurement of particle size, so the contents of all hindgut sites were pooled.

Particle retention at various portions of the gut was measured on a captive adult hoatzin. The bird was previously acclimated to a maintenance diet for more than 60 days (Grajal et al. 1989). The maintenance diet consisted of romaine lettuce, soybean protein powder, ground alfalfa pellets and fresh young shoots of Enterolobium cyclocarpum, Pithecellobium saman, Guazuma ulmifolia and Phthirusa cf. orinocensis. The hoatzin was force-fed a gel capsule with plastic markers of three sizes (10, 4 and 1 mm^2) in a single pulse dose (Warner 1981b). The inert plastic markers were pieces of brightly-

colored commercial flagging tape. This material has the advantage that its specific gravity is almost one (1.01), so it resembles the specific gravity of wet food particles in the fermentation chambers (Warner 1981b). The captive hoatzin was housed in an individual custom-made metabolic cage with removable floor trays and given food ad libitum. All feces were collected after the pulse dose, and all markers present in the feces were counted. After 24 h of administration of the single pulse dose, the bird was killed and the plastic particles at each gut portion were counted. Acclimating hoatzins to captivity is an expensive and time-consuming effort, so this experiment was not repeated with more than one bird.

The characteristics of the gut contents and mean particle size were compared at different sites of the gut using two-tailed statistical tests with an alpha level of 0.05. Individual birds were considered experimental units for the tests. Standard deviations are shown in parentheses.

All sacrificed birds were used for other complementary experiments on in vitro fermentation rates, microbial population studies, and general histology (Grajal et al. 1989, also see Chapter 5). Additionally, complete skeletons of these birds were prepared for museum collections and deposited at the MARNR Museum at Maracay, Aragua state, Venezuela and the Florida Museum of Natural History at Gainesville, Florida, U.S.A.

Results

Digestive tract morphology

Mean body mass of 24 adult hoatzins was 687.3 g (±77.1, Table 2.1). Although males were on average heavier than females (730.7 vs. 705.9 g, respectively,) the difference between sexes was not statistically significant.

Similarly, no significant differences in body mass were found between capture sites or times of capture. The fresh contents the large crop and posterior esophagus averaged a mass equivalent to 9% of total body mass, roughly equivalent to 77% of the mass of the total digestive tract contents (Fig. 2.1, Table 2.2).

The mouth region has been partially described by early authors (Banzhaf 1929, Böker 1929). The general structure of the bill was more Galliform than cuckoo-like, which may explain the classification of the hoatzin as a Galliform for many years (Banzhaf 1929, Huxley 1898). The bill had sharp edges that help in cutting leaves. The lanceolate tongue had sharp caudally directed papillae, like backward-pointing spines, which probably assist in swallowing large pieces of leaves. A pair of large sublingual mandibular salivary glands (glandula mandibularis externa) (sensu McLelland 1979) were evident. Although I did not measure the composition of the saliva from these glands, it was quite thick and sticky. Other salivary glands in the corner of the mouth (glandula anguli oris) and in the cheeks were relatively smaller (F. Michelangeli, pers. comm.). The upper esophagus was quite smooth, soft and elastic, with almost no muscle. Near the entrance of the crop, the upper esophagus started to show some inner longitudinal ridges and thick muscle tissue, resembling the upper crop.

The crop was a large muscular organ folded into two chambers and wrapped by mesenteries. The two crop chambers were connected through a constricted zone with circular muscles that resembled the pillars found in ruminant stomachs. The crop extended ventrally and was harbored in a concave depression of the sternum keel (Fig. 2.2). The muscle wall of the crop was thick, with several circular muscle layers. The interior lining was covered by a hard epithelium and showed parallel longitudinal ridges and

folds. The ridges were generally higher (up to 4 mm) on the ventral side of the crop, and shorter and stouter on the dorsal side of the crop. The terminal portion of the second crop chamber had the shortest ridges. The crop ended in a narrow pillar zone connecting to the posterior esophagus. The crop contents were a heterogeneous green mixture of fully recognizable leaves, partially broken leaves and unrecognizable plant material.

The posterior esophagus was also heavily muscular and quite rigid. Its hard inner lining also showed longitudinal ridges, but these ridges were shorter and less uniform. The posterior esophagus consisted of a series of small sacculated chambers. Most of these chambers were separated by pillars and constriction zones, sometimes completely circular or otherwise resembling semilunar folds. Most of these muscular folds and constrictions were longitudinally connected, resembling short haustrations. The contents in the posterior esophagus seemed to be drier than those in the crop and were less diverse in size. No complete leaves were recognized in the posterior esophagus, except some small leaves, such as Acacia spp. (approx. 4 x 2 mm).

The glandular stomach or proventriculus was small, barely wider, and less muscular than the connecting posterior esophagus. An abrupt change in pH (Table 2.2) suggested that the proventriculus is the secretory region of gastric acids. The gizzard was also small but muscular, with a hardened keratinous inner lining. Two transversal muscle types were found in the gizzard, but none was thicker than the muscles of the crop. No grit was present in any hoatzin gizzard, as expected, considering that the birds rarely go to the forest floor. The contents of the gizzard were thoroughly ground, and only a few leaf veins and petioles could be identified.

The small intestine was uniform in diameter. The soft and elastic intestinal walls were only covered with thin muscle layers. The small

intestine was never completely full, and the contents were generally distributed in lumps. The contents in this region were not green as in the rest of the anterior gut, but orange-brown. Almost no recognizable particles could be found. The plant matter of the small intestine was mixed with a thick, sticky mucous substance. The paired caeca were relatively small for a herbivorous bird (Gasaway et al. 1975, Inman 1973, McLelland 1979, Ziswiler and Farner 1979) and lined with thin muscle. The caeca were partially full with an homogeneous dark green-brown material with the consistency of thick pudding. The large intestine was short and not clearly differentiated from the small intestine. No obvious morphological differentiation between the large intestine and the cloaca was evident (Fig. 2.1). In two individuals, white mucous streaks were found at the end of the large intestine. Whether these streaks were thick mucous aggregations or refluxed uric acid was not determined.

Gut contents

The dry matter (%DM) of the crop contents was significantly lower than the average %DM of the young tender leaves that constitute the typical hoatzin diet (Grajal et al. 1989) (Mann-Whitney U, $P = 0.006$, $n = 5$). The %DM of the contents of the posterior esophagus were significantly higher than those of the crop (Mann-Whitney U, $P = 0.016$, $n = 5$) but similar to those of the proventriculus and the gizzard. The hindgut had the lowest %DM contents (Table 2.2).

Nutritional characteristics of gut contents changed along the gut (Table 2.2). Cell wall levels were significantly higher in the esophagus than in the crop (Mann-Whitney U, $P = 0.009$, $n = 5$). Cell wall levels were significantly different among all three measured gut sites (Kruskal-Wallis one way ANOVA,

P = 0.002, n = 5; Fisher PLSD post-hoc test). The hindgut had the lowest cell wall levels. Nitrogen and organic matter levels were significantly higher in the esophagus than in the crop and much lower in the hindgut (Mann-Witney U, both P = 0.009, n = 5).

Particle dynamics

Mean particle size was smaller in the caudal esophagus than in the crop (Table 2.2), although the difference was barely significant (Mann-Whitney U, P = 0.047, n = 5). Mean particle size was significantly smaller (and less variable) at the hindgut than at either foregut site (Mann-Whitney U, P = 0.009, n = 5). Mean particle size was significantly different at all three gut sites (Kruskal-Wallis one way ANOVA P = 0.004, n = 5; Fisher PLSD post-hoc test). The experiments on particle retention showed that the larger the particle, the longer it remains in the anterior fermentation organs (Table 3). After 24 hours, 92.5% of the large (10 mm^2) plastic markers remained at the crop and esophagus, none was found in the hindgut, and only a few (3.7%) were excreted. Interestingly, all the excreted 10 mm^2 plastic markers were tightly folded in half. A higher proportion of the 4 mm^2 plastic markers were excreted in the 24 h period and none of these markers was folded. The small 1 mm^2 markers were present almost everywhere in the gut. No plastic markers were found in the caeca.

Discussion

Digestive morphology

In the hoatzin, obligate folivory has produced remarkable anatomical specializations. The crop and esophagus are the primary organ for digestion and fermentation. As a consequence, the morphology of the gut is more similar to that of small mammals with foregut fermentation (Hofmann 1989) than to any known herbivorous bird (Fig. 2.1). Indeed, the crop and the esophagus are the functional equivalent of multi-chambered fermentation organs. The relative capacities at these sites are among the largest fermentation capacities of any bird (Dawson et al. 1989, Herd and Dawson 1984), and roughly equivalent to the relative capacity of mammals with foregut fermentation (Demment and Van Soest 1983, Demment and Van Soest 1985, Parra 1978) (see Fig. 2.3). Similarly, the pH and VFA levels are within the range of mammals with foregut fermentation (Grajal et al. 1989). Since VFA can be actively absorbed at the fermentation sites, the inner folds of the crop and esophagus increase area for VFA absorption and probably help in the selective passage of particles. The dark red color of the crop muscles suggests a high blood supply that probably enhances oxygen supply and absorption of VFA (F. Michelangeli, pers. comm.).

The crop and posterior esophagus probably are important sites for selective retention of the solid over the liquid fraction. The thick muscle tissues at the crop and esophagus probably squeeze the digesta, resulting in a gradual increase in the %DM from the crop to the esophagus. The low %DM of the crop contents, relative to the average hoatzin diet, suggests that saliva secretions into the first portion of the fermentation chambers are significant.

The abrupt decrease in %DM contents between the gizzard and the small intestine suggests an increased absorption of water and digestible nutrients in solution.

Gut contents

The large volume, pH, and VFA concentrations in the crop and posterior esophagus demonstrate that these are the main fermentation sites where most cell walls are broken down and microbially digested. Usually, as the cell walls are broken by physical abrasion and microbial fermentation, digestible cell contents disappear rapidly. This study, however, could not discern whether the cell contents are more heavily used by foregut microbes or moved on to the lower gut to be absorbed by the host. In addition, gastric digestion of hemicellulose can be important in the overall disappearance of the cell wall fraction (Dawson et al. 1989, Keys et al. 1969, Parra 1978). Finally, the pH and VFA levels in the paired caeca demonstrate additional fermentation in the hindgut. Caecal fermentation is probably important in water and nitrogen recycling and microbial production of essential vitamins (Mead 1989, Remington 1989). Higher microbial density can explain the significantly higher levels of nitrogen and organic matter in the esophagus.

Particle dynamics

The constrictions and sacculations of the crop and posterior esophagus are presumably important adaptations for selective particle retention. A large proportion of the large and medium plastic markers remained in the crop and esophagus after 24 hours. The observation that almost all the excreted 10 mm^2 plastic markers were folded supports the idea that there is a minimum size threshold for escape to the lower gut. The 4 mm^2 plastic markers behaved

similarly, but they were not folded. None of the markers entered the caeca, suggesting that caecal filling can be highly selective (Björnhag 1989). I suppose that even the 1 mm^2 plastic markers were too large to enter the caeca, which were filled with an homogeneous thin paste.

The relatively long retention time of large plastic markers in the foregut was probably artificially high, since the markers could not be broken into smaller particles or attacked by microbes. Normally, large food particles are broken by a combination of physical abrasion and microbial fracture of the cell walls. Evidently, these plastic markers were inert to these digestive processes. The markers were appropriate to measure selective passage for two reasons. First, the behavior of these markers closely resembled that of food particles, since plastic tape has specific gravity similar to normal food particles. Second, the standardized particle sizes allowed a quantitative count of particles at the gut sites.

The crop and esophagus are also important sites for reduction and homogenization of particle size. This is probably achieved by the combined action of muscular pressure, abrasion by the hardened lining of the crop and intense microbial attack on the cell walls. The result is a functional equivalent to the re-mastication that gives ruminants their name, but with the added advantage that fermentation and trituration occur at the same site. Particle size reduction is an important factor in overall plant material digestion. Indeed, particle size reduction in toothless vertebrates is a crucial component of cell wall and cell contents digestion, because smaller plant particles can be more easily attacked by fermenting bacteria (Bjorndal et al. 1990). Further particle reduction probably takes place in the mid-gut, where the combined effect of gastric digestion in the proventriculus and physical grinding in the gizzard result in significantly smaller particles at the hindgut.

Although the small sample sizes did not allow measurement of particle size in the caeca, the appearance of their contents suggests that the caeca are sites for selective entrance of fluid and small particles.

Conclusions

The hoatzin's strategy to deal with a leafy diet is unique, leading to some extreme morphological, physiological and behavioral adaptations (Strahl 1988). This bird is the only known non-mammalian vertebrate with a foregut fermentation digestive system. These results demonstrate that the hoatzin crop and posterior esophagus are the primary site for digestion of its leafy diet. The anatomy and function of the hoatzin gut are unique for birds. Indeed, they are more similar to those of mammals with foregut fermentation, with the difference that the hoatzin is almost an order of magnitude smaller than the smallest mammals with well-developed foregut fermentation. This is probably achieved by a unique set of morphological adaptations in the hoatzin gut. In the hoatzin, food is effectively broken down into smaller particles at the fermentation chambers, increasing digestive efficiency. The selective retention of solid food particles at the foregut sites has not been reported for birds (Warner 1981b). Indeed, most other birds eating a bulky diet are able to either regurgitate or pass refractory solids faster than the more digestible liquids (Björnhag 1989, Duke and Rhoades 1977, Levey 1976, Warner 1981b). The hoatzin digestive strategy, however, seems to use of both cell contents and cell wall as nutritional sources.

The relative capacity of the hoatzin's fermentation structures is similar to the capacity of mammals in which foregut fermentation supplies a significant amount of the metabolic requirements. The levels of VFA at the crop and esophagus are similar to those of foregut fermenting mammals,

suggesting that microbial fermentation at the foregut sites is important for the overall metabolism of hoatzins. The contents of the anterior crop chamber are dry compared to the dry matter of contents in ruminants or other foregut fermenting mammals (Parra 1978, Van Soest 1982). Although the salivary glands were not large, the saliva was thick and probably contained mucoproteins and buffering salts. It is not clear how hoatzins regulate pH levels at the foregut fermentation sites. The ridges at the interior lining of the crop increase the absorption area, diminishing the acidifying effect of VFA accumulation in the fermentation organs. High microbial populations at the posterior esophagus may explain the increase in organic matter and nitrogen concentrations from the crop to the posterior esophagus.

The presence of a well-developed foregut fermentation system in the hoatzin provides new insights into the morphological and functional constraints of foregut fermentation in vertebrates. Gut capacity, particle reduction, and selective retention are important characteristics for an efficient use of plant leaves as a food source. Indeed, relative gut capacity, particle reduction and dynamics, pH and VFA levels in the hoatzin are quite similar to mammals with foregut fermentation systems. These similarities across taxonomic classes suggest similar functional constraints and selective pressures on the evolution of foregut fermentation.

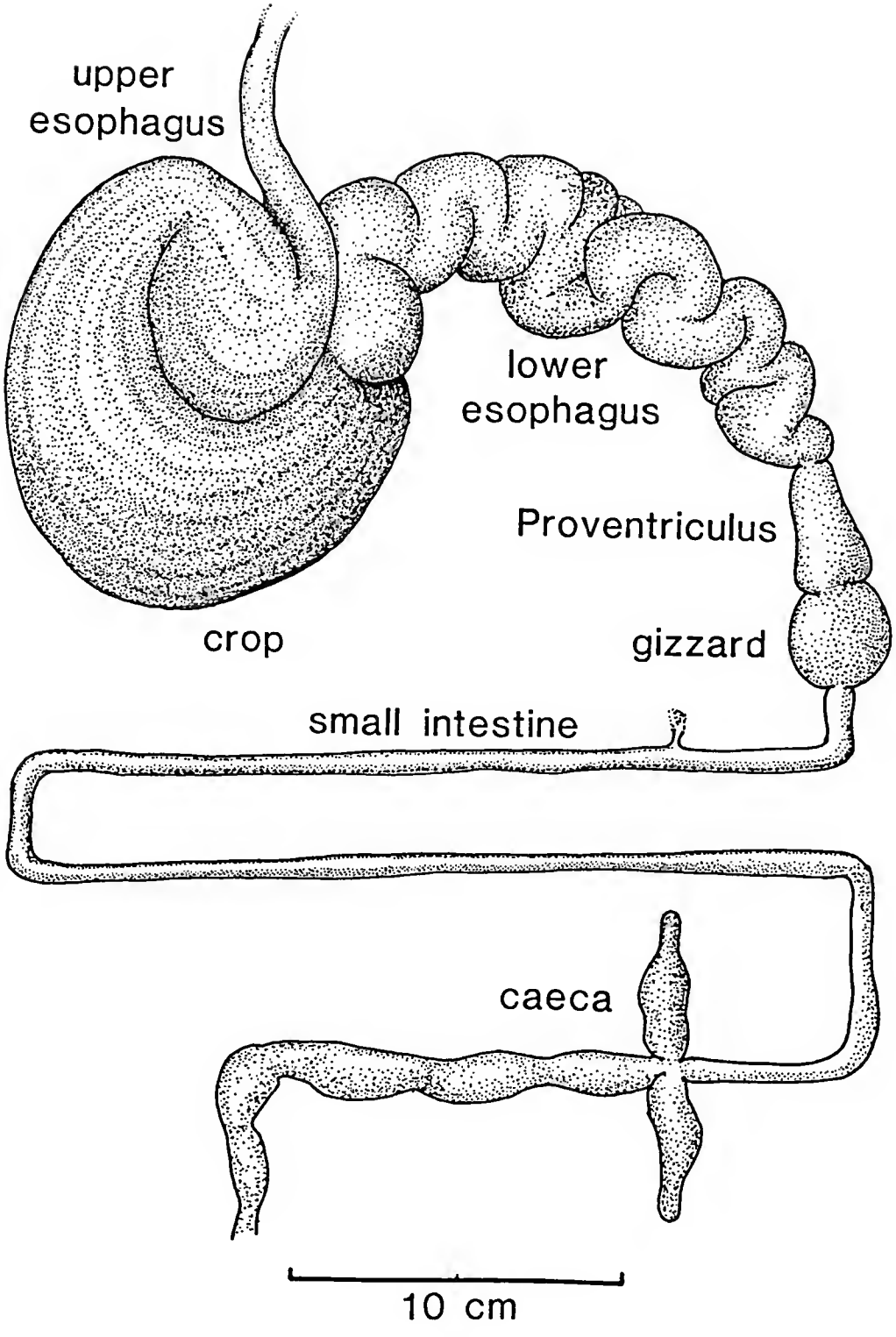

Figure 1) The digestive tract of the hoatzin. Its unique form and function is more similar to that of mammals with foregut fermentation than to any known bird.

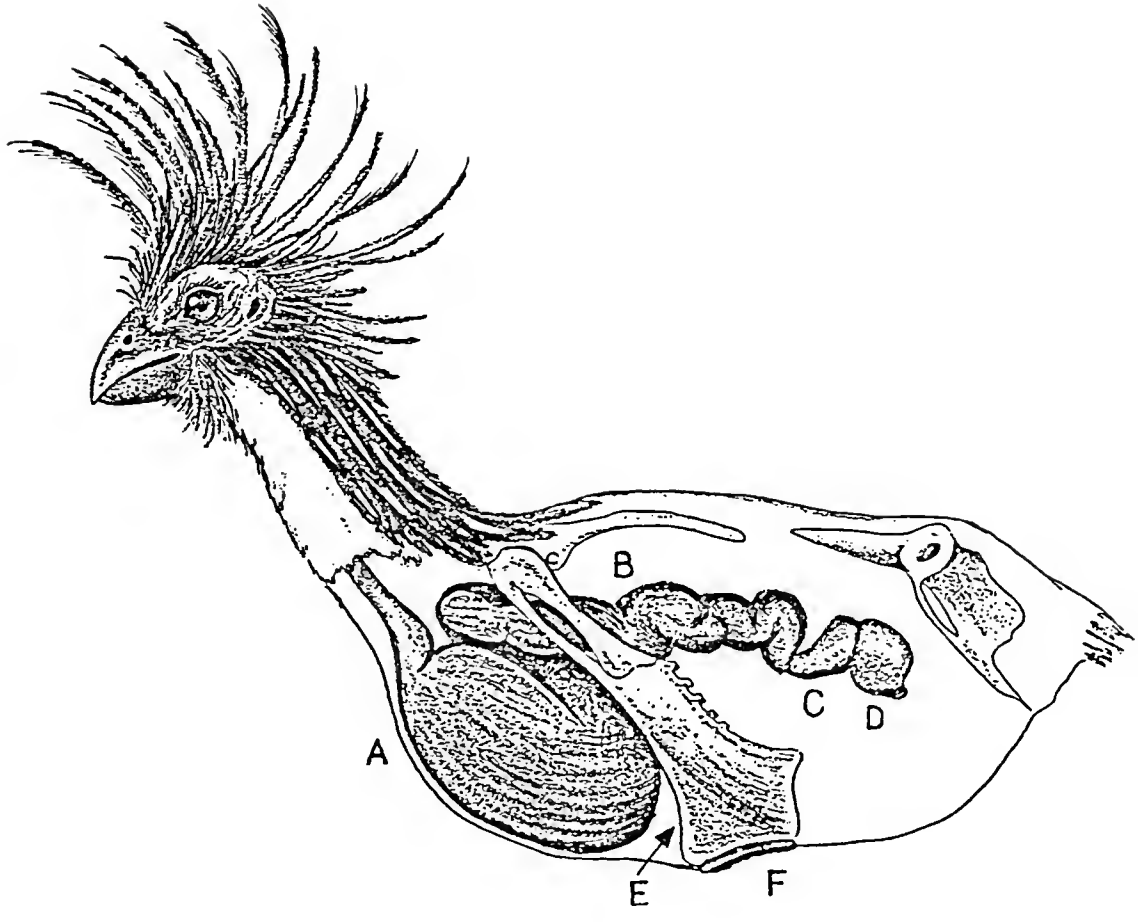

Figure 1) Schematic representation of the anterior gut of an adult hoatzin seen from the left, showing the crop (a), caudal esophagus (b), proventriculus (c), and gizzard (d). The anterior sternum is much reduced to room the voluminous fermentation chambers, with a drastic reduction of the area for flight muscle attachment to the sternal carina (e). A "resting" pad (f) at the end of the sternum is used while perching with a full crop.

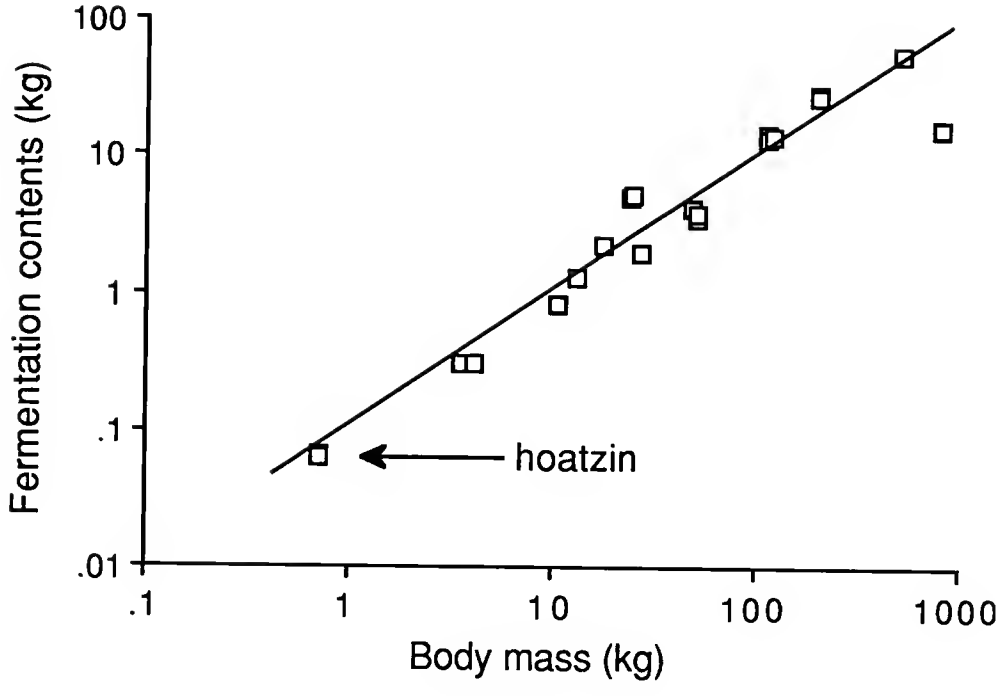

Figure 2.3) Relationship between body mass (kg) and fermentation contents (kg) of wild ruminants from Demment and Van Soest (1983). The line represents the regression log y = -1.02 + 0.998 log x (R^2 = 0.95). The fermentative capacity of the crop and esophagus of the hoatzin falls within the 95% confidence limits of the regression line.

Table 2.1) Mass (in g), sex and capture site of hoatzins used in this study. Capture sites correspond to the following geographic coordinates: Masaguaral (67° 35' W, 8° 34' N), Guárico River (67° 28' W, 8° 33' N), Suapure (66° 20' W, 6 ° 08'), Piñero (68° 04' W, 8° 82' N). Not all birds were sexed. Suapure data from unpublished observations by Rodrigo Parra.

Mass (g)	Date	Site	Sex
765	12-2-84	Suapure	male
785	12-2-84	Suapure	male
681	12-2-84	Suapure	female
653	12-2-84	Suapure	female
650	25-5-88	Masaguaral	
600	27-5-88	Masaguaral	
520	27-5-88	Masaguaral	
740	13-6-88	Masaguaral	
660	28-6-88	Masaguaral	
450	28-6-88	Masaguaral	
730	29-6-89	Guárico River	female
695	15-7-89	Guárico River	female
685	22-7-89	Guárico River	male
700	20-7-89	Guárico River	female
720	01-8-89	Guárico River	male
740	07-8-89	Guárico River	female
740	18-9-89	Guárico River	female
680	18-9-89	Guárico River	male
740	11-7-90	Piñero	male
640	11-7-90	Piñero	female
760	11-7-90	Piñero	female

Table 2.2) Characteristics of the gut contents of hoatzins. Sample sizes were n = 5 for all parameters except for relative capacity, which is presented as percentage of body mass (mean body mass for this sample was 712 g ± 56.6, n = 8). Mean values of organic matter, nitrogen and cell wall are presented on a dry matter basis. Large intestine values for mean particle size, organic matter, nitrogen and cell wall represent the pooled contents of the caeca, large intestine and lower small intestine. Volatile fatty acids (VFA) are presented in mmol/l of contents. Standard deviations are in parentheses.

	Posterior				Small		Large
	Crop	Esoph.	Provent.	Gizzard	Intest.	Caeca	Intest.
Length (cm)	25	15	3	3	63	3	15
Relative capacity	7.5	1.4	0.1	0.2	1.5	0.2	0.6
(% of body mass)	(1.2)	(0.3)	(0.0)	(0.0)	(0.3)	(0.1)	(0.2)
%DM	22.9	28.3	27.7	30.8	20.3	19.3	19.9
	(3.0)	(2.6)	(9.9)	(6.0)	(3.6)	(1.4)	(2.2)
Mean particle size	467.2	279.6					138.6
(microns)	(158.4)	(122.7)					(5.1)
%Organic matter	92.4	93.4					91.0
	(0.2)	(0.2)					(0.8)
%Nitrogen	4.4	4.7					4.1
	(0.1)	(0.1)					(0.1)
%Cell wall (NDF)	51.0	59.3					37.1
	(2.3)	(2.4)					(3.0)
pH	6.4	6.6	2.1			7.5	
	(0.4)	(0.3)	(0.3)			(0.1)	
VFA (mmol/l)	114.5	170.3				94.7	
	(62.3)	(121.0)				(42.1)	
%Acetic	68.1	69.8				77.4	
	(5.8)	(3.6)				(0.6)	
%Propionic	13.2	13.9				13.3	
	(4.8)	(1.3)				(0.6)	
%Butyric	8.3	7.7				-	
	(2.3)	(3.1)				-	
%Isobutyric	10.4	8.6				13.6	
	(1.6)	(1.8)				(9.5)	

Table 3) Percentage of plastic markers found at gut sites after 24 hours from a single pulse dose. The total number of markers given were 27 large (10 mm^2), 38 medium (4 mm^2), and 39 small (1 mm^2).

	Plastic marker type		
Gut site	10 mm^2	4 mm^2	1 mm^2
Crop	48.1	23.7	25.6
Posterior Esophagus	44.4	31.6	15.4
Proventriculus	3.7	2.6	5.1
Gizzard	0.0	5.3	2.6
Small Intestine	0.0	0.0	2.6
Caeca	0.0	0.0	0.0
Large Intestine	0.0	0.0	7.7
Excreted	3.7	36.8	41.0

CHAPTER 3
DIGESTIVE EFFICIENCY OF THE HOATZIN

Introduction

Plant leaves are difficult to digest, bulky and usually have defensive chemical compounds. The digestion of leaves generally results in a low rate of energy extraction that can conflict with the high energy demands of flight and endothermy. Consequently, few birds rely on plant fiber digestion for their nutritional needs. About 3% of extant bird species feed extensively on green leaves or buds (Morton 1978). The main reason for the rarity of herbivory in birds seems to be related to the conflict between eating a bulky diet of low nutritional value and the energy demands of flight and endothermy (Morton 1978). Although some birds can digest plant fiber, it is generally of little nutritional value. Therefore, most birds that eat significant amounts of plant material only extract the readily digestible cell contents, quickly excreting the bulk of the cell wall or fiber. Examples are herbivorous Anseriformes (geese and ducks) (Buchsbaum et al. 1986, Dawson et al. 1989, Marriot and Forbes 1970), some Galliformes (Inman 1973), takahe, and kakapo (Morton 1978). Large ratites may digest significant amounts of fiber (Herd and Dawson 1984, Mackie 1987, Withers 1983). Within the Galliformes, species of the family Tetraonidae (grouse and ptarmigan) can derive significant nutritional benefit from the fermentation of fiber in enlarged paired caeca (Gasaway 1976, Gasaway et al. 1976, Hill et al. 1968).

Most birds that digest significant amounts of fiber have fermentative chambers in the posterior part of the gut (hindgut) (e.g. grouse and

ostriches). Others, such as geese, ducks and emus have no specialized gut fermentative chambers (Buchsbaum et al. 1986, Dawson et al. 1989, Herd and Dawson 1984, McLelland 1979, Ziswiler and Farner 1979). Foregut fermentation is essentially restricted to mammals such as ruminants, colobid monkeys, kangaroos and tree sloths. The hoatzin, Opisthocomus hoazin, is unique among birds. It is one of the few known obligate avian folivores and the only known bird with a well-developed foregut fermentation system (Grajal et al. 1989). Although specialization to a folivorous diet was reported by early studies (Beebe 1909, Grimmer 1962), the hoatzin's nutritional ecology received little attention until recently (Grajal et al. 1989). In the hoatzin, the crop and caudal esophagus are enlarged (see Chapter 2), with a relative gut capacity similar to the fermentative structures of mammalian herbivores (Demment and Van Soest 1983, Parra 1978). Furthermore, the pH and concentrations of fermentation by-products such as volatile fatty acids (VFA) in the anterior part of the gastrointestinal tract are comparable to known foregut fermenters (Grajal et al. 1989). Therefore, hoatzins are the only known flying vertebrate with a well-developed foregut fermentation system. This digestive system is unique both in structure and function among birds.

Foregut fermentation in hoatzins is unexpected. Current allometric models of foregut fermentation predict a lower limit of $\approx$ 8 kg body mass for endotherms with foregut fermentation (Demment and Van Soest 1983, Demment and Van Soest 1985, Parra 1978). The rate of energy available from the fermentation of plant fiber does not fulfill the predicted total metabolic requirements for an endotherm below this critical mass. Hoatzins, however, are an order of magnitude lower than the predicted minimum body mass for a mammal with foregut fermentation (650 g).

To evaluate the function of this unique digestive strategy, it is necessary to estimate the digestive efficiency of the hoatzin. Additionally, a comparison of hoatzin digestive efficiency with other mammalian and avian herbivores can provide new insights into the evolution of foregut fermentation.

Hoatzin digestive efficiency was studied using balance trials under captive conditions with three experimental diets of various fiber levels. Previous attempts to keep these birds in captivity failed, probably due to nutritional imbalances (Grimmer 1962) and wide fluctuations in ambient temperature (Webb 1965). After extensive field work on the dietary and thermoregulatory constraints of wild populations (Grajal et al. 1989), I was able to keep hoatzins in captivity in 1986.

Materials and Methods

Animal husbandry

Hoatzins were captured along the Guárico River (67° 28' W, 8° 33' N), an affluent of the Orinoco River in central Venezuela. Two birds were used for the balance trials in 1986, two in 1988, and five in 1989. The birds were kept in outdoor aviaries at Fundo Pecuario Masaguaral, a private ranch in the central llanos of Venezuela. The birds were acclimated to captivity by a slow and progressive change from their natural diet to experimental diets. Hoatzins are extremely neophobic towards unknown foods, and acclimation required dedication and persistence. After an acclimation period of more than 60 days, the birds were moved to the Animal Production Institute of the Universidad Central de Venezuela (UCV) campus at Maracay. The birds were kept indoors in 1 x 1 x 2 m custom-made metabolic cages for a 20 day adjustment period before

the start of the experiments. The cages had removable floor trays for quantitative recovery of feces. Food during the adjustment period was offered ad libitum twice daily, in the morning and in late afternoon.

Diet composition

The experimental diets were a "salad" of romaine lettuce, sprinkled with a powdered mix of varying proportions of ground alfalfa hay pellets, ground Timothy grass hay, ground roasted soybeans, and a multi-vitamin and mineral complement. Three diets were offered (Table 3.1). All three diets were different not only in their fiber and protein content, but also in the quality of the fiber, with different lignin/cellulose and hemicellulose/cellulose ratios. Diet A was an acclimation diet with high protein and low fiber levels that maintained the animals in stable condition and helped to overcome the initial stress of confinement. Diet A, however, had a relatively poor quality fiber fraction, since most of the fiber came from soybean hulls and alfalfa hay. Consequently, Diet A had a very low (negligible) hemicellulose content. This resulted in low overall fiber digestibilities (see results below). The digestibility of this diet was studied in 1986. Diet B was used in 1988, and was designed to resemble the high nitrogen and low fiber portions of the hoatzin's natural diet. Consequently, Diet B had levels of hemicellulose and lignin similar to these portions of the hoatzin's natural diet. Diet C resembled the high fiber portions of the hoatzin's natural diet, and was used in 1989. The higher fiber content of diet C was achieved by increasing the grass hay contribution to the diet. This increased the hemicellulose content and decreased the lignification ratio. None of the three diets was identical to the estimated natural diet. Diet C, however, was the most similar in nitrogen level, overall fiber content and fiber composition.

Intake and digestibility

Digestibility experiments lasted 7 days, with a previous acclimation period of 10 days to the experimental diet. During the experiments, feces were collected daily on pre-weighed aluminum foil on the floor trays. Offered and rejected food were measured twice daily in separate food trays. Food and feces were dried in a forced air oven at 65°C for 48 h and cumulatively stored for later analysis. Sub-samples were dried at 105°C for absolute dry matter (DM) content. All samples were ground and chemically analyzed following the detergent methods of Goering and Van Soest (Goering and Van Soest 1970). Neutral detergent fiber (NDF) consisted of cellulose, lignin, and hemicellulose. Acid detergent fiber (ADF) consisted of cellulose and lignin. Lignin was determined by treating the ADF fraction with concentrated sulfuric acid. Hemicellulose was the difference between ADF and NDF. Cellulose was calculated by subtracting lignin and ash from the ADF fraction. Cell contents were estimated by subtracting NDF from the original sample dry weight. Organic matter was estimated as the difference between DM and ash after incineration at 500°C. Nitrogen was determined by the Kjeldahl method. Uric acid from whole feces was determined by elimination using the method of Tepstra and deHart (1973).

Since the experimental diets were composed of two substrates with different nutrient compositions and physical properties (fresh lettuce and a dry powder mix), substrate selection by the hoatzins was unavoidable. A separate experiment was performed to estimate the relative intake of each substrate by three hoatzins. The hoatzins were kept in the same metabolic cages and fed Diet C twice daily for 5 days immediately after the digestibility trials. All three mixed diets had similar physical properties, so Diet C was used as a representative. Lettuce and powder mix were separately weighed before

they were combined and offered. The rejected fraction was removed twice daily. All rejected lettuce was physically separated from the rejected powdered mix by washing under running water, and collecting the powder on a 0.3 mm^2 wire mesh sieve. The separated rejected lettuce and powder mix were dried in a forced air oven at 65°C for 48h and relative rejection of each substrate was calculated on a dry matter basis. The relative rejection percentages were used to estimate the average composition of the intake in the three diets (Table 3.1). Average intake of lettuce and powdered mix was found to be significantly different from the average lettuce and powdered mix ratio of the offered diet (see Results section).

Since hoatzins were actively selecting substrates of the offered diet, dry matter intake (DM intake) was estimated as [(gDM offered lettuce + gDM offered powdered mix) - gDM total rejected]. This formula was also used to calculate intake for each nutritional fraction, such as nitrogen or NDF. Apparent digestibility for each nutritional fraction was calculated as [(DM intake of the nutritional fraction - DM fecal excretion of the nutritional fraction) / (DM intake of the nutritional fraction)] x 100. Organic matter digestibility of the experimental diets by live hoatzins was compared to an <u>in vitro</u> organic matter digestibility (IVOMD) using cow ruminal inoculum (Alexander and McGowan 1966). The IVOMD provided a comparison of the organic matter digestibility of the experimental diets. Gross energy from lettuce, powdered mix, rejected food, and feces was measured with a Parr Bomb calorimeter. Metabolizable Energy Coefficients (MEC) were calculated as [(Gross energy of diet x DM intake - Gross energy of fecal excretion x DM fecal excretion) / (Gross energy of diet x DM intake)] x 100.

All statistical tests were two-tailed with an alpha level of 0.05. Individual birds were considered as the experimental units for each test.

Standard deviations are shown in parentheses. Digestibilities (expressed as percentages) were transformed to their square root and compared using unpaired t-tests, unless otherwise specified.

Results

The body mass of captured birds ranged from 550 to 690 g, with a mean of 616.5 g (±85.1) at the time of the experiment. All birds maintained body mass on the three experimental diets. Hoatzins at the end of the experiments were between 99% and 103% of their original body mass (mean 100.9% ± 1.8).

Intake and digestibility

Lettuce was actively selected over the powdered mix by captive hoatzins. On average, hoatzins rejected 66% (±8) of the offered powdered mix and 40% (±18) of the lettuce. The average intakes of dry matter lettuce and dry matter powdered mix were 58.9% and 41.1% (±17.6) from the total dry matter intake, respectively. The relative DM intakes of lettuce and powdered mix were significantly different (Wilcoxon matched pairs signed rank test, $P = 0.005$, $n = 3$). Total DM intakes (gDM/day) or mass-specific intakes (gDM/kg body mass day) were not significantly different among diets (Table 3.2) (unpaired t-tests). Similarly, gross energy intakes (KJ/day or KJ/kg body mass day) were not significantly different (unpaired t-tests).

Apparent digestibilities and metabolizable energy coefficients (MEC) are shown in Table 3.3. Dry matter digestibilities and cell contents digestibilities were significantly different among diets B and C ($P = 0.02$ and $P = 0.001$, respectively). Organic matter and nitrogen digestibilities were not significantly different among diets (unpaired t-tests). *In vitro* organic matter digestibilities of the experimental diets were similar to the *in vivo* average

organic matter digestibility for the three diets (Table 3.3). Diet A had lower MEC than Diet B and Diet C (unpaired t-tests, P = 0.03).

Neutral detergent fiber (NDF) digestibilities were lower for Diet A and Diet B than those for Diet C (unpaired t-tests, P = 0.003 and P = 0.006, respectively). Diets A and B did not show significantly different NDF digestibilities. In general, the higher the NDF content of the diet, the higher the NDF digestibility. Since cellulose digestibilities were only different among diets B and C (P = 0.04), and lignin digestibilities were not significantly different, most of the difference in NDF digestibilities was the result of differential hemicellulose digestibilities. Indeed, hemicellulose digestibilities were significantly different among all diets, with the highest digestibility for Diet C, and very low ("negative") hemicellulose digestibility for Diet A. This "negative" hemicellulose digestibility was an artifact, because Diet A had low hemicellulose levels, and the analytical methods failed to detect this fraction in such small amounts.

The extraction of uric acid from the feces demonstrated its importance in the calculation of nitrogen digestibilities. Average nitrogen digestibility without uric acid extraction was low (56.2% $\pm$11.4), while uric acid extraction showed a more realistic average nitrogen digestibility (78.3% $\pm$9.3) (Table 3.3). Although nitrogen digestibilities were not much different among diets, the differences between nitrogen digestibilities with and without uric acid extraction were highly significant (paired t-test, P = 0.002).

Discussion

Comparisons of the results on hoatzin intake and digestibilities with other studies of herbivorous birds or mammals are difficult to interpret because of major differences in experimental methods, diets, digestive strategies and body mass. Furthermore, most studies on ruminants are based on relatively large, domesticated grazers, such as sheep and cows.

Hoatzins seem to have mass-specific intake levels within the range of the emu, a large ratite herbivorous bird (Herd and Dawson 1984) and within the levels reported for grouse (Gasaway 1976, Gasaway et al. 1976, Inman 1973). Hoatzin intakes are lower than intake levels in geese (Marriot and Forbes 1970), probably because geese make little nutritional use of fiber. However, hoatzins have intakes levels lower than ruminant intakes under similar diets (Van Soest 1982).

Mass-specific DM intake levels in hoatzins were constant and independent of diet type. This relatively constant intake in the hoatzin under three different diet compositions is in contrast with the variable intakes of almost all herbivorous birds under different dietary fiber levels (Gasaway 1976, Herd and Dawson 1984, Hill et al. 1968, Miller 1984, Moss and Parkinson 1972, Moss and Trenholm 1987). This may suggest that hoatzin intake is regulated by gut fill rather than by cell wall level, at least for the range of cell wall levels offered. The range of cell wall levels in the experimental diets was within the range of similar experiments on herbivorous birds (Gasaway 1976, Herd and Dawson 1984, Hill et al. 1968, Miller 1984, Moss and Parkinson 1972, Moss and Trenholm 1987), but relatively limited compared to similar experiments on ruminants (Van Soest 1982). The cell wall range of the experimental diets, however, reflected natural variation in cell wall levels of the natural diet of hoatzins (Strahl and Parra 1985).

Energy intakes were similar among diets. This is explained by the similarities in DM intake and energy content of the experimental diets. The resulting MEC's were higher than any values of MEC reported for other herbivorous birds under diets with similar energy content (Karasov 1990).

Apparent digestibilities of many dietary fractions were high (Table 3.3). Digestibilities of cell contents, organic matter, and DM by hoatzins were as high as those reported for larger mammalian herbivores (Parra 1978, Van Soest 1982). Dry matter (DM) digestibilities in the hoatzin were higher than DM digestibilities by other herbivorous birds (Dawson and Herd 1983, Dawson et al. 1989, Gasaway 1976, Gasaway et al. 1976, Herd and Dawson 1984, Inman 1973, Moss and Trenholm 1987). High overall DM digestibilities also indicate that hoatzins are able to digest both the cell contents and cell walls of plant material to a greater extent than most avian herbivores. Furthermore, the high DM digestibilities may explain the relatively low DM intake of hoatzins when compared to other herbivorous birds and most mammals.

Total nitrogen digestibilities were not different among diets. The relatively low values of nitrogen digestibility are always a methodological artifact of balance trials on vertebrates that excrete feces and urinary products through a common cloaca (e.g., birds and reptiles). Uric acid and other urinary nitrogenous compounds are present in bird feces, inflating the amount of total nitrogen excretion and reducing the apparent digestibility of the nitrogenous fraction. The extraction of uric acid from feces showed significantly higher nitrogen digestibilities, well within the range of other herbivorous birds (Buchsbaum et al. 1986, Marriot and Forbes 1970).

Organic matter digestibilities were within the range reported for ruminants (Hoppe 1977a, Parra 1978, Van Soest 1982). The similarities between organic matter digestibility by live hoatzins and <u>in vitro</u> organic matter

digestibility (IVOMD) by cow ruminal inoculum shows that the hoatzin fermentation system is comparable to that of the ruminant in extracting and digesting the organic component of the diets. High organic matter digestibility is the result of the breakdown of the cell wall structural fiber, which in turn makes the cell contents available for digestion.

Digestibilities of all fiber fractions by hoatzins were equal to or higher than fiber digestibilities recorded for herbivorous birds (Buchsbaum et al. 1986, Dawson et al. 1989, Gasaway 1976, Gasaway et al. 1976, Herd and Dawson 1984, Inman 1973, Marriot and Forbes 1970, Moss and Trenholm 1987). Some studies (Dawson et al. 1989, Herd and Dawson 1984) show higher NDF digestibilities than hoatzins on Diet A, but lower than NDF digestibilities of Diets B or C. When compared to herbivorous mammals, these hoatzin fiber digestibilities are more similar to reported fiber digestibilities for ruminants and higher than for some non-ruminant mammalian herbivores under similar diets (Demment and Van Soest 1985, Hume and Dellow 1980, Parra 1978, Van Soest 1982).

Digestibilities of the NDF fraction were positively correlated to diet fiber content. The results for NDF digestibility of Diet A appear to be relatively low compared to digestibilities of the other two experimental diets or digestibilities recorded in ruminants (Parra 1978, Van Soest 1982). The low fiber digestibility of Diet A can be attributed to the poor fiber quality of this diet. A large portion of the NDF fraction was composed of relatively indigestible fiber, mainly soybean hulls and the lignified portions of alfalfa. Both soybean hulls and the lignified alfalfa are mainly composed of refractory fiber compounds, such as cutin and lignin, which are essentially indigestible (Van Soest 1969). In contrast, Diet C showed high NDF digestibilities.

Since cellulose, ADF or lignin digestibilities were not different among diets, the differences in NDF digestibilities can be explained by the differential digestibility of the hemicellulose fraction of each diet. Diet C had more hemicellulose than the other two diets, and consequently hemicellulose digestibilities of Diet C were the highest of the three. In fact, hemicellulose digestibilities by hoatzins are among the highest recorded for avian herbivores (Buchsbaum et al. 1986, Dawson et al. 1989, Herd and Dawson 1984). Hemicellulose can be microbially digested to the same extent as cellulose (Keys et al. 1969). In addition, hemicellulose may be hydrolyzed by gastric enzymes under low pH levels (Dawson et al. 1989, Herd and Dawson 1984, Keys et al. 1969, Parra 1978). The combination of microbial and gastric digestion may explain the high hemicellulose digestibility, and therefore its contribution to the higher NDF digestibility of Diet C.

High cellulose digestibility in hoatzins is not predicted by current models of cellulose digestibility as a function of body mass (Demment and Van Soest 1983, Parra 1978). Small herbivores have limited capacity to digest fiber because of the size limitations in gut capacity and high energy turnover of a small endotherm. Consequently, the relationship of body mass to cellulose digestion in mammalian herbivores has been described as $y = 14.5 + 5.4x$, where y is cellulose digestion and x is body mass (in kg $^{0.25}$) (Van Soest et al. 1983). This model predicts a cellulose digestibility of 19.4% for an herbivore of a mass of 0.65 kg, as the hoatzin. Although the hoatzin is not a grazer and assuming that the equation is valid for a folivorous bird, the empirical measurements of cellulose digestibility for the three diets were approximately 60%, well above the predicted values of the equation. Therefore, cellulose digestion in hoatzins is higher than predicted by body mass or by size limitations in gut capacity (Demment and Van Soest 1983, Parra 1978). Other

factors, including the unique gut structure and function (see Chapters 2 and 4), and the low rate of metabolism (see Chapter 6), contribute to the relatively high cellulolytic activity for a small herbivore such as the hoatzin. Additionally, the digesta are retained for long periods of time in the anterior chambers of the gastrointestinal tract, particularly in the crop and caudal esophagus (Grajal et al. 1989). Thus providing enough time for thorough microbial fermentation. Finally, food particles are reduced in size in the crop and esophagus, possibly by a combination of microbial attack and physical grinding by the crop's internal epithelium (Grajal et al. 1989, also see Chapter 2). Particle size reduction increases the surface area for microbial and enzymatic attack of the digesta.

The existence of foregut fermentation in an animal of this mass is intriguing. Foregut fermentation can decrease the digestive efficiency of a small selective browser, because the high quality proteins and carbohydrates of plant cell contents can be microbially fermented before the host absorbs these components in the intestine (Demment and Van Soest 1983, Demment and Van Soest 1985, Parra 1978). Additionally, fermentation products from microbial metabolism, mainly CO_2 and methane, can be a substantial energy loss for a host with high mass-specific energy requirements.

Most other avian herbivores on similar plant diets increase their rate of energy and nutrient extraction by trading fiber digestion for higher intake rates. In the hoatzin, foregut fermentation results in efficient digestion of both plant cell contents and cell walls. The question is then why hoatzins do not have higher intake rates (with the concomitant reduced fiber use) or why do they bother to digest fiber if other avian herbivores on similar diets do not. One reason may be the detoxification of plant secondary compounds. Foregut fermentation is advantageous as a detoxification mechanism because most

secondary compounds are readily degraded by gut bacteria (Barry and Blaney 1987, Freeland and Janzen 1974). Moreover, the internal lining of fermentative structures in ruminants is impermeable to most types of secondary compounds, allowing detoxification before the food reaches the absorptive tissues of the gastrointestinal tract (Freeland and Janzen 1974). Hoatzins in the Venezuelan llanos feed on plants that seem to have a wide array of secondary compounds (pers. obs.). Unfortunately, almost nothing is known about the secondary compound biochemistry of most tropical plants found in the hoatzin's diet. Another possible explanation may be the microbial synthesis of essential amino acids and vitamins that provide the host with balanced nutrition from an otherwise unbalanced diet (Purser 1970, Van Soest 1982).

Table 3.1) Chemical composition of the three experimental diets and the estimated composition of the natural diet of hoatzins at the study area. Values presented as percentage of dry matter (% DM) except for energy content (KJ/gDM). Natural diet values from (Strahl and Parra 1985).

	Experimental diet composition			Natural diet composition
	Diet A	Diet B	Diet C	
Dry matter (DM 65°C)	41.4	42.2	57.0	30.4
Organic matter (DM 100°C - ash)	84.9	85.7	87.7	70.1
Energy (KJ/gDM)	17.8	18.3	17.9	--
Nitrogen	4.1	4.6	2.9	2.9
Cell wall (NDF)	29.2	35.7	39.0	47.8
Cell contents (100-NDF)	70.8	64.3	61.0	52.2
Acid detergent fiber (ADF)	22.0	21.3	18.7	34.3
Cellulose	14.5	13.2	13.9	17.4
Hemicellulose (NDF-ADF)	7.2	14.4	20.3	13.5
Lignin	6.6	7.4	4.6	10.5
Lignin/cellulose ratio	0.46	0.56	0.33	0.60
Hemicellulose/cellulose ratio	0.50	1.09	1.46	0.78

Table 3.2: Mean intake, fecal excretion rates and average body mass of captive hoatzins for the three experimental diets. Standard deviations are in parentheses.

	Diet A	Diet B	Diet C
Dry matter intake (gDM/day)	43.6 (0.6)	40.8 (5.9)	35.2 (5.4)
Dry matter intake (gDM/kg body mass day)	64.9 (2.6)	66.4 (0.4)	60.6 (13.9)
Gross energy intake (KJ/day)	694.0 (10.9)	738.2 (106.2)	616.2 (97.5)
Gross energy intake (KJ/kg body mass day)	1032.6 (43.4)	1200.9 (6.8)	1059.4 (240.5)
DM fecal excretion (gDM/day)	13.7 (2.3)	8.5 (0.1)	9.71 (1.3)
Average body mass (g)	672.5 (17.7)	615.0 (91.9)	594.6 (100.6)
number of birds	2	2	5

Table 3.3) Apparent digestibilities, metabolizable energy coefficients (MEC) and in vitro organic matter digestibilities (IVOMD) of the three experimental diets. Standard deviations in parentheses. Significant differences between Diet A and C are marked by a (X), differences between Diet A and Diet B diets are marked by a (Y) and differences between Diet B and Diet C diets are marked by a (Z). (Unpaired t-tests, $P \leq 0.05$).

	Digestibilities (%)			
	Diet A	Diet B	Diet C	
Dry matter (DM 65°C)	69 (5)	79 (3)	72 (2)	(Z)
Organic matter (DM 100°C - ash)	72 (5)	80 (3)	74 (3)	
Nitrogen (without uric acid extraction)	9 (0.3)	10 (1)	9 (1)	
Nitrogen (with uric acid extraction)	12 (0.1)	13 (1)	13 (1)	
Cell wall (NDF)	35 (7)	41 (14)	71 (4)	(X)(Z)
Cell contents (100-NDF)	77 (3)	87 (2)	74 (2)	(Z)
Acid detergent fiber (ADF)	62 (9)	31 (5)	58 (6)	(Z)
Cellulose	61 (14)	47 (2)	63 (8)	(Z)
Hemicellulose (NDF-ADF)	negative -	68 (4)	78 (3)	(X)(Y)(Z)
Lignin	58 (4)	67 (11)	39 (11)	
MEC	66 (6)	80 (3)	75 (2)	(X)(Z)
In vitro organic matter digestibility	78	80	79	

CHAPTER 4
RETENTION TIMES AND PARTICLE PASSAGE RATES OF DIGESTA MARKERS IN THE HOATZIN GUT

Introduction

The passage rates of birds are generally short, with a few common trends (Karasov 1990, Warner 1981b). 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., grouse and ptarmigan) have longer retention times than herbivorous birds with little or no fermentation (e.g., geese, emu) (Karasov 1990, Warner 1981b).

Only a few studies have analyzed selective particle retention in birds; Some birds 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). 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 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 larger fiber particles are retained in the fermentation chambers for further digestion, while the digestible liquids and small solid particles are passed to the lower gut 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 1981a).

The hoatzin 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 caudal esophagus). The capacity of these foregut sections is approximately 10% of the adult hoatzin's body mass (Grajal et al. 1989, see Chapter 2). The morphology of these organs is unique. The crop is divided by a fold into two connected chambers, while the caudal esophagus is heavily sacculated, with multiple semilunar folds and constrictions. Particle size is significantly reduced at these fermentation sites (Grajal et al. 1989, see Chapter 2), suggesting a combined abrasive action by the internal lining of the muscular crop and intense microbial attack on the fiber components of the diet. The proventriculus and gizzard are much reduced in size. Some additional fermentation takes place in the small paired caeca.

In the hoatzin, digesta dynamics are probably 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 examined differential particle passage rates and retention times of digesta in the hoatzin.

Materials and Methods

The experiments were performed with two captive hoatzins in 1988 and five captive hoatzins in 1989. All birds had ad libitum access to a diet consisting of romaine lettuce with a powdered mix of ground alfalfa hay pellets, ground Timothy grass hay and ground roasted soybeans (see Chapter 3). Additionally, fresh young shoots of plants in their natural diet (e.g., Enterolobium cyclocarpum, Pithecellobium saman, Guazuma ulmifolia and Phthirusa cf. venezuelensis) were offered ad libitum twice daily. The birds were housed individually indoors in adjacent 1 x 1 x 2 m cages with removable floor trays for quantitative collection of feces. All trials started in the morning (730-1000 h) just before the routine morning feeding.

Hoatzins were force-fed a gel capsule with markers as a single pulse dose (Warner 1981b). Two markers, Cr-EDTA and ytterbium mordanted on fiber, were used to measure differential passage rates of liquid and fiber phases of the digesta, respectively. Plastic markers were used to measure differential particle size passage rates along the gut. Plastic markers consisted of two sizes ($1mm^2$ and $4mm^2$) of squares of brightly-colored pink and orange commercial flagging tape. The specific gravity of this flagging tape was 1.01, similar to the specific gravity of wet fiber plant fractions (Warner 1981b). The liquid phase was marked with a Cr-EDTA (chromium ethylene-diamine tetra-acetic acid) complex prepared following the procedure of Binnerts et al. (1968). The Cr concentration in fecal excretion was measured using atomic absorption spectrometry (Williams et al. 1962). Fiber from a mature grass hay was marked with ytterbium (Yb) oxide, using the procedure of Ellis et al. (1982). Before marking, the fiber was sieved to $1mm^2$ particles and purified using neutral detergent fiber extraction (Goering and Van Soest 1970). After the NDF extraction, the fiber was dried over absorbent filter paper at ambient

temperature for 24 h and then stored in a desiccator until needed for marking. The ytterbium concentration was measured by atomic absorption spectrometry using a nitrous oxide - acetylene flame.

The doses for the trials were estimated at approximately 16 mg Cr, 15 mg Yb (mordanted to 2 g fiber), 500 $1mm^2$ plastic markers and 200 $4mm^2$ plastic markers per bird. The exact dose of Cr and Yb for each animal was not known because the sizes of the gel capsules were slightly different, and some material remained in the bill and mouth of the birds. Therefore, recovery of these markers was calculated based on estimated doses. Feces were quantitatively collected on thick black plastic film liners on the cage's floor tray. The black plastic liners on which feces were collected provided a color contrast that enhanced the recovery of the tiny $1mm^2$ plastic markers. Feces were collected at regular intervals of 2-3 h the first day, 4-5 h the second day, 8 h the third day and 10 h the fourth day. For each batch of feces, the total number by size-class of plastic markers were counted and removed. All feces from each collection were removed from the plastic liners, dried in a forced-air oven at 65 °C to constant mass and kept individually in plastic bags until analyzed.

Feces from each sample were ashed at 600 °C. Some samples were too small (e.g., from the early feces), and were pooled for a larger sample corresponding to the later sample time. Both Cr and Yb from the ash were simultaneously extracted based on the procedure to extract Cr by Christian and Coup (1954) and modified for simultaneous extraction of Cr and Yb by Siddons et al. (1985). The Yb extraction was similar to that of Siddons et al. (1985), but instead of centrifugation after ash extraction, the extract was filtered through Whatman™ Nº 41 filter paper and stored until analyzed. Standards for both Cr and Yb were prepared using feces 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 feces. 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 feces at the ith defecation at time t_i after dosing (Blaxter et al. 1956). This method makes no assumptions about the frequency distribution of dye excretion (Warner 1981b), an important advantage because fecal excretion curves are generally not uniform in shape. Although a high proportion of marker recovery is desirable, this formula is advantageous because it does not depend on the total recovery of ingested marker, but rather on the amount excreted.

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 five birds (18 Sept.) and another trial consisted of Cr-EDTA and Yb markers (2 Oct.). All other markers were given once to each bird.

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. Mean retention time and transit time were transformed to the inverse of the square root to reduce heteroscedasticity (heterogeneous variances) in the ANOVA tests. Similarly, percentage recovery was transformed to the square root (Sokal and Rohlf 1981). Post hoc multiple comparisons were performed using the Games-Howell test (Games and Howell 1976). This test is a conservative and robust procedure under unequal sample sizes, heterogeneous variances and violations of normality (Jaccard et al. 1984). Standard deviations are shown in parentheses.

Results

Fecal excretion rates

Fecal excretion rates of hoatzins were relatively constant. No daily fluctuations were evident at the scale used. Nevertheless, handling of birds during the administration of the marker pulse lowered the fecal excretion rate for the first 4-6 h of the trials. This initial depression of the fecal excretion rate was present in most birds, but its length was different for each individual (Fig. 4.1). Solid markers first appeared well after the fecal excretion rates stabilized. The effect of initial low fecal excretion rates on liquid transit time remains unknown.

Marker recovery

The percentage recovery for all four markers was high (Table 4.1). No difference was found in mean percentage recovery among markers (One factor ANOVA, $F = 0.187$, d.f. = 3, $P = 0.91$). The Cr-EDTA concentration was below the sensitivity of the atomic absorption spectrometer in some samples, so a higher dose of this marker or larger intervals between feces collections would have been desirable. These results, however, did not affect the outcome of the experiment.

Mean retention times

One bird in 1989 (Y-chick) had longer retention times for all markers (One factor ANOVA, $F = 2.2$, d.f. = 5, $P = 0.12$, Games-Howell test). This bird was a growing fledging that developed flight feathers and increased body mass during the experiments. This was the only fledging bird in the group, so it was not included in further calculations.

Marker concentration curves were clearly skewed. Mean retention times for each marker are shown in Table 4.1. No difference in mean retention time was found among individual hoatzins, but the difference was highly significant among markers (Two factor ANOVA, $F = 2.22$, d.f. = 4, $P = 0.27$ for birds and $F = 14.07$, d.f. = 3, $P = 0.03$ for markers). The interactions between bird and marker were not significant ($F = 0.6$, d.f. = 7, $P = 0.74$). The liquid marker (Cr-EDTA) had a shorter retention time than all solid markers (Games-Howell test). Mean retention time was shortest for the liquid marker, followed by Yb and $1mm^2$ plastic particles. The longest retention time was recorded for $4mm^2$ plastic particles; more than twice the liquid retention time (Table 4.1). Small particles passed faster than large particles. The larger variation of mean retention times for the plastic markers obscured some of these differences. For example, no significant differences could be detected between $1mm^2$ and $4mm^2$ plastic markers. The effect of particle size was evident when significant differences in mean retention times were found between Yb and $4mm^2$ markers (Games-Howell test). Interestingly, there was no difference between Yb and $1mm^2$, as expected because the fiber marked with Yb was sieved to $1mm^2$ particles.

Transit times

Transit times were fast for Cr-EDTA (Table 4.1). This marker appeared in the first collection after the single pulse dose, so the smallest detectable transit time was on average 2.57h (± 0.54). Transit times were similar for Yb and $1mm^2$ plastic particles. The longest (and most variable) transit time was recorded for $4mm^2$ plastic particles (Table 4.1). Overall, transit times were significantly among markers (One factor ANOVA, $F = 32.93$, d.f. = 3, $P = 0.0001$),

but multiple comparisons (Games-Howell test) show that the only significant difference was between Cr-EDTA and the particulate markers.

Discussion

All markers seemed satisfactory to measure mean retention times as well as selective particle size retention. The marker recovery rate was relatively high (Sklan et al. 1975), and the markers did not seem to affect the birds. The fact that mean retention times of $1mm^2$ plastic and $1mm^2$ Yb particles were not different, demonstrates that small plastic markers can provide a quick 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 that measured with chemical markers. This variability is probably related to the behavior of the plastic markers in the gut. Although food particles are chemically and physically attacked by microbes, enzymes, and the grinding action at the crop, esophagus and gizzard, plastic markers remain completely inert and do not change in composition or size.

Mean retention times in hoatzins are among the longest ever recorded for a herbivorous bird and are similar to those of ruminants and arboreal folivores (Karasov 1990, Warner 1981b). 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 (Björnhag 1989, Clemens et al. 1975a, Duke 1988) and herbivorous mammals (Clemens et al. 1975b, Warner 1981b). The effect of the initial low defecation rates produced by the handling of birds upon liquid transit time could not be discerned with

the methodology used. Since hoatzins rarely drink, it was not possible to administer the liquid marker without handling the animals.

Liquids passed faster than solid particles. Selective retention of solid particles against the liquid phase has been reported in mammals with foregut fermentation (Grovum and Williams 1973, Hume and Dellow 1980, Warner 1981a, Warner 1981b). Within this group, macropodid marsupials can separate the two phases to a higher degree than can ruminants (Hume and Dellow 1980, Warner 1981a). The separation of liquid and solid markers in the hoatzin does not reach the extent seen in macropod mammals; it is more similar to marker separations seen in ruminants. Faster passage of the liquid fraction can be important for a small vertebrate with foregut fermentation, such as the hoatzin. This pattern allows the passage of the more digestible substrates to the lower gut 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 away and digested in the lower gut. This may result in a detrimental reduction of the time allowed for microbial population turnover. Microscopic observations of the crop contents of hoatzins indicate that most bacteria are firmly attached to the cell walls (F. Michelangeli, pers comm.). As a result, most bacteria seem to 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 (e.g., 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), while most herbivorous and frugivorous birds show the opposite trend - larger particles

are excreted significantly faster than the smaller particles and the liquid fraction (Björnhag and Sperber 1977, Gasaway et al. 1975, Levey and Grajal 1991, Warner 1981b). This pattern is especially prevalent in birds with caecal fermentation (e.g., family Tetraonidae), in which the caeca seem to selectively retain small particles and liquids and reject the undigestible larger food particles (Björnhag 1989, Björnhag and Sperber 1977). The differential particle selectivity probably increases 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.

Long retention times in the hoatzin increase the nutritional use of both cell contents and cell walls. In contrast, most herbivorous birds use the readily digestible nutrients of plant cell contents at the expense of more thorough nutritional use of cell walls. This is important, because microbial fermentation of readily digestible cell contents can insert an additional trophic level between the food and the host. Thus, microbial metabolic losses (e.g., methane, CO_2 and heat) can decrease the overall energy available to a herbivorous bird. These losses can be significant for a small vertebrate. In the hoatzin, these microbial metabolic losses may be offset by other nutritional benefits such as an increased nutritional use of cell walls, VFA (volatile fatty acid) production and detoxification of secondary compounds.

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. On an experimental diet with 39% neutral detergent fiber (NDF) and 3% nitrogen, hoatzins digested 63% cellulose, 78% hemicellulose and 71% NDF (see Chapter 3). Moreover, the high VFA

production rate at the crop and esophagus provides energy for about 60% of the basal rate of metabolism of adult hoatzins (see Chapters 5 and 6). These high fiber digestibilities probably result from the combined effect of long retention times, intense microbial fermentation, and selection of a highly fermentable diet. Indeed, hoatzins in their natural habitat select plant parts that are low in cell wall, lignin and high in protein and water content (Grajal et al. 1989). The selectivity of high quality plant parts is possible because hoatzins fly and therefore can track resources that are patchy in space and time.

These patterns of differential passage rates optimize the energy and nutrient extraction from the hoatzin's leafy diet (Grajal et al. 1989, also see Chapter 3). Foregut fermentation is possible because hoatzins select particular plant parts that are low in fiber and high in protein, maintain a high rate of microbial fermentation, and have very long retention times of the digesta. Foregut fermentation and the long digesta retention times in the hoatzin result in a unique evolutionary adaptation that provides an efficient use of a herbivorous diet by a flying bird.

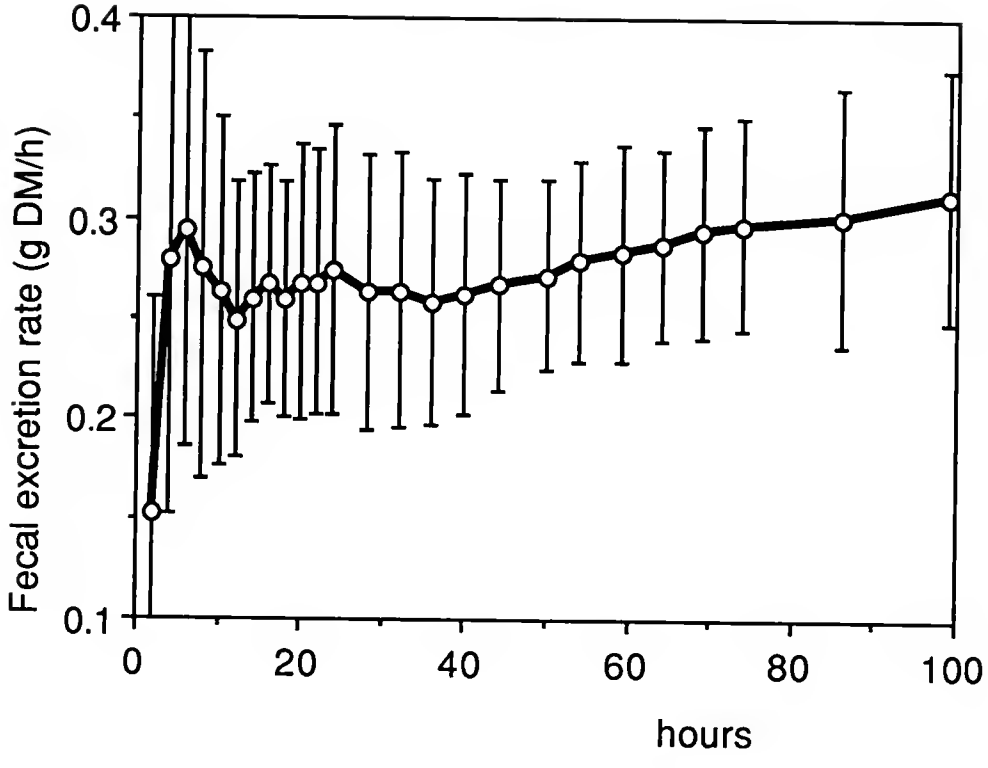

Figure 4.1) Average change in fecal excretion rate (in grams dry matter/hour) over time. Fecal excretion rates were low at the start and then remained relatively constant throughout the experiments. Bars represent standard deviations (n = 4).

Table 4.1) Average mean retention time (MRT), transit time (TT), percent recovery and sample size for liquid and solid markers. Liquid marker was Cr-EDTA (chromium ethylene-diamine tetra acetic acid). Solid markers were ytterbium (Yb) mordanted to $1mm^2$ particles of hay fiber and two sizes ($1mm^2$ and $4mm^2$) of cuts of commercial plastic flagging tape. All markers were orally given as a single pulse dose in a gel capsule. Standard deviations are shown in parentheses. Significant differences ($P < 0.05$) among markers are denoted by an asterisc.

	Liquid	Solid			
	Cr-EDTA	Yb	$1mm^2$	$4mm^2$	
MRT (hours)	17.9 (3.4)	24.4 (2.3)	33.3 (16.8)	44.4 (15.4)	(*)
TT (hours)	2.6 (0.5)	8.3 (2.9)	7.5 (1.0)	10.7 (4.6)	(*)
Recovery (%)	66.7 (17.6)	73.0 (15.9)	70.15 (8.4)	67.3 (4.3)	
(n)	7	4	4	3	

CHAPTER 5
FERMENTATION RATE IN THE CROP AND ESOPHAGUS OF THE HOATZIN

Introduction

The hoatzin is the only known bird with an active foregut fermentation digestive system and the only instance of such a digestive system outside the mammals (Grajal et al. 1989). This distant relative of the cuckoos (Sibley and Ahlquist 1973, Sibley et al. 1988) inhabits gallery forests, forest swamps and oxbow lakes of the Orinoco and Amazon drainages (Strahl 1988). Unusual characteristics such as functional wing claws in the first and second digits of the wings of young hoatzins were first seen as evidence of a "missing link" between the ancient Archaeopteryx and modern birds (Banzhaf 1929, Garrod 1879, Huxley 1898, Parker 1891).

The nutritional ecology and digestive physiology received little attention until recently (Grajal et al. 1989, Strahl 1988, Strahl and Schmitz 1990). Hoatzins are one of the few avian obligate folivores: up to 87% of their diet is composed of green leaves of plants (Grajal et al. 1989). Nutritional analyses have shown that preferred plant parts (shoots, buds and new leaves) are lower in fiber content and higher in nitrogen and water than non-preferred parts (Grajal et al. 1989, Strahl 1985).

The structure and function of the gastrointestinal tract of the hoatzin is unique. Although early descriptions included the disproportion in the sizes of the crop and the proventriculus (or gastric stomach), no fermentation activity was suggested (Böker 1929, Gadow 1891). In fact, the greatly enlarged crop and caudal esophagus have a relative gut capacity similar to the fermentative

structures of mammalian herbivores (Demment and Van Soest 1985, Parra 1978).

In herbivores with gut fermentation chambers, volatile fatty acids (VFA) represent the most important microbial by-products in terms of energy benefits to the host. In the hoatzin, VFA concentrations and pH levels in the anterior part of the gastrointestinal tract are comparable to mammals with foregut fermentation (Grajal et al. 1989). Additional fermentation takes place in the paired caeca of the lower gut. Microbial densities in the crop and caudal esophagus are the same order of magnitude as in ruminants and other mammals with foregut fermentation (Grajal et al. 1989). At least three species of protozoans have been found at the foregut sites (F. Michelangeli, pers. comm.). Studies with captive hoatzins showed that fiber digestibilities were among the highest ever recorded for herbivorous birds under equivalent diets (Grajal et al. 1989, see also Chapter 3).

Foregut fermentation in hoatzins is a theoretical anomaly. Present models predict a limit of 6-10 kg of body mass below which foregut fermentation cannot fulfill the energetic needs of an endotherm (Demment and Van Soest 1985, Parra 1978). At this mass, the predicted total metabolic requirements surpass the rate of energy available from plant fiber fermentation. The small mass of the hoatzin (650 g) is an order of magnitude lower than the predicted minimum body mass for a foregut fermenter. A study of the foregut fermentation system of hoatzins can enhance the understanding of the physiological limits of foregut fermentation.

This study examines the contribution of foregut microbial fermentation to the metabolism of hoatzins. The basal rate of metabolism in hoatzins is relatively low for a bird of its size (see Chapter 6) -about 68% of the expected value from allometric models (Kleiber 1961, McNab 1988). A measurement of

the rate of VFA production can estimate the contribution of fermentation to the overall metabolic expenditure of live hoatzins. One experimental approach to determine this contribution is to measure in vitro fermentation activity. Standard techniques have been developed to study domestic herbivores (i.e., cows and sheep) (Alexander and McGowan 1966, Tilley and Terry 1963). These techniques, however, can not be used in small (< 3kg) herbivores, because the small sample size is a limitation. Therefore, a "miniature" in vitro technique was designed to culture fermenting microbes from a small sample (<150g fresh mass compared to 2,500 g for the standard technique).

The objectives of this study were a) to determine the rate and extent of microbial fermentation in the hoatzin using in vitro techniques, b) to determine the energy contribution of microbial fermentation to the metabolism of the live hoatzin, c) to compare the fiber fermentation capabilities of microbial inocula from hoatzin crop and cow rumen incubated under the same in vitro conditions and d) to determine the replicability and reliability of the "miniature" in vitro technique.

Materials and Methods

Laboratory study: Fermentation in captive hoatzins

Hoatzins were captured along the Guárico River (67° 28' W, 8° 33' N), a northern affluent of the Orinoco River in central Venezuela. The birds were kept in outdoor aviaries at Fundo Pecuario Masaguaral, a private ranch and biological station in the central llanos of Venezuela. The birds were acclimated to captivity by a slow and progressive change from their natural diet to an artificial diet. The latter was a "salad" of romaine lettuce, sprinkled with a powdered mix of ground alfalfa hay pellets, ground Timothy grass hay,

ground roasted soybeans and a vitamin-mineral supplement. The nutritional composition of this diet was (on a dry matter basis): 84.9% organic matter, 4.1% nitrogen, 29.2% neutral detergent fiber (NDF), 22% acid detergent fiber (ADF), 14.5% cellulose, 7.2% hemicellulose, 6.6% lignin.

After more than 60 days of acclimation, the birds were moved to large outdoor aviaries at the Animal Production Institute of the Universidad Central de Venezuela campus at Maracay. The acclimated hoatzins were used for other studies on passage rates and in vivo digestibilities (see Chapter 3). One bird was used 10 December 1986, and two birds were used 18 July 1988. The birds were killed and the gut rapidly removed, with the foregut sections (crop and caudal esophagus) separated by string knots. These fermenting sections were weighed, and the contents were rapidly passed to a previously weighed baby food blender vase with a continuous flow of pre-heated CO_2 . The container was kept closed in a water bath at 39°C. A 1:5 (w/w) dilution was made by adding the necessary volume of pre-heated buffered artificial saliva (McDougall 1948) to the vase. A 2.5% of a solution of 2.64% ammonium sulfate solution was added, as a source of nitrogen for fermenting bacteria.

The blender was turned on at high speed for 5 seconds and then stopped for 10 seconds. This sequence was repeated three times to allow the separation of fermenting bacteria from the substrate. This solution was then filtered in a special anaerobic filter assemblage (Fig. 5.1) through a double layer of cheesecloth. This filter was designed to maintain anaerobic and isothermic conditions and to achieve a high filtering efficiency from a small sample. After filtration, the inoculum was gently shaken every other minute and kept under a continuous flow of pre-heated CO_2 in a water bath at 39°C during inoculation.

The fermenting substrate for the laboratory in vitro experiments was a finely (1mm) ground sample of alfalfa pellets (2.9% nitrogen and 36.1% NDF). Approximately 100 mg of the sample were added to 20 ml Hungate tubes with screw-on caps with internal rubber stoppers. To avoid caking of the dry substrate during inoculation, two drops of artificial saliva were used to moisten the alfalfa substrate 30 min before inoculation. The Hungate tubes were individually gassed with CO_2 before inoculation and then 10 ml of the inoculum were added to each tube with a repeating inoculation syringe. The mixture was gently stirred and thoroughly flushed with a flow of CO_2 and then tightly capped. Blank samples consisted of 10 ml of inoculum in tubes without substrate. The tubes were gently shaken by hand 4 times every 24 h, and each time the screw-on caps were slightly unscrewed to release the gas buildup.

At each time period of 0, 1, 2, 2.5, 4, 5, 6, 12, 18, 24, 48 and 72 h after inoculation, two tubes and a blank were taken from the incubation and the fermentation stopped. Fermentation in tubes corresponding to 0, 1, 2, 2.5, 4 and 5 h was stopped with approximately 1 ml of concentrated sulfuric acid. These tubes were used to estimate the production rate of VFA by extrapolation from a linear regression model (zero-time method) (Carroll and Hungate 1954). Daily energy available from VFA (in KJ/day) was calculated using the model by Prins et al. (1984) as

$$\text{Daily energy available} = W \times FC \times DM \times Y \times E \times (1/0.6) \times 24 \times 10^{-4}$$

where W is the body mass in kg, FC is the mass of fermentation contents as percentage of body mass in kg, DM is the dry matter content of the fermentation organ contents, Y is the in vitro fermentation rate in mmol VFA/gDM h, E is the energy equivalent of the mix of acetic, propionic, butyric

and other VFA found in the individual birds, using the energy equivalents of VFA given by (Blaxter 1962) and 0.6 is the average utilization efficiency of the metabolizable energy from VFA, assuming 85% efficiency for maintenance and 35% efficiency for other "production" activities, such as mating, stress or social interactions (Blaxter 1962).

The fermentation in the other tubes was stopped with 2 ml of toluene. These tubes were analyzed for in vitro cell wall digestibility (Van Soest 1982). Fiber content (as neutral detergent fiber) of the alfalfa substrate and amount of fiber digested by crop microbes was measured using standard detergent fiber analysis (Goering and Van Soest 1970). Fiber digestibility was estimated as (dry matter mass of fiber in the tube before fermentation - dry matter mass of fiber in the tube after fermentation) x 100/dry matter mass of fiber in the tube before fermentation.

Field study: Fermentation in wild hoatzins

This study was designed to measure the VFA production rate of wild hoatzins eating their natural diet. Between 11-12 July 1990, three adult hoatzins were shot at Hato Piñero (68° 04' W, 8° 56' N), a cattle ranch and biological station on the Cojedes River, another northern affluent of the Orinoco River. The dead birds were immediately taken to a close-by field laboratory and the foregut contents removed. The time between death and the start of inoculation was no more than 35 min. The same buffering saliva as in the laboratory study was used for a 1:5 (w/w) dilution. Most of the separation and filtration procedures were similar to the laboratory study, but instead of a blender, the container with the gut contents and the saliva was vigorously shaken by hand for 20-40 seconds to separate the attached bacteria from the surface of the cell walls.

The fermenting substrate for the field in vitro experiments was a finely (1 mm) ground mix of leaves representing the natural diet of hoatzins at the study site. The substrate mix was composed of 12.8% Guazuma ulmifolia leaves, 7.2% G. ulmifolia buds, 6.8% Phthirusa cf. orinocensis, 27.8 % Enterolobium cyclocarpum, and 45.3% Lonchocarpus cruciarubierae. The nutritional composition of this combination was 92.9% organic matter, 2.6% nitrogen, 39.3% NDF, 24.5% ADF, 13.7% cellulose, 15.3 hemicellulose, 10.8% lignin. This diet was taxonomically similar to the estimated average natural diet composition eaten by wild hoatzins (Strahl and Parra 1985). Approximately 100 mg of the sample was added to 20 ml Hungate tubes with screw-on caps with internal rubber stoppers. Small (5ml) syringes were punched on the rubber caps every hour to alleviate the gas pressure buildup inside the tubes once fermentation started. Inoculation proceeded as in the laboratory study. At 0, 1, 2, 2.5, 4 and 5 h after inoculation, two tubes and a blank were taken from the incubation and the fermentation stopped with approximately 1 ml of a 0.5 M solution of sulfuric acid.

Comparison of hoatzin and cow in vitro digestibilities

This study compared the fiber fermenting capabilities of hoatzin crop contents and cow rumen contents. The hoatzin crop contents were treated in the same way as in the laboratory study. The ruminal contents were extracted from the ventral part of the rumen of a fistulated Holstein cow eating mature grass hay. The ruminal contents were kept under isothermic and anaerobic conditions until filtering. After removing part of the ruminal liquid through a double layer of cheese cloth, the contents were treated in the same way as with the hoatzin crop contents. A dried, ground sample of alfalfa hay (3.1% nitrogen and 39.5% NDF) was used as the fermenting substrate. Again, 100 mg

of the sample were added to 20 ml Hungate tubes with screw-on caps with internal rubber stoppers. At 2, 6, 12, 24, 48 and 72 h after inoculation, two tubes and a blank were taken from the incubation and the fermentation stopped by adding 2 ml of toluene and stored in a refrigerator at -4°C until fiber analyses were done. The rates of in vitro fiber digestibility were estimated as the slope of regression curves of the natural logarithm (ln) transformation of apparent digestibilities on hours of fermentation.

Comparison of the miniature and standard in vitro techniques

This study was designed to compare the level of fiber digestibility of the miniature in vitro technique to that of the standard technique. Inoculum from a fistulated cow eating grass hay was used to compare both in vitro techniques. The substrate used was a ground mature grass hay (61.65% NDF). Both experiments consisted of 10 tubes (replicates), and were run simultaneously. For the miniature technique, 100 mg of the sample were added to the same Hungate tubes as in the previous experiments. The standard in vitro technique was a version of the Tilley and Terry method (Tilley and Terry 1963), but without the acid pepsin digestion stage. For the standard technique, 500 mg of the sample were added to 100 ml glass centrifuge tubes, capped with Bunsen gas release valves. The buffering solution was the same as in the other experiments. In both in vitro techniques, the dilution was 1:5 (w/w) of fermentation contents to buffering saliva solution. Both in vitro fermentations were stopped after 24 h by adding 3 ml of Toluene. Then the tubes were stored in a refrigerator at -4°C until NDF analyses were done.

Gas-liquid chromatography

Volatile fatty acid (VFA) concentrations were measured using gas-liquid chromatography. Samples were acidified in the field with 2 drops of concentrated sulfuric acid, frozen in solid CO_2, and later stored in a freezer at -10°C until analyzed. For the chromatographic analysis, the samples were thawed and centrifuged at 10,000 r.p.m. for four minutes. A volume of 1.35 ml of the supernatant was acidified with 0.15 ml of 20% phosphoric acid to a concentration of 2% (V/V). The sample was injected into a glass column of 10% SP-1000 on 100/120 Chromosorb W/AW (Supelco Inc.). The column was maintained at 140°C with nitrogen as the carrier gas at 40 ml/min. The injector was set at 160°C and the flame ionization detector was set at 200°C.

Statistical analyses

All statistical tests were two-tailed with an alpha level of 0.05. Individual in vitro tubes were considered as the experimental units for the in vitro regressions. Individual birds were considered as the experimental unit for comparisons of digestibilities or VFA production rates. Digestibilities (expressed as percentages) were transformed to their square root and compared using unpaired t-tests, unless otherwise specified. Standard deviations are shown in parentheses.

Results

VFA production rate in captive and wild hoatzins

The concentration of VFA increased linearly with incubation time both in the captive and in the wild hoatzin *in vitro* fermentations (Fig. 5.2). These results suggest there was no significant interference by the accumulated end-products from fermentation during the 5-6 h of the trial. The VFA production rates were estimated from the slopes of the regression lines of VFA concentration over time. The overall *in vitro* VFA production rate was significantly higher in wild hoatzins than in captive hoatzins (t-test for the comparison of two regression coefficients, $t = 3.437$, d.f. = 47, $P = 0.001$) (Zar 1984). Additionally, the proportions of individual VFA were different in each experiment, reflecting differences in the fermentative capabilities of captive and wild hoatzins, probably due to the different diets of captive and wild hoatzins, and different substrates in the tubes of each experiment (Blaxter 1962) (Table 5.1). For example, the acetic:propionic ratio was higher in captive hoatzins than in wild hoatzins (Table 5.1). In fact, production rates of all individual VFA were higher in the fermentation from wild hoatzins than from captive hoatzins. The production rate from captive hoatzins was, on average, 53 mmol/kgDM h or 21 KJ/day while for wild hoatzins the average production rate was 136 mmol/kgDM h or 102 KJ/day. These energy contributions represent about 14% of the basal rate of metabolism of captive hoatzins and 62% of the basal rate of metabolism of wild hoatzins.

In vitro fiber fermentation and comparison with cow ruminal fermentation

Fiber fermentations by hoatzin and cow inoculum are represented in Fig. 5.3. The resulting fermentation regressions for the first 24 h of incubation were: $y = 3.841 + 0.017x$ for hoatzin inoculum ($R^2 = 0.81$, slope standard error = 0.003) and $y = 3.475 + 0.022x$ for cow inoculum ($R^2 = 0.63$, slope standard error = 0.007). The fermentation rates (regression coefficients) were not significantly different, but the intercept was significantly higher for hoatzin than for cow inocula (t-test for the comparison of intercepts of two regression lines, $t = 2732.4$, d.f. = 13, $P < 0.001$) (Zar 1984). In vitro fiber digestibilities stabilized after 48 h of incubation, both for cow rumen contents and hoatzin crop contents. Total fiber digestibilities after 48 h were not significantly different between hoatzin and cow inoculum (unpaired t-test).

Comparison of the miniature and standard in vitro techniques

In vitro fiber digestibility measured by the miniature technique was higher than that measured by the standard technique (unpaired t-test, n = 10, p = 0.038). Average fiber digestibility was 37.7% (±3.92) for the small tubes and 34.6% (±1.97) for the large tubes. Although the average digestibilities were not very different, the small variability among the large tubes made the difference significant.

Discussion

VFA production rate in captive and wild hoatzins

The VFA production rate of captive hoatzins was lower than the range of VFA production rates reported from mammals with foregut fermentation (Table 5.2). In wild hoatzins, however, the VFA production rates are similar to low rates measured for domestic ruminants, but below the rates of ruminants that feed selectively on young plant parts (concentrate selectors *sensu* Hofmann 1989) (Table 5.2). Therefore, VFA production rate in the hoatzin provides a significant proportion of its energy requirments. In fact, VFA production rate in hoatzins is the highest recorded for a bird. For example, VFA production rate in captive hoatzins is higher than the average production rate for willow ptarmigan, while VFA production rate in wild hoatzins is almost twice of the maximum rate measured for willow ptarmigan (McBee and West 1969) (Table 5.2).

These fermentation rates are probably the result of the fermentation of both cell walls (fiber) and cell contents. In the hoatzin, VFA production rate does not meet all its energy requirements, but the contribution of VFA production to the hoatzin's metabolism is quite significant. The energy contribution of fermentation to the metabolism of hoatzins is much larger than in any other known bird with fiber fermentation (Annison et al. 1968, Clemens et al. 1975, McBee and West 1969), and similar to that of other foregut fermenting mammals (Dreschen-Kaden 1977, Hoppe et al. 1983, Parra 1978).

The difference between *in vitro* fermentation in captive and wild hoatzins is related to the differences in their diets and probably reflect different microbial communities. Similar differences have been reported in other comparisons between captive and wild herbivores (Foley et al. 1989,

Hoppe 1977, Hoppe et al. 1977, Hume 1977). Moreover, different fermentation rates and proportions of individual VFA generally reflect the different dietary compositions (Blaxter 1962). For example, diets with a less lignified cell wall are generally fermented at a faster rate than highly lignified mature plant material (Smith et al. 1972, Smith et al. 1971). As a consequence, the proportions of individual VFA are also different. The ratio of acetic to propionic acid in the fermentation from captive hoatzins was about 5.47, while this ratio was 1.99 in wild hoatzins. The higher proportion of propionic acid in the fermentation from wild hoatzins is an important contribution to the energy balance of wild hoatzins, because propionic acid is one of the main precursors of glucose (Blaxter 1962, Miller 1979). The composition and high production rate of VFA in wild hoatzins reflect not only a more fermentable diet but possibly a better developed microbial community.

In vitro fiber fermentation

The fiber fermenting capabilities of the hoatzin microbial community are remarkable, especially for a small herbivore. Fiber fermentation from hoatzin fermentation contents was not different during the first 48 h than from cow fermentation contents. The extrapolation from the regression models, however, indicates that fiber fermentation from hoatzin fermentation contents started at a higher level. These differences probably reflect the effect of the different diets upon the composition of the microbial community of the two species. The offered substrate was more similar to the diet of captive hoatzins than to the diet of the fistulated cow. Therefore, the hoatzin inoculum may have adapted to a familiar substrate more rapidly and fermented it more rapidly than the cow inoculum did. After 48 h, both fiber digestibilities

become nearly asymptotic, probably due to substrate disappearance and accumulation of unusable end-products.

Miniature and standard in vitro techniques

Although the difference in digestibilities measured in small and large tubes was not great, it was significant. The miniature in vitro technique overestimated fiber digestibility when compared to the standard technique. The difference between small and large tubes is not easy to explain. One methodological difference is that the large tubes had Bunsen gas release valves, while the small tubes were sealed and the gas buildup pressure was released only three times during the 24 h fermentation. As a consequence, it is possible that the accumulation of gas pressure in the miniature technique affected in vitro fiber fermentation. The use of small (5ml) syringes punched in the rubber caps can alleviate the gas pressure buildup inside the small tubes.

This study demonstrated that the miniature in vitro technique can be performed with acceptable accuracy, permitting comparisons using the same miniature technique without a loss of precision or repeatability. Comparisons with the standard technique, however, should take into consideration that the miniature technique seems to marginally overestimate fiber digestibilities.

Conclusions

Microbial foregut fermentation in the hoatzin provides a unique nutritional use of cell wall and cell contents for a bird. Cell wall fermentation has two main advantages: first it makes the highly digestible cell contents available to the host and the microbial community. Second, cell wall microbial fermentation produces VFA that in turn make a significant contribution to the

energy requirements of the hoatzin. Moreover, in vitro estimates of the contribution of fermentation to the metabolism of the whole animal usually underestimate this contribution (Blaxter 1962). In fact, the energy contribution of fermentation in the live hoatzin is probably higher, because additional fermentation takes place in the paired caeca (Grajal et al. 1989) and optimal conditions for fermentation are better maintained in the live animal.

Fermentation in the hoatzin is unlike that of any other herbivorous bird, and more similar to that of selective browsing ruminants. As a consequence, the fermentation rate from hoatzin crop microbial communities is higher than in any other bird and more similar to that of ruminants with low fermentation rates. Fermentation in hoatzins optimizes the nutritional use of both the fiber and cell content fractions of their leafy diet. Additionally, VFA production rate can sustain a large portion of the energy requirements of the hoatzin. Finally, foregut microbial fermentation in the hoatzin may provide other nutritional advantages, such as microbial production of vitamins and amino acids and the detoxification of plant secondary compounds. These latter aspects need further study.

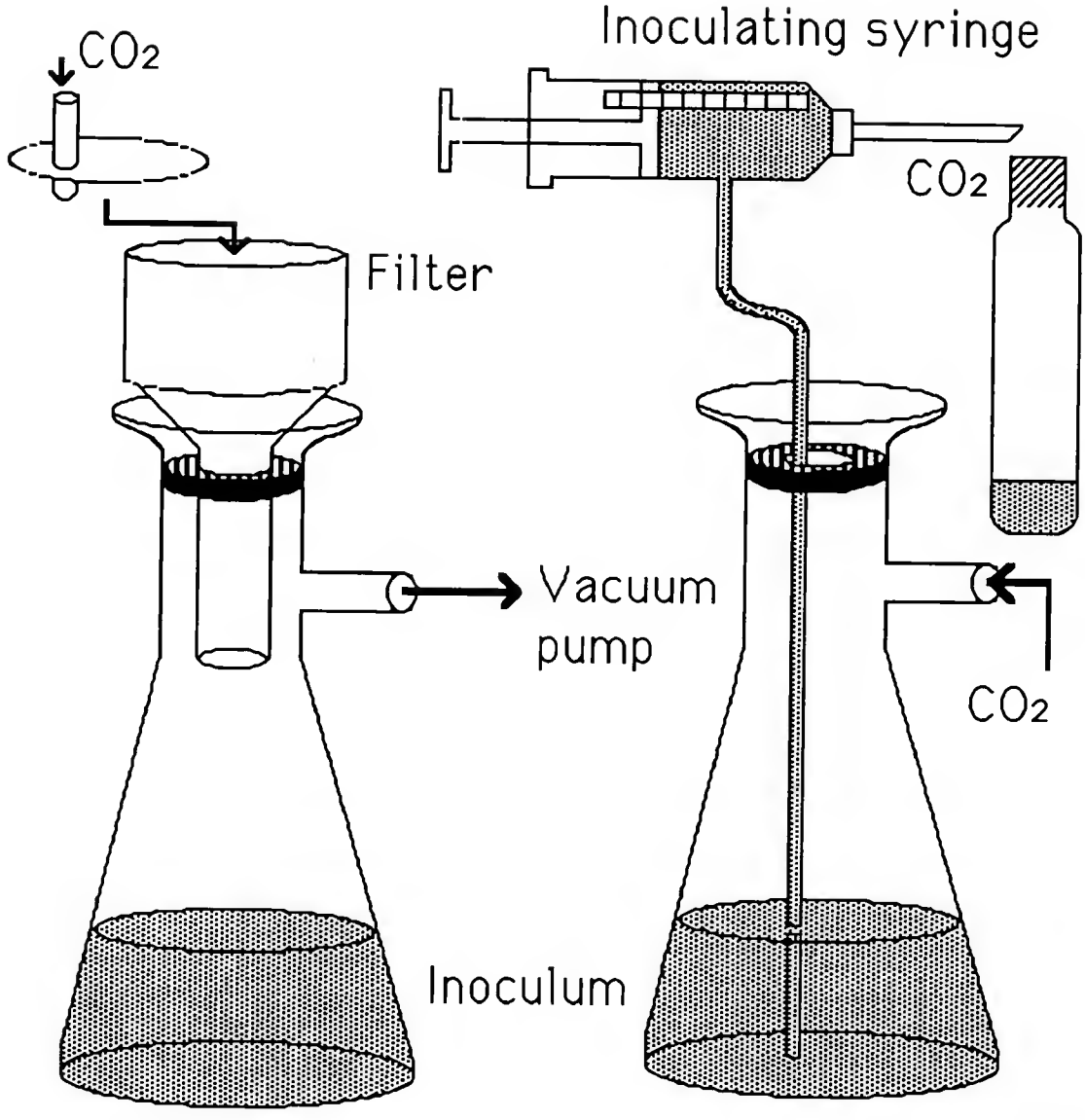

Figure 5.1) Diagram of <u>in vitro</u> filter system used to achieve a high filtering efficiency while keeping anaerobic and isothermic conditions. When the filter clogged, the lid was used as a piston to press the cheesecloth with the contents and increase the amount of filtrate.

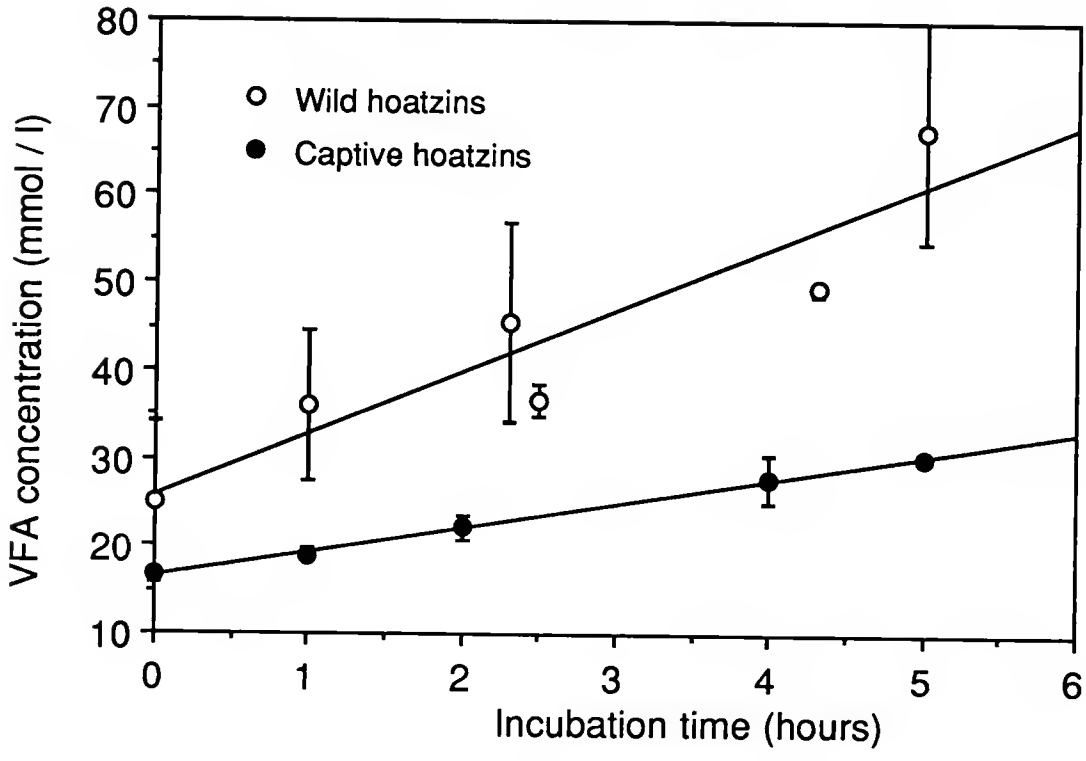

Figure 5.2) Change in VFA concentration (in mmol/l of fermentation contents) with time from the crop and caudal esophagus inoculum of captive and wild hoatzins.

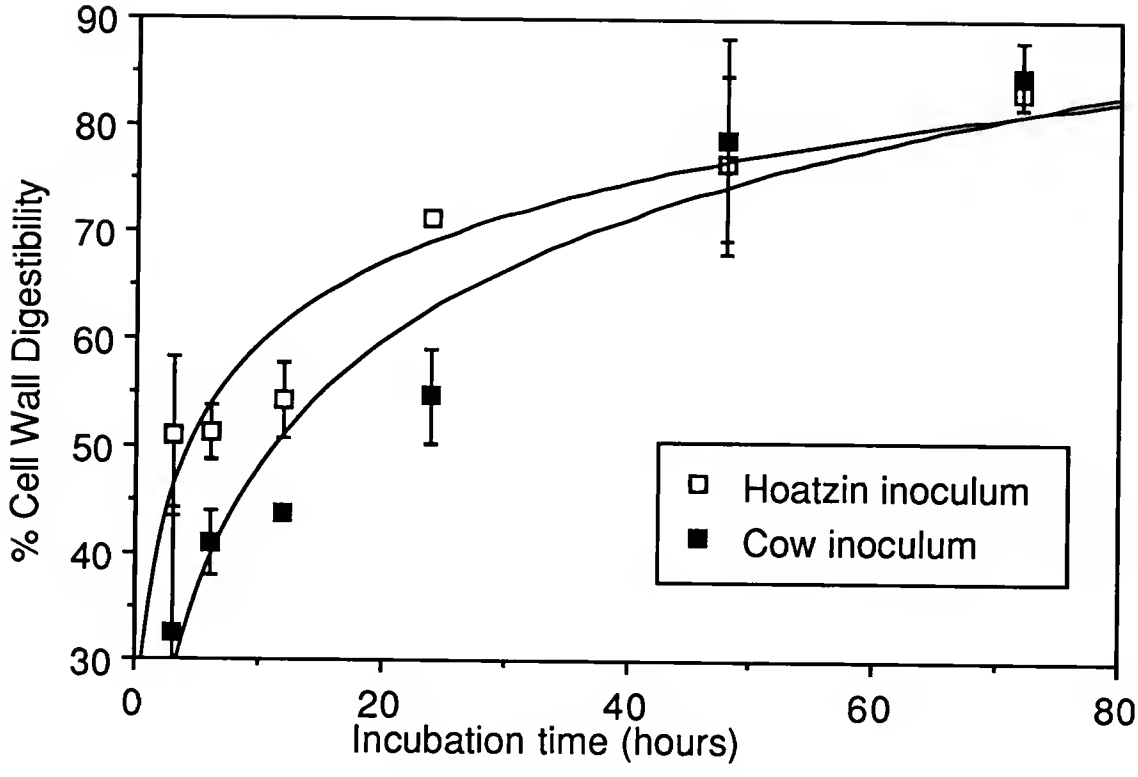

Figure 5.3) Cell wall fermentation capabilities of cow ruminal microbial inoculum and hoatzin crop microbial inoculum using a miniature <u>in vitro</u> technique. Curves represent logarithmic regression models: $y = 9.2 + 38.7 \log x$ ($R^2 = 0.92$) for cow inoculum and $y = 33.7 + 25.5 \log x$ ($R^2 = 0.91$) for hoatzin inoculum.

Table 5.1) Total VFA production rates (in mmol/kg dry matter of fermentation contents h) and proportional contribution of each VFA for wild and captive hoatzins. The proportional contribution of individual VFA production rates to the basal rate of metabolism (% BRM) was calculated using the model by Prins et al. (1984) with energy equivalents of individual VFA from Blaxter (1962). Fermentation contents refer to contents of the crop and caudal esophagus.

	CAPTIVE HOATZINS		WILD HOATZINS		
	1	2	1	2	3
Body mass (kg)	0.64	0.65	0.75	0.64	0.76
Ferm. contents (kg)	0.034	0.040	0.052	0.045	0.066
VFA production rate					
% Acetic	79.5	80.1	61.5	54.7	36.5
% Propionic	18.2	12.2	22.2	30.1	17.9
% Isobutyric	0.0	0.7	0.6	0.6	23.4
% Butyric	0.6	5.7	11.0	11.2	6.2
% Isovaleric	1.3	1.0	4.0	0.8	2.8
% Valeric	0.4	0.3	0.7	2.6	3.1
TOTAL (mmol/kgDM h)	55.41	50.49	139.12	152.29	116.14
% of BRM	13.23	14.65	54.23	61.46	69.70

Table 5.2) Comparative table showing fermentation rates (as maximum reported VFA production rate in mmol/kgDM of fermentation contents h) of selected mammals with foregut and hindgut fermentation, herbivorous birds, and hoatzins in this study. Feeding strategies represent categories defined by (Hofmann 1989), as CS = concentrate selector, IF = intermediate (mixed) feeder and GR = grass and roughage eater.

DIGESTIVE STRATEGY Species	VFA PRODUCTION RATE	MAIN FEEDING STRATEGY	FERMENTATION SITE	REFERENCE
Mammals with foregut fermentation				
Suni	629	CS	rumen	(Hungate et al. 1959)
Kirk's Dikdik	542	CS	rumen	(Hoppe et al. 1983)
Colobid monkey	475	CS	forestomach	(Bauchop and Martucci 1968)
Thompson's gazelle	420	IF	rumen	(Hoppe et al. 1977)
Grant's gazelle	356	IF	rumen	(Hoppe et al. 1977)
Greater Kudu	175	CS	rumen	(Giesecke and Gylswyk 1975)
Quokka	135	IF	forestomach	(Moir et al. 1956)
Zebu cattle	126	GR	rumen	(Hungate et al. 1959)
Mammals with hindgut fermentation				
Rabbit	205	IF	caecum	(Hoover and Clarke 1972)
Howler monkey	250	CS	caecum	(Milton and McBee 1983)
Birds				
Willow Ptarmigan	74	CS	caeca	(McBee and West 1969)
Captive hoatzin	53	CS	crop	this study
Wild hoatzin	136	CS	crop	this study

CHAPTER 6
RATE OF METABOLISM IN THE HOATZIN

Introduction

Basal rates of metabolism in birds are correlated to body mass and food habits (McNab 1988). Among all possible food habits, folivory is rare in birds (Morton 1978). This rarity seems to result from a conflict between the processing and digestion of a bulky diet and the energy requirements for flight (Sibly 1981). Fermentation of leaves of plants requires large fermentation chambers in the gastrointestinal tract where anaerobic microbes break down cell walls. This study examines the rate of metabolism of the hoatzin, Opisthocomus hoazin, a unique folivorous bird with a well-developed foregut fermentation system.

The hoatzin is one of the most folivorous of all birds: up to 85% of its natural diet consists of plant leaves (Grajal et al. 1989). It is the only known vertebrate with a foregut fermentation digestive system outside the mammals. Moreover, it is the smallest vertebrate with such a digestive system. Folivory in the hoatzin has resulted in dramatic morphological, physiological and behavioral adaptations (Grajal et al. 1989, Strahl 1988). The sternal carina is reduced to accommodate the voluminous crop and caudal esophagus, where fermentation occurs. As a result, there is little area for flight muscle attachment. Indeed, hoatzins are not powerful fliers, preferring to hop from branch to branch. Other life history characteristics, such as functional wing claws in young hoatzins, might be related to the energy constraints of the hoatzin's folivorous habits (Grajal et al. 1989).

In mammals, arboreal folivorous food habits are accompanied by low basal rates of metabolism (McNab 1978). It is not clear, however, how folivorous food habits affect basal rates of metabolism in birds. This is partly caused by the lack of a universally acceptable standard basal rate of metabolism for birds. For example, birds of the order Passeriformes seem to have significantly higher basal rates of metabolism than other (non-passerine) birds (Dawson and Hudson 1970, Lasiewski and Dawson 1967). Furthermore, Aschoff and Pohl (1970) found that birds have higher basal rates of metabolism during the active phase of the daily cycle. These data sets reflect potential biases because most measured birds are from temperate habitats and come from a narrow taxonomic spectrum when compared to world bird diversity. Moreover, a confounding factor in these data sets is that most measured Passeriformes are of small body mass (<300g), while measured non-passerines are of medium or large body mass (McNab 1988, Prinzinger and Hänssler 1980). Indeed, when small non-passerines are included in the allometric models, no appreciable differences in basal rates of metabolism can be found between Passeriformes and non-passerines (Prinzinger and Hänssler 1980).

As a result, comparisons of the basal rate of metabolism of folivorous birds are difficult. For example, folivorous Grouse and Ptarmigan (family Tetraonidae) have higher rates of metabolism than mammals of similar size (Kendeigh et al. 1977). On the other hand, partially folivorous tropical mousebirds (Colius) have relatively low rates of metabolism when compared to other birds (Bartholomew and Trost 1970, Prinzinger et al. 1981). This study explores the possible relationship between rate of metabolism and folivorous food habits in birds in general and in hoatzins in particular. If this relationship is similar between mammals and birds, then it would be expected

that an avian arboreal folivore, such as the hoatzin, would have the relatively low rate of metabolism found in arboreal folivorous mammals.

Materials and Methods

Three adult hoatzins were captured at the Guárico River, in the North-Central Llanos of Venezuela (67° 35'N, 8° 34' W). The birds were of unknown age and sex, since no external sexual dimorphism is present in this species. The birds were progressively acclimated from their natural diet to an artificial diet composed of romaine lettuce and a mix of alfalfa pellets, soybean protein concentrate and a vitamin supplement (Grajal et al. 1989). After 35 days, the birds were acclimated to captivity and maintained a stable body mass. During the study, 2-9 October 1989, the hoatzins were housed in 1 x 1 x 0.5 m wire cages in a temperature-controlled room (28°C ± 3°C) and controlled light cycle (12:12 hours). In the wild, hoatzins are most active late in the morning and late in the afternoon. Outside these activity peaks, wild hoatzins spend most of their time resting (pers. obs.). Consequently, the hoatzins were fed twice daily during their normal activity peaks, while measurements of their metabolism were performed at other times. No attempts were made to starve the hoatzins, since their gut has to be close to full capacity at all times for a regular and substantial rate of fermentation.

Rate of oxygen consumption (VO_2) was measured using a controlled temperature, negative pressure, open flow system with an Applied Electrochemistry S-3A Oxygen Analyzer. Water vapor and carbon dioxide were removed with drierite and ascarite before entering the respirometry chamber. Two temperature-controlled chambers of 36 and 42 l were used for 32 measurements. Each hoatzin was measured in both chambers for the same ambient temperature. Flow rates were adjusted to the volume of each chamber

and ranged from 90 to 200 ml/min. Measurements lasted 2-5 hours, and were terminated when a low and constant VO_2 was obtained. Body temperatures (Tb) were measured before and after the VO_2 determination, using an electronic telethermometer with a thermocouple probe inserted 3-5 cm in the cloaca for 15-30 seconds. Hoatzin Tb and body mass were measured immediately before and after each VO_2 measurement.

Thermal conductance was calculated as the mean conductance from individual conductances $C_m = VO_2 / (Tb - Ta)$ at each measurement, in which C_m is the thermal conductance (McNab 1980) and Ta is the ambient temperature (air temperature inside the chamber). To compare the hoatzin basal rate of metabolism with expected values of mass-specific basal rate of metabolism for endotherms, the regression model from Kleiber (1961) was used: $VO_2 / M = 3.4\ M^{-0.25}\ cm^3\ O_2\ g^{-1}\ h^{-1}$. Additionally, the basal rate of metabolism in hoatzins was compared to the mass-specific non-passerine model of Prinzinger and Hänssler (1980): $VO_2 / M = 6.8\ M^{-0.28}\ cm^3\ O_2\ g^{-1}\ h^{-1}$. Minimal resting mass-specific conductance was calculated from Aschoff (1981) $C_m / M = 0.95\ M^{-0.53}\ cm^3\ O_2\ g^{-1}\ h^{-1}\ °C^{-1}$.

Average environmental temperature in the natural habitat of the hoatzin in the Llanos of Venezuela is 27°C with a year minimum of 19°C and a maximum of 43°C (Troth 1979). Therefore, the temperatures at which we tested the oxygen consumption of hoatzins are within the range of temperatures experienced by hoatzins in their natural habitat.

Results

The relationships of body temperature and rate of metabolism with ambient temperature are shown in Fig. 6.1. No differences in rate of metabolism between day and night measurements were found. The three hoatzins maintained a constant body temperature of 38.5°C (s.d. ± 1.2°C, n = 32) at Ta between 12°C and 36°C. At ambient temperatures over 36.5°C, the hoatzins were restless and became dangerously hyperthermic (Fig. 6.1).

The rate of metabolism for hoatzins within the thermoneutral zone was 0.48 cm^3 O_2 g^{-1} h^{-1}, about 69.8% of the expected value for an endotherm with a body mass of 598 g (Kleiber 1961) and 43% of the expected basal rate of metabolism for a non-passerine bird (Prinzinger and Hänssler 1980). The thermoneutral zone was between 26.5 and 36.5°C. Thermal conductance below the thermoneutral zone was 0.039 cm^3 O_2 g^{-1} h^{-1} $°C^{-1}$ (s.d. = 0.006, n = 18), which represents 105% of the expected value for birds (Aschoff 1981). Conductance decreased to 89% (0.033 cm^3 O_2 g^{-1} h^{-1} $°C^{-1}$) at Ta below 18°C.

Discussion

Hoatzins maintain homeothermy over a wide range of environmental temperatures. In the hoatzin, constant temperature is probably important for the optimization of microbial and enzymatic activity (Grajal et al. 1989). Furthermore, in wild hoatzins, constant body temperature is probably maintained using special behavioral patterns. Overall energy expenditure is probably reduced by long periods of inactivity and thermoregulatory behaviors, such as selection of shady or cool microhabitats at high Ta . Moreover, hoatzins adopt a specialized sunbasking posture during early morning or after heavy rains, opening the wings, ruffling the rump feathers, and exposing the dark skin underneath (pers. obs., Strahl 1985). Hoatzins

change conductance at low Ta, suggesting they are able to mix chemical and physical thermoregulation (McNab 1980). Lower conductance at Ta below 18°C may be another technique for energy conservation as a result of decreased peripheral circulation and changes in feather position (McNab 1989).

The low basal rate of metabolism of the hoatzin agrees with the general pattern of folivorous arboreal mammals (McNab 1978, McNab 1983) and is much lower than rates of metabolism for non-passerine birds (Prinzinger and Hänssler, 1980). The small number of studies on the rate of metabolism of folivorous birds, however, prevent broad generalizations (McNab 1988). Other highly folivorous birds, such as Grouse and Ptarmigan (family Tetraonidae) have comparatively high rates of metabolism (Kendeigh et al. 1977). These results are difficult to compare with the rate of metabolism in hoatzins due to several factors: Hoatzins are exclusively tropical, eat mostly young leaves and shoots of angiosperm plants and fermentation occurs in the foregut. In contrast, Grouse occur only in temperate boreal forests and tundra habitats, eat a variety of plants, including lichens, conifer needles and seeds, and their fermentation site is located in the hindgut (Davis 1987, Gasaway 1976b, Gasaway 1976c, Martin and Martin 1984). Additionally, other folivorous birds do not have significant fiber fermentation capabilities and still can maintain normal to high levels of metabolism (Crawford and Schmidt-Nielsen 1967, Kendeigh et al. 1977). These contrasting trends probably reflect the diversity of digestive strategies of herbivorous birds (see Chapter 7).

In the hoatzin, a unique combination of selective pressures probably resulted in a well-developed foregut fermentation digestive system and accompanying low basal rates of metabolism. These selective pressures reflect the energy conflicts between homeothermy and flight ability on one side, and leaf fiber and secondary compound concentration on the other. Leaves are

bulky, and their fermentation releases assimilable energy at a slow rate, when compared to other food items. Most folivorous birds avoid these energy constraints by an increase in processing rate. For example, folivorous birds with little or no fermentation process their bulky diet at high rates (Buchsbaum et al. 1986, Dawson and Herd 1983, Dawson et al. 1989, Mackie 1987, Mattocks 1971). Faster processing rates, however, result in decreased digestive efficiency (Van Soest 1982). In avian herbivores, fast food processing decreases per unit dry matter digestibility but can increase the total rate of energy intake. In fact, simple gastric digestion of cell contents and sometimes of the hemicellulose fraction of the cell walls seem to supply most of the energy needs of some of these birds (Buchsbaum et al. 1986, Dawson et al. 1989, Herd and Dawson 1984, Mackie 1987).

Evidently, the digestive patterns of these avian folivores are not always possible. Most dicotiledoneous plants have lower levels of hemicellulose than grasses (Agricultural Research Council 1980, Van Soest 1969) and usually contain a large variety of secondary compounds (Freeland and Janzen 1974). Fermentation in the hoatzin has resulted in significantly slower passage rate and comparatively lower intake rate than nonfermenting folivorous birds (Grajal et al. 1989, also see Chapters 3 and 4). This digestive pattern provides several advantages: a) enhanced fiber digestion (Van Soest 1982), b) reduced dependence on continuous foraging to meet energy requirements (with more possibilities for food selectivity) and c) detoxification of plant defensive chemical compounds (Freeland and Janzen 1974). On the other hand, effective fermentation of leaves requires gut fermentation chambers of large capacity and delayed passage rates (Parra 1978). In the hoatzin, the enlarged capacity of the crop and esophagus has caused extensive anatomical and behavioral

modifications that reduce flight performance (Böker 1929, Gadow 1891, Grajal et al. 1989, L'Hermenier 1837).

Foregut fermentation reduces the hoatzin's dependence on continuous foraging and increases the time for microbial attack of fiber and secondary compounds. Microbial metabolic losses of fermentation can reduce the rate of energy available for assimilation to the host. Therefore, the low basal rate of metabolism in the hoatzin is probably the result of an evolutionary tradeoff between the benefits of enhanced fiber digestion, greater selectivity, detoxification of secondary compounds, and the costs of a low rate of energy availability and reduced flight ability. As a consequence, the low basal rate of metabolism is probably one of the most important physiological adaptations for the evolution of a foregut fermenting system in a flying bird.

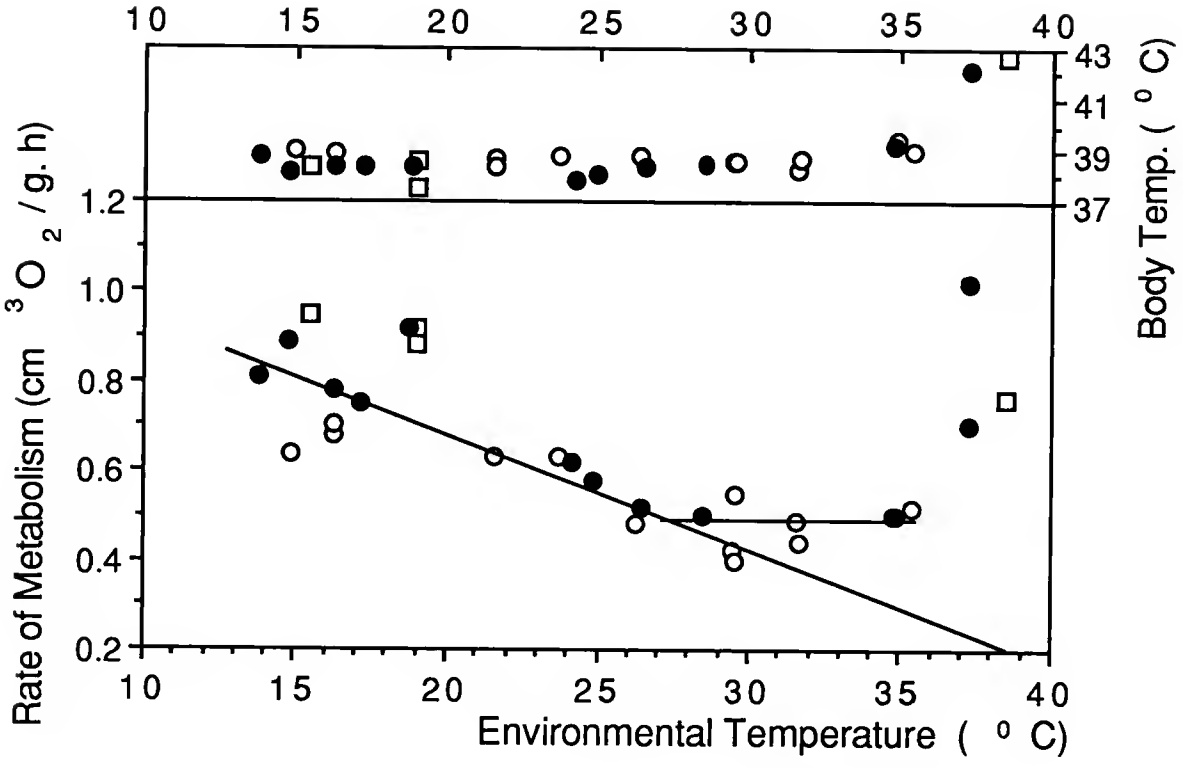

Figure 6.1). Mass-specific rates of metabolism and body temperatures of three adult hoatzins (each individual denoted by a different symbol) as a function of ambient temperature.

CHAPTER 6
GENERAL DISCUSSION

Evolution of Foregut Fermentation

The hoatzin's way to deal with a leafy diet is unique, leading to some extreme morphological, physiological and behavioral adaptations. The hoatzin crop and posterior esophagus are the primary site for digestion of its leafy diet. This foregut fermentation system is unique among birds, and it is more similar to foregut fermentation systems in mammals. The hoatzin, however, is almost an order of magnitude smaller than the smallest mammal with a well developed foregut fermentation.

The evolution of foregut fermentation has been interpreted as a digestive strategy that takes advantage of diets low in nitrogen and high in fiber (Demment and Van Soest 1985, Hume and Warner 1980, Janis 1976, Parra 1978). Indeed, the rapid radiation of foregut fermenting Artiodactyls and Macropods probably occurred with a simultaneous expansion of grasslands during the Miocene and Pliocene (Janis 1976). As a consequence, most evolutionary explanations of the presence of foregut fermentation have emphasized the advantages of cell wall digestion as an important selective force in the evolution of foregut fermentation systems (Janis 1976). Although some of the most advanced foregut fermenters, such as ruminants, do indeed take advantage of a highly fibrous diet, foregut fermentation has evolved independently in other taxa that are not grassland dwellers, but tropical forest inhabitants. This group includes tree-kangaroos (Hume 1978, Hume 1982), tree-sloths (Bauchop 1978, Montgomery and Sunquist 1978), colobid monkeys

(Bauchop 1978, Bauchop and Martucci 1968, Ohwaki et al. 1974), tragulids (primitive Artiodactyls) (Langer 1974) and the hoatzin (this study, Grajal et al. 1989). Hume and Warner (1980) proposed that the presence of foregut fermentation in these forest animals is probably not related to the nutritional use of grasses, but to the use of tropical forest plants. Tropical forest plants are usually available year-round, but generally have high levels of secondary compounds (McKey et al. 1981, McLeod 1974, Moreno-Black and Bent 1982, Robbins et al. 1987). Foregut fermentation can be an adaptive foraging strategy in these habitats, because foregut microbes can detoxify secondary compounds before they reach the lower gut where absoption takes place (Barry and Blaney 1987, Freeland and Janzen 1974, Mackie 1987). In addition, foregut fermentation can enhance the quality of nitrogen levels in the diet and allow the use of plant fiber as a nutrient source.

Therefore, the presence of foregut fermentation in another small, arboreal no-mammalian vertebrate, seems to indicate that foregut fermentation has evolved several times not only as a response to the use of tropical forest plants as a resource and not to the nutritional use of grasses. Other lines of evidence suggest that indeed most foregut fermentation systems have evolved from ancestral forest forms that have later radiated into grasslands. Indeed, Tragulids are tropical forest Artiodactyls, and considered "primitive" ruminants because they have retained ancestral characteristics of the original ruminants (Langer 1974).

Herbivory in Birds

Given the advantages of foregut fermentation, it is not clear why hoatzins are the only birds with this digestive system. Indeed, foregut fermentation may not be advantageous for birds. Microbial fermentation of readily digestible cell contents inserts 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 host. Given the high energy cost of flight and endothermy, such metabolic losses may not be acceptable for most birds (Morton 1978).

These and other costs may explain why only 3% of the extant species of birds consume plant leaves as a significant proportion of their diet (Morton 1978). Most herbivorous birds increase the digestion of cell contents at the expense of a reduced nutritional use of cell walls. Although a more thorough investigation of digestive patterns of herbivorous birds is needed, some general trends may explain how herbivorous birds deal with different kinds of leafy diets and habitat constraints. Some of these major categories include:

1) Caecal fermenters: This group includes some well-studied taxa (e.g., ptarmigan and grouse of the family Tetraonidae) and others not so well studied (e.g., screamers of the family Anhimidae, and large ratites such as ostriches). Ptarmigan and grouse eat some of the most refractory diets for a bird, including pine needles, twigs, and catkins (Davis 1987, Gasaway 1976b, Hill et al. 1968, Leopold 1953, Moss 1974, Moss 1977, Moss and Parkinson 1972, Pendergrast and Boag 1971, Ponce 1985, Ponce 1987, Pulliainen et al. 1968, Pulliainen and Tunkkari 1983). The digestive pattern of these advanced herbivores can be summarized as an optimization of the nutritional use of cell contents until food arrives in the enlarged caeca. The caeca fill selectively with highly fermentable smaller particles and liquid, while most of the

largely undigested cell wall is excreted (Duke 1989, McLelland 1979, Ziswiler and Farner 1979). Although significant amounts of fiber are digested by caecal fermenters (Gasaway 1976a, Inman 1973, Moss 1973, Moss and Trenholm 1987, Pulliainen et al. 1968, Suomalainen and Arhimo 1945), cell wall digestion may not be the digestive goal (Björnhag 1989, Remington 1989). Caecal fermentation provides important benefits in addition to some fiber fermentation. For example, caecal fermentation enhances the use of urinary nitrogen for microbial growth (Skadhauge, 1976) and provides the energy benefits of microbial VFA production (Björnhag 1989, Gasaway 1976b, Gasaway 1976c, Gasaway et al. 1976, Mackie 1987, Remington 1989, Skadhauge 1976, Withers 1983). Another advantage of caecal fermentation is that it allows wider dietary niches. For example, when high quality food sources as seeds and fruits are seasonally available, caecal fermenters can reduce the ingestion of leaves and take advantage of these higher quality food items (Davis 1987, Ponce 1985, Ponce 1987). A similar dietary plasticity is found in most other avian herbivores (probably except hoatzins), but in caecal fermenters it is combined with an efficient fermentation system.

2) Non-fermenting grazers and aquatic plant eaters: This group includes many members of the family Anatidae (ducks and geese) and some coots and gallinules of the family Rallidae, including some flightless species (Buchsbaum et al. 1986, Dawson et al. 1989, Kingsford 1989, Mulholland and Percival 1982, Reid 1974). Most of these birds have little or no fermentation (Clemens et al. 1975), with fast passage rates and high food intakes (Björnhag and Sperber 1977, Buchsbaum et al. 1986, Burton et al. 1979, Dawson et al. 1989, Ebbinge et al. 1975, Halse 1984, Marriot and Forbes 1970, Miller 1984, Muztar et al. 1977). Most eat grass or aquatic plants with low nitrogen and high fiber levels (Ebbinge et al. 1975, Esler 1989, Hardin et al. 1984, Kingsford 1989,

Montalbano et al. 1979, Mulholland and Percival 1982, Owen 1975, Reid 1974). The rate of nutrient and energy uptake is optimized by increasing food intake at the expense of a thorough digestion of both cell contents and cell walls. Some fiber is digested, mainly by acid degradation of hemicellulose at the stomach and some microbial fermentation (Buchsbaum et al. 1986, Dawson et al. 1989, Marriot and Forbes 1970).

3) Frugivores-folivores: This is a heterogeneous group with varying proportions of leaves in the diet. In South America, Passeriformes of the family Phytotomidae and Saltatoridae ingest large amounts of leaves during parts of the year (pers. obs.), but not detailed studies are available yet. Similarly, birds of the family Colidae are partially folivorous (Bartholomew and Trost 1970, Prinzinger et al. 1981). The New Zealand fruit pigeon subsists for more than eight months of the year exclusively on leaves of a few plants in riparian forests of temperate New Zealand (Clout et al. 1986). Finally, some Galliformes include large proportions of leaves in their diets, including members of the families Phasianidae (Young et al. 1991) and Cracidae (largely unstudied). It is unclear why these frugivores ingest leaves or what is the proportional composition of their diet. Leaves may supplement the low nitrogen or mineral deficiencies of a frugivorous diet (Morton 1978).

4) Fiber manipulators: This is the niche of another oddity among avian herbivores. The kakapo or owl parrot, Strigops habroptilus, uses its dexterous bill and tongue to literally "chew" tussocks, grass blades and rhizomes, squeezing the cell contents and leaving dried clumps of squeezed plant leaves (Stivens 1964). Whether fermentation takes place in the lower gut or in the enlarged crop remains unclear (Böker 1929). Sadly, this species is one of the rarest birds in the world, and its survival as a species depends on active management by humans (Merton 1977). Therefore, research on this bizarre

avian herbivore is unlikely in the near future. Other parrots, such as the Antipodes green parakeet, Cyanoramphus unicolor, ingests significant amounts of plant leaves using their special bill and tongue to select and manipulate plant parts, but no detailed study is available (Taylor 1971).

5) Large flightless herbivores: These are mostly ratites, the ostrich in Africa, emus in Australia, and rheas in South America and probably the New Zealand giant flightless gallinule, the takahe (Dawson and Herd 1983, Herd and Dawson 1984, Mackie 1987, Reid 1974, Withers 1983). Emus digest significant amounts of fiber without any special retention mechanism or enlarged fermentation compartment (Herd and Dawson 1984). Ostriches and Rheas have enlarged caeca where fermentation takes place, but no definitive studies have been done on these avian herbivores (Mackie 1987, McLelland 1979, Withers 1983).

6) Foregut fermentation: The only known folivorous bird with foregut fermentation is the hoatzin. The digestive strategy is characterized by long retention times, selective particle retention, fiber fermentation, low rate of metabolism, poor flight ability, and a voluminous foregut. Some pre-conditions are necessary for the evolution of foregut fermentation, including a large crop and some kind of glandular tissue inside the crop that allows buffering of the crop contents. Given these constraints, the New Zealand Pigeon and the kakapo may have some kind of foregut fermentation, because both are largely herbivorous (Clout et al. 1986, Stivens 1964), have large crops (Böker 1929) and both pigeons and parrots have secretory tissues in their crops (McLelland 1979). Both species remain largely unstudied.

Foregut fermentation is advantageous for the hoatzin because it provides a more effective use of both cell walls and cell contents. Microbial fermentation also produces volatile fatty acids that can be readily used by the

hoatzin to support more than 60% of its basal metabolic requirements. Foregut fermentation can be an efficient way to detoxify plant secondary compounds before they enter the absorptive sites of the lower gut (Freeland and Janzen 1974, Mackie 1987). Finally, microbes produce vitamins and amino acids that can balance an otherwise unbalanced diet. Flight adds a new dimension to selective feeding in a vertebrate herbivore. It provides access to resources that are dynamic in time and space, especially in tropical environments. Therefore, hoatzins can track resources that otherwise would not be available to non-volant vertebrate herbivores.

Suggestions for Future Studies

Future studies should focus on the evolution of fermentation systems in vertebrates. Particularly, how these species deal with plant secondary compounds and the relative importance of plant fiber and secondary compounds on niche width. Therefore, future studies on hoatzins, avian herbivores, and foregut fermenting folivores should include:

-. More nutritional ecology and digestive ecology of many poorly studied avian herbivores discussed above.

-. The effect of foregut fermentation on the detoxification of secondary compounds, in particular in tropical habitats.

-. The advantages of foregut fermentation in the hoatzin for the balancing of minerals and other micronutrients.

-. The implications of digestive strategies on niche width of herbivorous birds and its effect on other life history characteristics.

Coda

Hoatzins at this moment are not threatened as a species although local populations within its range have disappeared. For example the hoatzin is the national bird in Guyana, but the species is on the brink of extinction in that country (S. Strahl, pers. comm.). On the other hand, hoatzins are relatively common in suitable habitat along major rivers. The destruction of tropical wet forests is a major threat to the survival of hoatzins. During the four years of this study, I have personally witnessed the conversion of thousands of hectares of gallery forests into rice paddies.

The hoatzin is one of the most obvious birds of the Amazon and Orinoco basins, and even then it is one of the most interesting birds in the world. So apart from this unique creature, who can say what other evolutionary wonders will be revealed by the study of the almost 3,000 species of birds of tropical America?

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BIOGRAPHICAL SKETCH

Alejandro Grajal was born in Madrid, Spain, 21 December 1957. His family moved to Caracas, Venezuela, in 1963, where he received most of his formal education. He received his high school degree in science in 1975. Then he attended the Universidad Simón Bolívar in Caracas, Venezuela, where he received the Licence in biology, mention in ecology in 1981. His thesis work was entitled "Comparisons between Decapod crustaceans communities associated with three species of corals of the genus Acropora in the Los Roques Archipelago," under the supervision of Dr. R. Laughlin. This research was supported by the Venezuelan National Council for Science and Technology (CONICIT) and the Los Roques Scientific Foundation.

After graduation, he spent several years as field assistant for various projects, including a study of the communal nesting and homing of female green iguanas (Iguana iguana) with the Smithsonian Institution during 1982-84, and the social behavior of the hoatzin (Opisthocomus hoazin) with Dr. S. D. Strahl, during 1984. He also worked as a nature guide for Tropical Scientific Tours Co. during 1985-86.

He has taken field courses on biology and ecology of the coral reef and in marine ecology, both offered by the Los Roques Scientific Foundation, Venezuela. He also took a course on scientific illustration at Universidad Simón Bolívar, a tropical botany course at the Fairchild Botanical Gardens through the University of Florida, and a special training internship on rearing and care of captive birds at the Bronx Zoo in New York.

In 1987, he entered the graduate program of the Department of Zoology at the University of Florida. His studies were funded by a scholarship from the Organization of American States (OAS) (1987-88), a Graduate loan-fellowship from the Venezuelan National Council for Science and Technology (CONICIT) (1988-89) and teaching and research assistantships from the Department of Zoology, University of Florida.

His Ph.D. dissertation project focused on the nutritional ecology and digestive physiology of the hoatzin, (Opisthocomus hoazin), a unique avian folivore. This work was funded with grants from the Alexander Wetmore Memorial Fund Research Grants of the American Ornithologists' Union, Sigma Xi Grants-in-Aid, New York Zoological Society, Nixon Griffis Fund Award, Chicago Zoological Society Zoo Research Award and the Venezuelan National Council for Science and Technology (CONICIT), Venezuela. He also studied the evolutionary implications of seed size, passage rate and fruit preferences by cedar waxwings (Bombycilla cedrorum) at the University of Florida with Dr. D. Levey.

He has been very interested in biological conservation. He received a scholarship from the Pew Charitable Fund in Integrated Approaches to Training in Conservation and Sustainable Development. This scholarship included an internship in July 1990 on public communications at the Education Department of the New York Zoological Society and a series of surveys of the publics surrounding the Henri Pittier National Park in Northern Venezuela. His general interests are related to animal-plant interactions, herbivory, frugivory, vertebrate energetics, and comparative studies of digestive systems. He understands biological conservation as a biological problem with social and economic consequences, so he has been working on public communications, the interactions of people on national

parks, and the effect of communication media on the conservation of biodiversity. As a scientific illustrator, he has published several posters, books and prints as a means to create the necessary awareness for conservation issues, especially among young people.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1991

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