ASSIMILATION OF ENERGY, PROTEIN AND FATTY ACIDS BY THE SPECTACLED CAIMAN *CAIMAN CROCODILUS CROCODILUS* L.

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ABSTRACT

At $30\pm1°C$, caimans (*Caiman crocodilus*) ate a mean satiation ration of 8.2% body weight. Mean total gut clearance time (TGCT) was 136 h; mean gastric emptying time (GET) was 97 h. These data indicate that caimans eat considerably less food than salt-water crocodiles (*Crocodylus porosus*) of similar size, not because their meal size was less, but because they take much longer to process food. Assimilation efficiencies for protein, energy (cals) and dry mass were 91.8%, 68.2% and 62.0% on a diet of sheeps' hearts and 90.2%, 69.2% and 64.7% in caimans fed on fish. These efficiencies are all significantly lower than those found in salt-water crocodiles. Hard particulate material is retained within the stomach by a powerful pyloric sphincter, but caimans appear not to eat gastroliths deliberately. Evidence is presented to show that polyunsaturated fatty acids (PUFAs), especially 22:5ω3, are assimilated and incorporated into tissues. Fish-fed caimans showed more 20:5ω3 and 22:6ω3 in their tissue than liver-fed animals. It has previously been suggested that species of the Family Crocodylidae (believed to be of largely marine ancestry) can assimilate PUFAs while members of the Family Alligatoridae (probably of prolonged freshwater ancestry) cannot. The results of this study deny this clearcut distinction and indicate that any differences in lipid metabolism amongst crocodillians are likely to reflect ecological considerations rather than taxonomic patterns.

INTRODUCTION

It has been suggested that living true crocodiles (Family: Crocodylidae) have evolved from predominately marine ancestors, while alligators and caimans (Family: Alligatoridae) have a long history of life in freshwater ecosystems. The main evidence for this hypothesis lies in the observations of Taplin & Grigg (1981) and Taplin *et al.*, (1982), who found that true crocodiles have lingual glands which secrete salt, while members of the Alligatoridae do not. However, there is some supporting evidence from nutritional studies. Garnett (1985) suggested that the salt-water crocodile (*Crocodylus porosus*) has a nutritional requirement for the long chain polyunsaturated fatty acids (PUFAs) found in marine fish, while Ferguson (1981) and Lance (1982) both indicate that alligators (*Alligator mississippiensis*) are unable to assimilate these marine fatty acids. the main aim of the study reeported here was to determine whether another member of the Alligatoridae, the spectacled caiman, *Caiman crocodilus*, could incorporate long chain fatty acids into its tissues. In addition, it was decided to investigate appetite, gut transit, plus protein and energy assimilation in the caiman, for comparison with the data recently presented for the salt-water crocodile by Davenport *et al.*, (1990).

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE

Ten spectacled caimans were obtained from biological suppliers. At the time of the study the animals weighed between 240 and 600 g. They were held in running fresh water at the experimental temperature of $30\pm1°C$. Five of the animals (used in gut transit studies) were fed on sheep heart and ox liver (offered daily) while the other five were divided into two groups. Two were fed on pigs' liver, while the other three were fed on whole chopped herring. Feeding was continued for one month prior to experimentation.

APPETITE AND TRANSIT

Satiation ration was estimated in the caimans by depriving them of food for 4 days and then offering food from preweighed rations until they were satiated.

Gut transit of food was investigated in two ways. Five animals were each given a meal of minced ox liver containing chromic oxide (2% w/w). Thereafter they were fed normally on unlabelled ox liver. The animals were held separately and their faeces collected and inspected twice per day. The presence of green (i.e. chromic oxide labelled) faeces was recorded. This experiment allowed calculation of total gut clearance time (TGCT) and an estimate of gastric emptying time (GET). Another two animals were fed a meal labelled with 2% (w/w) barium sulphate plus numbers of barium/polystyrene spheroids (1 mm diameter) and lead glass beads (0.4 mm diameter). X-rays were obtained before and immediately after the meal; further X-rays were taken after 4, 8, 12, 19, 24, 52, 74, 96, 144, 168 and 192 h. After the radio-opaque meal they were offered unlabelled food each day. This experiment provided information about the behaviour of material within the various parts of the gut.

ASSIMILATION OF NUTRIENTS

Assimilation of energy, protein and dry mass was studied in the group which had been fed initially upon sheeps' heart and later upon whole herring. Fatty acid assimilation was investigated in the two groups fed upon pigs' liver and whole herring repectively. Each group was fed upon chromic-oxide labelled food (2% w/w) which had been finely minced and reminced so that the food and label were evenly mixed. Labelled food was offered to the animals every 48 h. Uneaten food was removed after 4 h. Animals were fed for a week (a period longer than the TGCT) on labelled food and then faecal samples were collected from each animal (by placing them in individual tanks with

broad mesh floors). Animals were inspected several times per day; only freshly voided faeces were collected. Feeding and faecal collection continued until adequate amounts of material were available for chemical analysis. The five animals being studied for fatty acid assimilation were then killed humanely; tissue samples were taken from the tail muscle and a large abdominal fat body found in all the caimans. For comparative purposes, tail muscle and adipose tissue samples from three specimens of *Crocodylus porosus* (stored frozen from the earlier study of Davenport *et al.*, 1990) were also analysed for fatty acid composition.

Food and faecal samples were dried at 70°C to constant weight. Chromic oxide content was measured by the method of McGinnis & Kasting (1964). Energy content was determined by the wet oxidation method of Ivlev (1935). Protein content was estimated by measuring total nitrogen (micro-Kjeldahl technique). Lipids were extracted from dried food, faecal and tissue samples and the total lipid content determined gravimetrically as described by Folch *et al.*, (1957). Once the lipid content had been determined, fatty acid composition was studied. Samples were transmethylated prior to fatty acid analysis by heating with an excess of boron trifluoride methanol reagent (15% BF_3) for 1 h at 100°C. Fatty acid methyl esters (FAME) were extracted into pentane, evaporated to dryness with oxygen-free nitrogen and dissolved in hexane with a known amount of tricosanoic acid meythl ester as standard. Analysis was carried out by capillary gas chromatography, using a Carlo Erba 6180 gas chromatograph fitted with a flame ionization detector and a DB Wax 30 W capillary column, 25 m, 0.32 mm, I.D. and an on-column cold trap injector. FAME were identified using commercially available standards, graphical techniques and systematic separation factors (Ackman, 1972). Lipid classes were investigated by thin layer chromatography, using pre-cooled plates (silica-gel 60). The plates were then developed in petroleum spirit: diethyl ether (95:5 v/v) and visualised with iodine vapour. Samples were co-eluted with a methyl ester standard (178-1; Sigma Chemicals).

RESULTS

APPETITE AND GUT TRANSIT

At 30±1°C, the caimans ate a mean satiation ration of sheeps' heart of 8.2% body weight (SD 4.3%). The mean TGCT (time between chromic oxide-labelled meal and appearance of last labelled faeces) was 136 h; the mean GET (time between appearance of first and last labelled faeces) was 97 h. X-radiography showed that food moved through the gut in a similar fashion to the salt-water crocodile (Davenport, *et al.*, 1990). The stomach is large and drum shaped; the meal is totally confined within it until about 4 h after the meal when some barium sulphate label moves into the duodenum. Barium sulphate labelled material passes rapidly through the small intestine into the large intestine and rectum (some material reaching the rectum in as little as 12 h), but the barium spheroids and glass beads remain within the stomach, lying close to the pyloric sphincter. Clearly, as in the salt-water crocodile, a powerful sphincter sorts particulate material; only fluid and fine material passes into the small intestine. In the X-ray plates it was noticeable that the larger barium spheroids lay anterior to the glass beads which were packed next to the pyloric sphincter. After 52 h no barium sulphate

was present in the small intestine, but the particulate material was again dispersed throughout the stomach (the caimans having eaten non-labelled material which now filled the stomach). Barium sulphate and a few particles filled the rectum. After 74 h no barium sulphate could be seen in any part of the gut and about 25% of the spheroids and glass beads had passed into the colon. There was no evidence that the pyloric sorting mechanism could differentiate between spheroids and beads. As each new meal was eaten a few particles left the stomach; one animal had lost all particles after 168 h, but the other still had substantial numbers in the stomach after 192 h.

ASSIMILATION

Energy, protein and dry mass. Assimilation efficiencies were calculated as described by Maynard & Loosli (1969):

$$\% \text{ efficiency} = \frac{[Cr_2O_3]:[\text{nutrient in food}]}{[Cr_2O_3]:[\text{nutrient in faeces}]} \times 100$$

On a diet of sheeps' heart the assimilation efficiencies for protein, energy (cals) and dry mass were 91.8% (SD 3.6%), 68.2% (SD 5.6%) and 62.0% (SD 7.4%) respectively. On a diet of fish (herring) the mean assimilation efficiency for protein was 90.2% (SD 2.0%), for energy (cals) 69.2% (SD 4.1%) and for dry mass 64.7% (SD 4.6%). There was clearly no significant difference in rate of assimilation of these nutrients from the contrasting diets.

Fatty acids. Fatty acid data for diets, faeces and tissue samples are presented in Tables 1 and 2. As expected there were marked differences between the fatty acid compositions of the diets. Pigs' liver contained a far higher proportion of the C18 fatty acids than the herring diet with approximately ten times the proportion of linoleic acid (18:2ω6) and seven times the percentage of 18:0. The fish diet was rich in long chain fatty acids, particularly 22:6ω3. Rather surprisingly, the pigs liver contained some 22:6ω3 (perhaps because the pigs had been fed on fish meal at some stage), but at much lower concentrations than the herring.

In considering the faecal data, it must be remembered that they represent proportions of the unassimilated lipid, plus any fatty acids excreted by the caiman or synthesized by gut bacteria. Although both diets contained hardly any of the short chain saturated fatty acid 12:0, substantial amounts of faecal lipid were made up of this fatty acid which was probably of bacterial origin. Of particular interest are the low levels of 22:6ω3 in the faeces, indicating assimilation of this PUFA, particularly in the fish-fed animals.

There are some noticeable differences between the fatty acid composition of tissues of caimans fed on contrasting diets, providing further indication that dietary incorporation of fatty acids has a role to play in the species. Fish-fed caimans showed more 20:5ω3 and 22:6ω3 in their tissues than liver-fed animals, indicating that these fatty acids are incorporated from the diet. However, in the case of 22:6ω3, the tissue levels are still well below dietary concentrations (especially in the fish-fed animals), suggesting that this fatty acid has a low saturation point.

In Table 3, the tissue fatty acid composition of fish-fed caimans and salt-water crocodiles are compared. The tissue compositions are remarkably similar, and there are no signifi-

	Fatty acid content (mean % total lipid)			
Fatty acid	Diet	Faeces	Tail muscle	Fat body
12:0	T	52.5	8.6	0.4
14:0	0.1	T	0.6	1.2
15:0	T	T	T	0.2
16:0	13.5	5.4	7.0	9.0
16:1ω7	0.7	0.2	4.4	0.7
17:0	0.7	0.1	0.7	0.6
18:0	21.7	10.2	9.2	13.4
18:1ω9	11.4	3.9	11.5	21.2
18:1ω7	2.2	1.4	4.2	4.6
18:2ω6	10.3	2.7	4.2	7.1
19:0	0.4	-	-	T
18:3ω3	0.6	0.2	0.4	0.6
18:4ω3	T	0.1	0.1	0.3
20:0	0.1	T	T	T
20:1ω9	0.2	T	1.4	1.7
20:4ω6	11.6	2.9	4.2	3.0
20:5ω3	1.0	0.3	0.6	0.5
22:1ω11	0.1	1.3	0.3	0.1
22:5ω3	2.5	1.2	1.0	0.9
22:6ω3	3.6	1.4	1.6	2.1

T = trace quantities; - indicates none detected

TABLE 1: Fatty acid data for caimans fed on pigs' liver

	Fatty acid content (mean % total lipid)			
Fatty acid	Diet	Faeces	Tail muscle	Fat body
12:0	0.7	29.6	3.2	2.5
14:0	2.1	0.1	0.2	1.7
15:0	0.3	T	T	0.2
16:0	16.7	5.8	5.0	5.7
16:1ω7	3.8	0.4	0.3	1.2
17:0	0.4	T	0.2	0.4
18:0	3.5	0.9	6.9	14.2
18:1ω9	4.6	0.7	7.2	23.9
18:1ω7	2.5	0.4	1.0	5.3.
18:2ω6	0.8	0.2	2.2	5.9
19:0	-	-	-	0.1
18:3ω3	0.5	T	0.2	0.6
18:4ω3	1.4	0.3	0.2	0.2
20:0	0.1	1.1	0.8	0.4
20:1ω9	0.1	2.4	0.1	0.5
20:4ω6	2.6	2.4	2.0	5.4
20:5ω3	8.5	1.4	7.5	0.9
22:1ω11	0.7	0.6	0.2	1.3
22:5ω3	0.3	0.3	0.5	1.1
22:6ω3	19.8	1.6	3.7	3.6

T= trace quantities; - indicates none detected

TABLE 2: Fatty acid data for caimans fed on whole herring

Fatty acid	A) *Caiman crocodilus*		B) *Crocodylus porosus*	
	Tail muscle	Fat body	Tail muscle	Fat body
12:0	3.2	2.5	8.5	1.3
14:0	0.2	1.7	1.4	T
15:0	T	0.2	T	T
16:0	5.0	5.7	3.9	1.5
16:1ω7	0.3	1.2ᵢ	0.4	1.5
17:0	0.2	0.4	T	0.1
18:0	6.9	14.2	2.1	2.1
18:1ω9	7.2	23.9	4.5	1.3
18:1ω7	1.0	5.3	1.3	1.5
18:2ω6	2.2	5.9	3.7	0.3
19:0	-	0.1	-	1.0
18:3ω3	0.2	0.6	0.4	T
18:4ω3	0.2	0.2	0.3	T
20:0	0.8	0.4	2.1	0.2
20:1ω9	0.1	0.5	4.5	T
20:4ω6	2.0	5.4	4.8	6.7
20:5ω3	7.5	0.9	0.7	T
22:1ω11	0.2	1.3	3.0	0.3
22:5ω3	0.5	1.1	O.5	1.4
22:6ω3	3.7	3.6	4.8	2.3

Fatty acid content (mean % total lipid)

T = trace quantities; - indicates none detected

TABLE 3. Comparison of fatty acid composition of tissues of caimans and salt-water crocodiles fed on fish diets.

cant differences between the levels of 22:5 and 22:6 fatty acids, suggesting that there is no systematic difference in PUFA requirement or uptake between the species. Only in the C18 fatty acids are there differences, with the caimans demonstrating greater incorporation.

DISCUSSION

The appetite of caimans (ca. 8% body wt. satiation ration) is similar to that of salt-water crocodiles (10% body weight satiation ration), but the mean TGCT of 136h is appreciably longer than that of *Crocodylus porosus* (97 h) and the GET is more than twice as long (97 h vs. 40 h). Taken together these observations indicate that young caimans eat considerably less food than young salt-water crocodiles of similar size. Assimilation rates for protein, energy and dry mass were all significantly lower in caimans (91.8, 68.2, 62.0% respectively) than in juvenile salt-water crocodiles (97.4, 85.2, 77.5% respectively), so a picture emerges of caimans eating less, taking longer to digest food and being less efficient in doing so. Generally speaking it would seem that salt-water crocodiles are faster moving, more active animals which must have a high metabolic rate and initial growth rate since they reach a much larger final size than the caimans; selection pressure in favour of fast and efficient food processing has presumably been greater in *Crocodylus porosus*. Perhaps the use of gastroliths by salt-water crocodiles is also related to the relative efficiency of digestion: Davenport *et al.*, (1990) found that salt-water crocodiles would deliberately eat small stones, and suggested that this would help in the breakup of insect and crustacean prey organisms. However, the caimans studied here showed little tendency towards this habit and would not eat stones, though small amounts of wood and paint flakes from their holding tanks were found in the stomachs of caimans post mortem (much less than in *Crocodylus porosus* held in the same tanks). This finding is consistent with the observations of Skoczylas (1976) and Schaler & Crawshaw (1982) who found no stones or other hard material in the stomachs of wild *Caiman crocodilus*.

The fatty acid data show that caimans, like salt-water crocodiles, are capable of assimilating PUFAs from the diet and incorporating them into the tissues. Garnett (1985) stated that *Crocodylus porosus* needed PUFAs in its diet because it could not desaturate and elongate short chain saturated fatty acids, a situation similar to that of marine fish (Ackman *et al.*, 1968). It seems likely that spectacled caimans also have a requirement for 'marine' PUFAs, presumably satisfied by the crustacean component in their diet. The findings of Ferguson (1981) and Lance (1982) that alligators are not capable of absorbing marine PUFAs clearly do not apply to all members of the family Alligatoridae, and in any case merit further study since some young alligators eat considerable quantities of the marine/estuarine blue crab *Callinectes sapidus* (Chabreck, 1971) which must be rich in PUFAs. In both caimans and salt-water crocodiles, relatively small quantities of PUFAs are incorporated into tissues by comparison with the concentration in the flesh of marine fish, even though substantial quantities of PUFAs are assimilated across the gut wall. The excess PUFAs, assimilated but not incorporated into structural materials, are presumably used as an energy source, or as the precursors of more complex molecules.

ACKNOWLEDGEMENTS

REFERENCES

Ackman, R. G.. (1972). The analysis of fatty acids and related materials by gas-liqiud chromatography. In *Progress in the chemistry of fats and other lipids,* 165-285. Holan, R. T. (ed). Oxford: Pergamon Press.

Ackman, R. G., Tocker, C. S. & McCachlan, J. (1968). Marine phytoplankton fatty acids. *Journal of the Fisheries Research Board of Canada* **25**, 1603-1620.

Chabreck, R. H. (1971). The food and feeding habits of alligators from fresh and saline environments in Louisiana. *Proceedings of the Annual Conference of the Southeastern Game and Fish Commission* **20**, 102-110.

Davenport, J., Grove, D. J., Cannon, J., Ellis, T. R. & Stables, R. (1990). Food capture, appetite, digestion rate and efficiency in hatchling and juvenile *Crocodylus porosus. J. Zool.* **220**, 569-592.

Ferguson, M. W. J. (1981). The value of the American alligator (*Alligator mississippiensis*) as a model for research in craniofacial development. *Journal of Craniofacial, Genetic and Developmental Biology* **1**, 123-144.

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497-509.

Garnett, S. (1985). Fatty acid nutrition of the estuarine crocodile *Crocodylus porosus. Comp. Biochem. Physiol.* **81B**, 1033-1035.

Ivlev, V. S. (1935). Eine mikromethode zur bestimmung des kaloreinghalts von nahrstoffen. *Biochem. Z.* **275**, 49-55.

Lance, V. (1982). Trace elements, Vitamin E, and Glutathione peroxidase in the American alligator, *Alligator mississippienesis. Proceedings of the Annual Alligator production Conference* **2**, 17-30.

McGinnis, A. L. & Kasting, R. (1964). Comparison of gravimetric and chromic oxide methods for measuring percentage utilization and consumption of food by phytophagus insects. *J. Insect Physiol.* **10**, 989-995.

Maynard, L. A. and Loosli, K. J. (1969). *Animal nutrition.* 6th Edition. New York: McGraw Hill Book Co.

Schaler G. B. & Crawshaw, P. G. (1982). Fishing behaviour of the Paraguayan caiman (*Caiman crocodilus*). *Copeia* **1982**, 66-72.

Skoczylas, R. (1978). Physiology of the digestive tract. In *Biology of the Reptilia: Physiology B*, pages 589-717. Gans, C. (ed). London and New York: Academic Press.

Taplin, L. E. and Grigg, G. C. (1981). Salt glands in the tongue of the estuarine crocodile *Crocodylus porosus. Science* **212**, 1045-1047.

Taplin, L. E., Grigg, G. C., Harlow, P., Ellis, T. M. & Dunson, W. A. (1982). Lingual salt glands in *Crocodylus acutus* and *C. johnstoni* and their absence from *Alligator mississippiensis* and *Caiman crocodilus. J. Comp. Physiol.* **B 149**, 43-47.