Netherlands Journal of Agricultural Science 38 (1990) 67-76

Influence of feeding level on the population of C-terminal-gastrin-immunoreactive cells in the digestive tract of young *Clarias gariepinus* (Burchell 1822)

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Received 22 August 1989; accepted 13 November 1989

Abstract

C-terminal-gastrin-immunoreactive cells, probably representing cholecystokinin (CCK)cells, were predominantly found in the first flexure of the anterior part of the intestine of young African catfish (*Clarias gariepinus*). Gastrin-containing cells could not be demonstrated in the stomach using the same technique and antibodies, suggesting the presence of different C-terminus of gastrin in this species compared with higher vertebrates. The number of CCK-like cells did hardly change with time in fish receiving a low feeding level. However, in fish receiving a high feeding level the number of CCK-cells increased from the sixth week on and was significantly higher than the number in fish with the low feeding level at the age of 8 weeks. The significance of this result is discussed.

Keywords: digestive tract, C-t-gastrin-immunoreactive cell, immunohistochemistry, hormones, feeding level, *Clarias gariepinus*

Introduction

Although the digestive tract of *Clarias gariepinus* is well described by Stroband & Kroon (1981), no data are available on the gut endocrine system of this species. This is in contract to what is known of this system in other fish species. Recently, immunocytochemical studies, using antisera against mammalian hormones, demonstrated the presence of several mammalian hormones in the digestive tract of fish which have no stomach (Rombout et al., 1986). With the same technique most mammalian hormones were detected in the digestive tract of fish which do have a stomach (Abad et al., 1987). Gastrin and cholecystokinin(CCK)-like hormones, demonstrated with antisera against C-terminal (C-t) gastrin, appeared to be present

in all fish species investigated. Hormones are very important for the release of acid, enzymes and bile into the digestive system of mammals (Modlin et al., 1981) and fish (van Noorden et al., 1980) and for the control of gut motility in fish (Jonsson et al., 1987).

The African catfish is able to digest infrequent and irregular meals effectively. This is caused by a relatively rapid secretion of digestive enzymes after feeding (Uys et al., 1987). The question rose whether there is a relationship between the number of hormoneproducing cells in the digestive tract, the quantity of digestive enzymes and feeding level to optimize the digestive capacity. The answer could be used for the development of an improved feeding regime in the culture of the African catfish to obtain an improved growth. Therefore, a study into this relationship was initiated. As a part of it the presence, number and the localization of C-t-gastrin-immunoreactive cells in the digestive tract of *C.gariepinus* at different feeding levels have been investigated.

Material and methods

Fish

Larvae of *C.gariepinus* were obtained by artificial reproduction (Hogendoorn & Vismans, 1980) and fed nauplii of *Artemia* during the first 9 days after hatching. Ultimately a commercial trout feed was given. Seven days after hatching, the larvae were divided at random over two aquaria, each aquarium containing 300 fish. From day 10 after hatching onwards, a commercial trout feed was given. On day 14 after hatching, each aquarium was allotted to a high or a low feeding level. Mortality was recorded daily.

Feed and feeding levels

Direct after hatching of *C.gariepinus* Instar I nauplii of *Artemia* were fed until satiation. From day 10 after hatching, Scharflinger conveyor belt feeders supplied food continuously between 09.00 and 24.00 h. Different crumb sizes were used for different sizes of fish. The food composition as given by the manufacturer is listed in Table 1. Food rations from day 14 were 9 and 27 % of fresh body weight per day at the low and high feeding level, respectively. The low level is comparable to the level defined by Hogendoorn et al. (1983) as physiological optimal, providing for the minimal feed conversion. The high feeding level is considered to be ad libitum feeding and may therefore be defined as the level giving maximum growth.

Experimental conditions

The two 70 l aquaria in which the fish were kept were both part of the same recirculation system with a total volume of 2380 l. Ammonia and nitrite values were kept below 0.5 mg l^{-1} and nitrate below 100 mg l^{-1}. Water temperature was 25 °C. Dissolved oxygen ranged from 83 to 98 % saturation. The pH fluctuated between 6.50 and 7.65.

Table 1. Crumb sizes used for different weight ranges of fish, and feed composition.

Weight range (g)	Mean crumb size (mm)
0.05-0.5	0.5
0.5 -1.0	1.2
1.0 -final weight	1.7
Composition of the commercial diet used	
Dry matter (%)	90.5
Crude protein (%)	51.0
Crude fat (%)	9.0
Ash (%)	10.5
Fibre (%)	1.5
Carbohydrates (%)	18.5
Vitamin A (IE kg^{-1})	20.000
Vitamin D_3 (IE kg^{-1})	2.000
Vitamin E (mg kg^{-1})	50
Vitamin C (mg kg^{-1})	1.000

Experimental procedure

The experiment lasted 8 weeks. Once a week, 10 randomly chosen fish were sampled and weighed to determine the average growth curve of the fish in relation to the respective feeding levels. Four fish out of the samples (on day 21, 28, 35, 42, 49 and 56) were used for immunohistochemistry.

Immunohistochemistry

Sampled fish were killed by decapitation and fixed in toto in Bouin's fluid (Romeis, 1968). From fish weighing more than 0.5 g the gastro-intestinal tract was dissected and embedded, while smaller fish were embedded in toto in Paraplast Plus (Sherwood). Tissue was serially sectioned at 5 μm and mounted on poly-L-lysine (MW: 350 000; Sigma)-coated slides. After dewaxing and rehydratation, sections were rinsed several times in 0.01 M phosphate-buffered 0.15 M saline (PBS; pH 7.2). The peroxidase-anti-peroxidase (PAP) method (Sternberger,1979) was used to demonstrate gastrin and CCK-like peptides in gut endocrine cells. In the first step, rabbit serum (1 : 3000; provided by Prof. Dr Bosman, Leiden/Maastricht, Netherlands) was used; it was raised against synthetic human gastrin and was demonstrated to react specifically with the C-terminal part of the gastrin and CCK (Rombout et al., 1986; Abad et al., 1987). A swine-anti-rabbit serum (1:50; Dakopatts, Denmark) and a rabbit PAP-complex (1:100; Dakopatts) was used in the second and third steps, respectively. All necessary controls (Grube, 1980; Rombout et al., 1986; Scopsi et al., 1986) were carried out to avoid non-specific binding of antibodies to anionic or cationic constituents of endocrine cells. Quantative evaluations were made on

cross sections of 4 specimens per sample date and feeding level. C-t-gastrin-immunoreactive cells were counted in the anterior part of the gut, just behind the stomach, where most of these cells are located. The number of immunoreactive cells was calculated per 1000 epithelial cells using a quadrangle of 0.5 × 0.5 mm with 100 squares of 0.05 × 0.05 mm at a magnification of 200×. All immunoreactive cells in the quadrangle were counted, whereas epithelial cells were calculated based on the counting of 10 squares on the diagonal. This procedure was repeated 2-5 times, depending on the fish size.

Statistics

Differences in growth and number of C-t-gastrin-immunoreactive cells between feeding levels on the same dates were analysed using the Student's *T* test. The relationship between the number of C-t-gastrin-immunoreactive cells and growth and the feeding level were analysed using Anova (Snedecor & Cochran,1976).

Results

Both feeding levels declined during the experimental period as illustrated in Figure 1. The mean growth of this fish is given in Figure 2. The high feeding level fish (HFL) grow faster than the low feeding level fish (LFL) from day 35 onwards ($P<0.01$). Between day 42 and day 49 a peak in the mortality of 15 % occurs in the HFL fish, while the mortality of the LFL was 1 % during the same period. This mortality was caused by the ruptured intestine syndrome (RIS) (Boon et al., 1987).

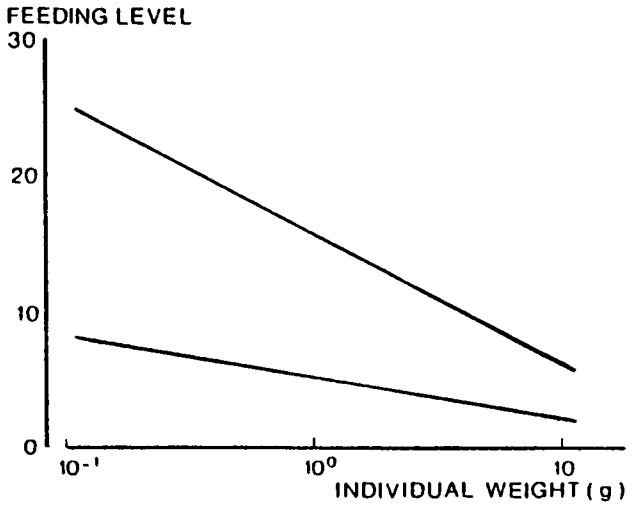

Fig. 1. *Clarias gariepinus*. Relationship between feeding level (% of fresh body weight per day) and mean fresh body weight (g).

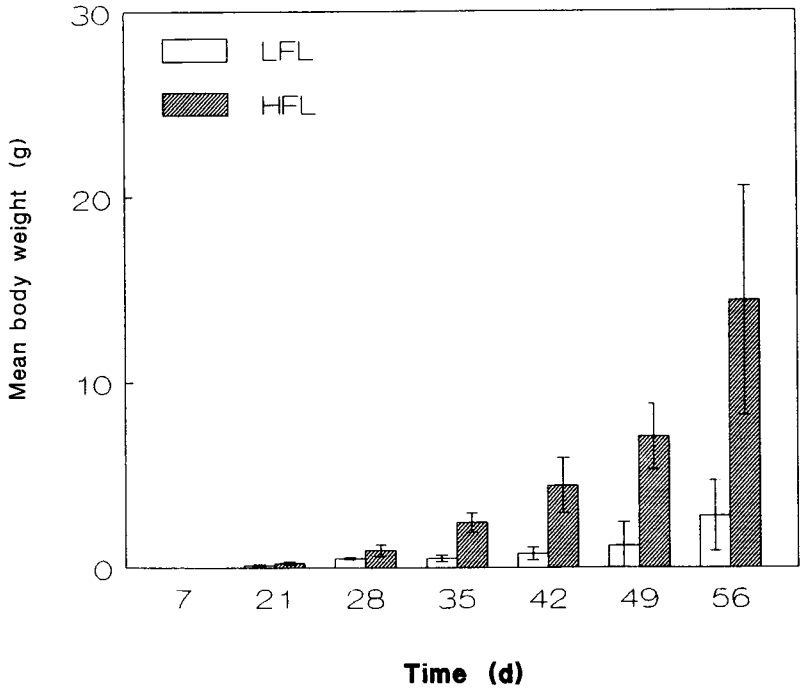

Fig. 2. *Clarias gariepinus*. Mean fresh body weight (+/− SD) (n = 10) per feeding level versus time. LFL = low feeding level, HFL = high feeding level.

Many C-t-gastrin-immunoreactive cells were found in the anterior part of the intestine and some were present in the middle part of the gut, but they were not found in the stomach of *C.gariepinus* (Fig.3a-c). Most of the immunoreactive cells appeared to be present in the first flexure of the gut and seemed to be of the open type, i.e. having a secretory granule containing base and a long narrow extension towards the intestinal lumen (Fig.4). The number of C-t-gastrin-immunoreactive cells per 1000 epithelial cells related to age and feeding level is shown in Figure 5. The difference in cell numbers between the feeding levels is significant on day 56 ($P<0.05$).

Discussion

The growth of the fish of both feeding levels was comparable to those given by Hogendoorn et al. (1983). This is an indication that the digestive activity of the digestive tract was normal. C-t-gastrin-immunoreactive cells were predominantly found in the anterior part of the intestine of *Clarias gariepinus*, whereas they were absent in the stomach. A similar distribution pattern was found in *Sparus auratus* by Abad et al. (1987), using different antisera (Langer et al., 1979; Holmgren et al., 1982). On the other hand, several authors described the presence of gastrin-immunoreactive cells in the stomach of some teleost species (Larsson & Reh-

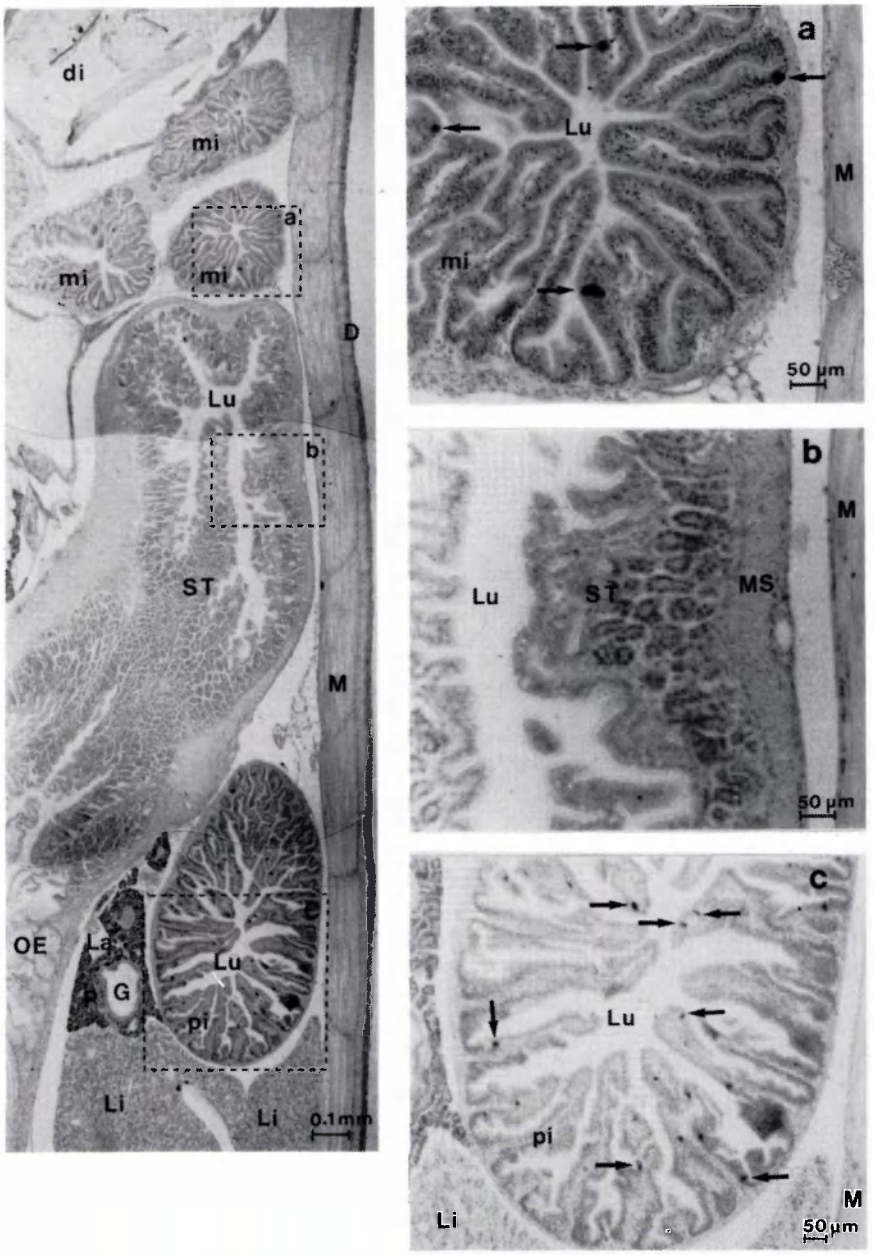

Fig. 3. An overview of a sagittal section of the digestive tract of young *Clarias gariepinus*. C-t-gastrin-immunoreactive cells can be distinguished in the anterior (a) and middle part (c) of the intestine but not in the stomach (b). OE = oesophagus, P = pancreas, G = gall bladder, Li = liver, ST = stomach, M = muscle, D = dermis, pi = anterior intestine, mi = middle intestine, di = posterior intestine, La = islet of Langerhans, Lu = lumen, MS = muscular layer of the stomach. Black arrows: C-t-gastrin-immunoreactive cells. Magnification: overview 40×; selected parts of the gut and stomach 120×.

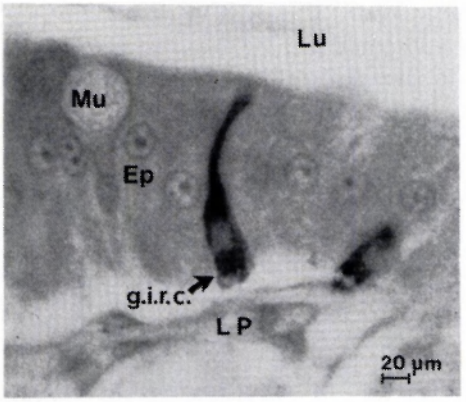

Fig. 4. *Clarias gariepinus*. C-t-gastrin-immunoreactive cell in the epithelium of the anterior part of the gut. Ep = epithelial cell, g.i.r.c. = C-t-gastrin-immunoreactive cell, LP = lamina propia, Lu = intestinal lumen, Mu = mucus cell. Magnification 1000×.

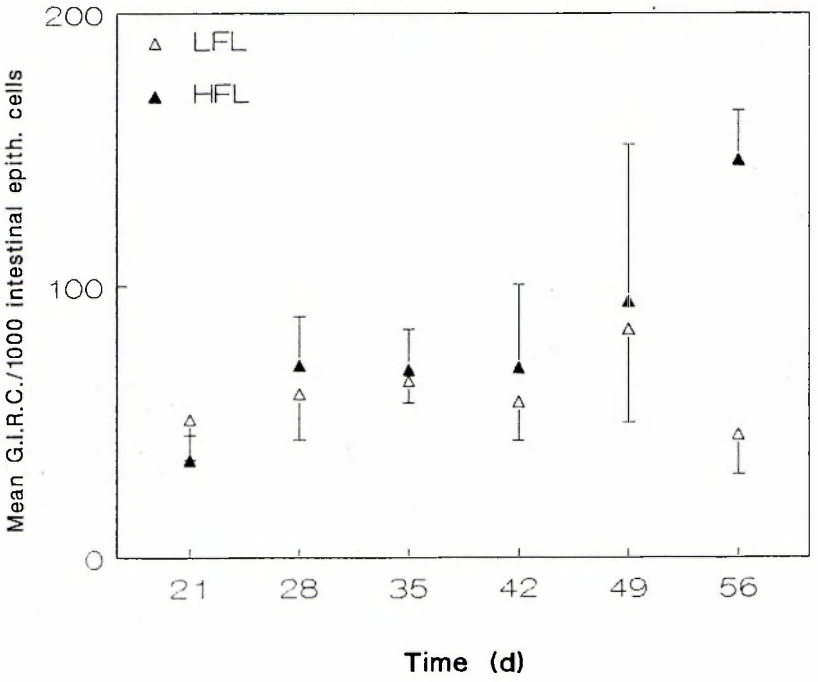

Fig. 5. *Clarias gariepinus*. Relationships between the number of C-t-gastrin-immunoreactive cells (GIRC) per 1000 intestinal epithelial cells and time at two different feeding levels. HFL = high feeding level, LFL = low feeding level.

feld,1977; Noaillac-Depeyre & Hollande,1981; Reifel et al., 1983; Jonsson et al., 1987). In *S.auratus* gastrin-immunoreactive cells could also be demonstrated in the stomach, using another anti-gastrin serum (Elbal & Agulleiro,1986). From these data it may be concluded that gastrin-producing cells present in the stomach of some teleosts do not contain exactly the same C-terminus as found in mammalian gastrin and cholecystokinin (CCK). Probably, the immunoreactive cells described in this study contain a CCK-like peptide. The predominant localization of the C-t-gastrin-reactive cells in the first flexure of the gut, is also described for the anterior part of the gut of *Barbus conchonius* (Rombout & Taverne-Thiele, 1982). The presence of a CCK-like rather than a gastrin-like hormone is proposed to be more likely in the gut of this stomachless fish.

The present study also describes a significant change in the number of these CCK-like cells during the development of young fish under different feeding conditions. In the low feeding level fish (LFL) the number of CCK-like cells is hardly changed. In the high feeding level fish (HFL) the number of CCK-like cells showed an increase after sixth weeks. In spite of the low number of fish per sample per group ($n=4$) the influence of the feeding level on the number of C-t-gastrin-immunoreactive cells was significant at the age of 8 weeks. In the present study it is still unknown whether the high number of CCK-like cells will be related to a higher amount of secreted CCK into the blood stream and hence a stronger release of digestive enzymes and bile into the gut. However, in starved and refed rats significant changes in the epithelium of the gastric crypts and plasma gastrin levels have been reported (Goodland et al., 1983).

Whether the increase in number of the hormone-producing cells in the digestive tract is an adaptation to a high feed intake, resulting into a better digestion and hence a higher body weight gain, remains to be investigated. In some gastro-enteric diseases the CCK level in the blood is lower than normal despite the increase in the number of CCK-like cells in the gut. This may be caused by an insufficient hormone release by these cells (Sundler et al., 1983). Consequently, higher numbers of detectable CCK cells are not always correlated to an increased digestive capacity and growth.

The increase of C-terminal-gastrin-immunoreactive cells may contribute to the explanation of the health problems in the HFL, which apparently results in an increased mortality between day 42 and 49. In African catfish the high level of C-terminal-gastrin-immunoreactive cells may be related to a high level of digestive hormones. This is supported by the relationship between a high specific growth rate of African catfish over the weight range 0.5-1.0 g and the incidence of the ruptured intestine syndrome (RIS) (Boon et al., 1987). It is suggested that a high plasma level of digestive hormones in some specimen is followed by an autodigestion of the gut mucosa inducing the first phase of the RIS. Therefore, LFL and HFL fish should be tested for the level of CCK, according to the method of Sundler et al. (1983). This could give more insight in the problems concerning the RIS of African catfish.

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