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SHORT-CHAIN FATTY ACID TRANSPORT IN THE INTESTINE OF A HERBIVOROUS TELEOST

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SUMMARY

Short-chain fatty acid (SCFA) concentrations of intestinal contents from the herbivorous tilapia, *Oreochromis mossambicus*, were analysed by gas and high-performance liquid chromatography. Concentrations of acetate ranging from 3 to 18 mmol l^{-1} were found along the full length of the intestinal tract, as were trace amounts of propionate. Characteristics of [^{3}H]acetate transport by the intestinal brush-border membrane were analysed in isolated brush-border membrane vesicles (BBMV). Influx of [^{3}H]acetate into BBMV occurred by apparent anion exchange and was not enhanced by inwardly directed gradients of sodium or potassium, indicating the lack of sodium-dependent coupling for acetate transport. Competition and stimulation studies with various organic and inorganic anions revealed the likelihood of a transport system in which [^{3}H]acetate is exchanged specifically for intracellular bicarbonate or other SCFA. Other anions tested had only limited or negligible effects on [^{3}H]acetate uptake. Kinetic analysis over a concentration range of external acetate from 0·1 to 35 mmol l^{-1} yielded a carrier transport K_m of 6·4 mmol l^{-1} and a J_{max} of 5·3 nmol mg protein^{-1} 10 s^{-1}. Transport of [^{3}H]acetate was not significantly affected by inhibitory drugs specific for anion exchangers. This study suggests that there is a specific antiport system for anions that is shared by bicarbonate and short-chain volatile fatty acids in the intestinal brush-border membrane of this herbivorous teleost.

INTRODUCTION

Short-chain fatty acids (SCFA), such as acetic, propionic and butyric acids, are major end-products of dietary fibre fermentation. In herbivores and omnivores the primary sources of catabolism are plant polysaccharides, including celluloses, hemicelluloses and pectins. Other sources include starches, amino acids and mucus, up to 80% of which may consist of polysaccharides (Engelhardt & Rechkemmer, 1983). SCFA constitute the major fraction of anions in the caecum and colon of a wide range of vertebrates. Concentrations of total SCFA from 50 to 200 mmol l^{-1}

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have been reported in the intestinal lumen, and in most cases these concentrations remain at constant high levels throughout the bowel length (Engelhardt & Rechkemmer, 1985). There have been few studies on SCFA uptake and assimilation by animal digestive systems, in spite of the proposed nutritive importance and wide-ranging occurrence of this substrate class among both vertebrates and invertebrates. Short-chain fatty acid production may account for as little as 7 % of the maintenance energy requirements in mammals, such as the dog, with simple large intestines and low dietary fibre intake. In herbivorous mammals, such as ruminants, with voluminous large intestines, SCFA production accounts for as much as 80 % of maintenance energy requirements (Engelhardt & Rechkemmer, 1985).

SCFA production and transport have a pronounced effect on water and electrolyte absorption in non-mammalian as well as mammalian systems. Physiological levels of acetate increase water and sodium transport significantly in the lower intestine of the chicken (Rice & Skadhauge, 1982). The presence of SCFA in the hindguts of rat, domestic pig, goat and man has been shown to expedite sodium and water absorption as well as net bicarbonate accumulation (Umesaki, Yajima, Yokohura & Mutai, 1979; Argenzio & Whipp, 1979; Argenzio, Miller & Engelhardt, 1975; Ruppin *et al.* 1980). It has been estimated that 95–99 % of SCFA in the hindgut is absorbed at rates which exceed those of other solutes present in the colonic contents (Engelhardt & Rechkemmer, 1985). It is generally held that SCFA transport in mammalian systems is a non-mediated process the rate of which increases linearly with an increase in concentration. No evidence has been reported for a saturable intestinal SCFA transport mechanism at physiological luminal concentrations (Engelhardt & Rechkemmer, 1983, 1985).

Several invertebrates, including a lumbricid earthworm, a freshwater oligochaete and a cestode tapeworm, have been demonstrated to transport SCFA across the integument (Richards & Arme, 1980; Hipp, Mustafa, Bickel & Hoffman, 1986; Arme & Read, 1968). Mediated transport at low substrate concentrations of SCFA, from 0·01 to 2·0 mmol l^{-1}, has been reported for these organisms, with diffusion occurring at higher concentrations. In addition, full or partial inhibition of uptake of individual SCFA by a complement of other SCFA has been demonstrated, suggesting the possibility of a common carrier for SCFA of various sizes. Transport of SCFA across the hindgut has been reported for such cellulose-ingesting insects as the cockroach, termite and locust (Hogun, Slaytor & O'Brien, 1985; Baumeister, Meredith, Julien & Phillips, 1981). As with the worms, carrier transport was reported at low concentrations of SCFA substrate, whereas diffusional transport occurred at higher concentrations. The presence of acetyl-CoA synthetase and acetylthiokinase has been demonstrated in insect gut wall tissues, suggesting that there are mechanisms whereby SCFA produced by cellulose fermentation could be assimilated for energy purposes.

Currently, there is only limited information on lipid transport mechanisms in fish intestine. The present investigation is an examination of the transport mechanisms for [^{3}H]acetate in intestinal brush-border membrane vesicles of the herbivorous euryhaline teleost, *Oreochromis mossambicus*. Results suggest that in this membrane

there is a specific anion antiport process that is shared by luminal SCFA and enterocyte bicarbonate.

MATERIALS AND METHODS

Preparation of brush-border membrane vesicles

Adult tilapia, *Oreochromis mossambicus*, were collected with hand nets from the local marine environments on Oahu, HA. They were kept for 12–24 h in the laboratory in clean aerated sea water to evacuate gut contents. Brush-border membrane vesicles from fish intestine were prepared using the method developed by Kessler *et al.* (1978) as described in Reshkin & Ahearn (1987). Animals were killed by a blow to the head. The upper intestine, defined as the proximal half of the full intestinal tract, was carefully extracted, slit lengthwise and plunged into a solution of ice-cold 300 mmol l^{-1} mannitol, 60 mmol l^{-1} Hepes/Tris, pH 7·4. When all intestines had been extracted and weighed, they were transferred to a solution of 60 mmol l^{-1} mannitol, 12 mmol l^{-1} Hepes/Tris, 6 mmol l^{-1} EGTA, 0·1 mmol l^{-1} phenylmethylsulphonylfluoride (PMSF), pH 7·4. Magnesium chloride was added to this solution to a final concentration of 15 mmol l^{-1}. This mixture was homogenized with a Brinkman Polytron for 15 s and kept on ice for 15 min with occasional agitation. The foamy lipid layer was removed by suction from the surface of the mixture and discarded. The remaining homogenate was centrifuged for 15 min at 3000 ***g*** in a Sorvall RC-5C high-speed centrifuge. The resulting pellet was discarded and the supernatant was centrifuged for 30 min at 27 000 ***g***. The pellet from the high-speed spin was resuspended in the same buffer as before, but without PMSF, with a Potter–Elvehjem homogenizer (10 strokes). The magnesium chloride precipitation, followed by low-speed and high-speed spins, was then repeated for the resuspended pellet. The final pellet following the high-speed centrifugation was resuspended in the appropriate buffer for the ensuing transport experiment, and centrifuged for 30 min at 27 000 ***g***. The resulting pellet was resuspended in the transport buffer at a protein concentration of approximately 13 mg ml^{-1} by 15 passages through a 22 gauge syringe needle. Protein concentrations were determined with the Bio-Rad protein assay. Protein standards were a mixture of human albumin (5 g dl^{-1}) and human globulin (3 g dl^{-1}).

Enzyme assays

The purity of brush-border membrane vesicles was assessed by comparing activities of protein marker enzymes from samples of the initial homogenate and the resuspended final pellet. These comparisons showed final pellet enrichments of alkaline phosphatase and leucine aminopeptidase, enzymes associated with the brush border, of 18- and 9-fold, respectively (Reshkin & Ahearn, 1987). Further, enzyme assays for Na^+,K^+-ATPase and cytochrome *c* oxidase, enzymes associated with the basolateral membrane and the mitochondria, respectively, showed enrichments of only 0·8 and 0·2, respectively. These results suggest a relatively pure brush-border preparation with minimum contamination by other cellular constituents. All enzyme

assays were determined spectrophotometrically. The activity of alkaline phosphatase was measured with Sigma Chemical assay kit no. 104, Na^+,K^+-stimulated ATPase activity was measured by the coupled assay reaction of Berner, Kinne & Murer (1976), and cytochrome *c* oxidase activity was measured by the procedures of Cooperstein & Lazerow (1951).

Transport measurements

Measurement of [^{3}H]acetate transport by brush-border membrane vesicles was conducted at room temperature (22°C) using the Millipore filtration technique of Hopfer, Nelson, Perrotto & Isselbacher (1973). Prepared membrane vesicles were stored on ice for not longer than 3 h prior to incubation. Short uptake incubation periods were made possible by a rapid uptake apparatus (Innovativ Labor, AG; Adliswil, Switzerland). Samples of vesicle suspension (5 μl) were automatically combined with 10 or 20 volumes of radiolabelled incubation medium to initiate uptake. At the termination of the incubation interval, a 2 ml sample of ice-cold stop solution was automatically injected to stop [^{3}H]acetate uptake. The vesicle suspension was immediately filtered through a Millipore filter (0·65 μm) and washed with an additional 8 ml of cold stop solution. Vesicle and incubation medium compositions varied according to the nature of the experiment and are indicated in the figure legends. Stop solutions were similar to the incubation media, minus the concentration of labelled or unlabelled acetate. Filters were placed in 5 ml of Beckman Ready Solv scintillation cocktail and counted in a Beckman LS-8100 liquid scintillation spectrometer. Each experiment was run at least in duplicate and all data points were expressed as means ± standard error of 3–5 replicates.

Volatile fatty acid analysis

Quantitative analysis of acetic acid concentrations in intestinal segments of freshly collected fish were undertaken utilizing gas chromatography (GC) and high-performance liquid chromatography (HPLC).

For GC analyses, fish were killed by carbon dioxide asphyxiation. Intestinal tracts were removed intact and ligated into equal proximal and distal sections. Contents from the corresponding sections of several fish ($N = 5$) were pooled and extracted by the metaphosphoric acid precipitation method (Supelco Inc., 1975). Following centrifugation for 30 min at 480 ***g***, the supernatant was syringe-filtered through a 0·2 μm Millipore filter and samples (10 μl) were then injected directly into a Hewlett Packard 5890 gas chromatograph equipped with flame ionization detection. The column (2 m × 4 mm i.d., glass) was prepacked with Chromosorb 101 (Supelco Inc.) and nitrogen was used as the carrier gas.

For HPLC analyses, intestinal contents were collected and pooled as above, centrifuged and filtered through a 0·2 μm Millipore filter. Samples (20 μl) of the resulting supernatant were injected directly onto a BioRad HP X 87H organic acid analysis column and separated isocratically using 0·005 mol l^{-1} H_2SO_4 at a flow rate of 0·5 ml min^{-1}. Peaks were analysed through a BioRad variable wavelength ultraviolet detector and quantified using a Hewlett Packard 3392A integrator.

Materials

[^{3}H]acetate was obtained from New England Nuclear Corporation (Boston, MA). Propionic acid, L-lactic acid and pyruvic acid were obtained from United States Biochemical Corporation (Cleveland, OH). Butyric acid was obtained from MCB Chemical (Los Angeles, CA). All other reagent grade chemicals and the ionophores, valinomycin and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), were obtained from Sigma Chemical (St Louis, MO).

RESULTS

Free fatty acid analysis of luminal contents

Gas chromatographic analysis of short-chain free fatty acid concentrations in the lumen of tilapia intestine revealed an increase in acetate concentration along the intestinal tract and the occurrence of trace amounts of propionate in each segment (Table 1). Acetate concentration along the intestinal tract of tilapia was determined by HPLC to have similar, although slightly higher, concentrations (Table 1). In addition, the middle and lower thirds also contained trace amounts of propionate. These results suggest the possible occurrence of fermentative processes in the gut of this herbivore.

Cation-independence of [^{3}H]acetate uptake

To examine the effect of an inward sodium gradient on the uptake of 0·1 mmol l^{-1} [^{3}H]acetate, vesicles loaded with either potassium gluconate or potassium acetate were exposed to inward 100 mmol l^{-1} gradients of Na^+ or K^+. Fig. 1 shows little difference between [^{3}H]acetate uptake in Na^+ and K^+ media by vesicles preloaded with potassium gluconate, suggesting the probable lack of coupling between the

Table 1. *Volatile fatty acid analysis of intestinal contents from pooled samplings (N = 5) of seawater-adapted tilapia*

Intestinal segment	Acetate concentration (mmol l^{-1})	Propionate presence
Gas chromatography (two replicates)		
Upper	2·66 ± 0·32	trace
Middle	6·20 ± 0·41	trace
Lower	11·66 ± 1·21	trace
High-performance liquid chromatography (three replicates)		
Upper	14·63 ± 1·34	
Middle	17·25 ± 1·51	trace
Lower	18·19 ± 1·61	trace

Samples were prepared using a metaphosphoric acid precipitation technique followed by high-speed centrifugation. Samples were syringe-filtered through 0·20 μm Millipore filters.

Results are presented as the mean of the replicates ± standard error.

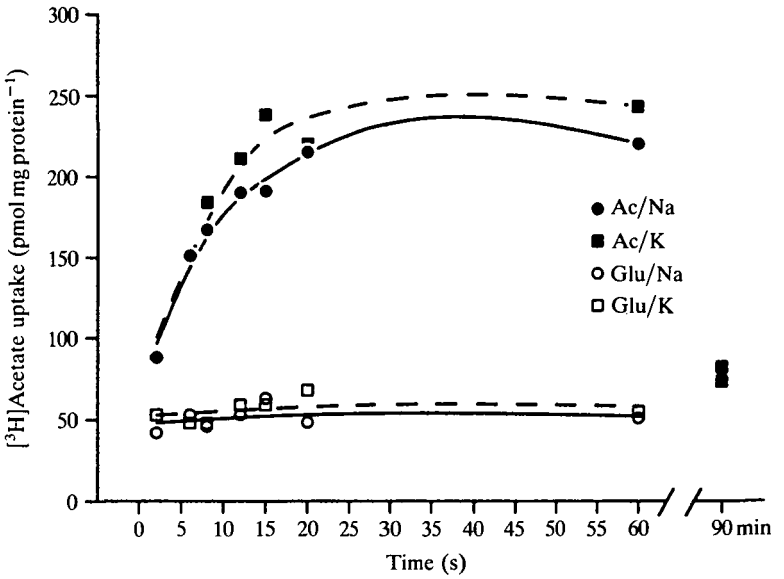

Fig. 1. Time-course determinations of Na^+-independent uptake (open symbols) and anion exchange (closed symbols) of [^{3}H]acetate in BBMV of tilapia anterior intestine. Symbols show media compositions (internal/external). Internal vesicle media consisted of (in mmol l^{-1}): Ac, tetramethylammonium (TMA) gluconate, 100; potassium acetate, 100; Glu, TMA gluconate, 100; potassium gluconate, 100. External media consisted of (in mmol l^{-1}): Na, potassium gluconate, 100; sodium gluconate, 100; K, potassium gluconate, 200. All solutions contained 12 mmol l^{-1} Hepes/Tris, pH 7·5. Lines were fit by an iterative curve-fitting computer program. Results are shown as the mean of three replicates. Error bars (2–10 %) have been omitted for clarity.

entry of acetate and Na^+ in these vesicles. Similar equilibrium uptake values occurred in the two ion treatments, indicating that apparent vesicle volume was not significantly affected by varying the ionic composition of the media. When vesicles were preloaded with potassium acetate, the uptake of [^{3}H]acetate was significantly enhanced in both Na^+ and K^+ media compared with that illustrated in either of these media by potassium-gluconate-loaded vesicles. However, little difference between Na^+ and K^+ treatments was observed in these potassium-acetate-loaded vesicles. These results suggest that although the transfer of [^{3}H]acetate across tilapia BBMV is apparently Na^+-independent, its rate is enhanced by exchange with internal anions.

Effects of preloaded anions on [^{3}H]acetate uptake

Our results suggest that [^{3}H]acetate transport by tilapia BBMV might occur by exchange with internal anions, and that in the absence of preloaded substrates the uptake of this short-chain fatty acid was markedly reduced (Fig. 1). To further characterize this apparent anion antiport process, 0·1 mmol l^{-1} [^{3}H]acetate uptake was measured in vesicles that were preloaded with several different anions

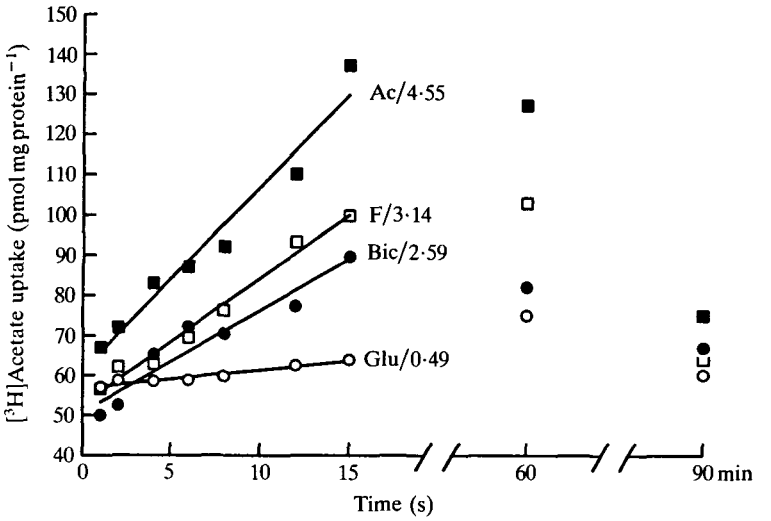

Fig. 2. Time course of 0·1 mmol l^{-1} [^{3}H]acetate uptake by upper intestinal brush-border membrane vesicles of tilapia preloaded with 100 mmol l^{-1} acetate (Ac), formate (F), bicarbonate (Bic) or gluconate (Glu); all sodium salts. In addition the vesicles contained 12 mmol l^{-1} Hepes/Tris (pH 7·5) and 20 μmol l^{-1} CCCP. Incubation media contained 100 mmol l^{-1} sodium gluconate, 0·1 mmol l^{-1} [^{3}H]acetate, 12 mmol l^{-1} Hepes/Tris (pH 7·5) and 20 μmol l^{-1} CCCP. Numbers indicate the slope value (pmol mg protein^{-1} s^{-1}) for each uptake curve from 1 to 15 s. Symbols represent the mean of three replicates. Error bars have been deleted for clarity.

(100 mmol l^{-1} acetate, formate, gluconate or HCO_3^-). Fig. 2 shows that [^{3}H]acetate uptake in such vesicles was a linear function of time from 1 to 15 s of incubation. The slopes of these uptake curves therefore represent a good approximation of acetate influx under each experimental condition. In addition, all equilibrium uptake values were similar, suggesting that the different treatments did not affect the apparent volume properties of the vesicles. The initial rate of [^{3}H]acetate uptake (influx) in vesicles preloaded with gluconate was not significantly different from zero (slope = 0·49 ± 0·28 pmol mg protein^{-1} s^{-1}; $P = 0·10$), indicating that over the first 15 s of incubation, little labelled acetate was accumulated by these vesicles apart from that binding to the vesicle membranes. Significant ($P < 0·05$) trans-stimulation of [^{3}H]acetate uptake occurred in vesicles preloaded with HCO_3^- (slope = 2·59 ± 0·31 pmol mg protein^{-1} s^{-1}), formate (slope = 3·14 ± 0·39 pmol mg protein^{-1} s^{-1}) and acetate (slope = 4·55 ± 0·49 pmol mg protein^{-1} s^{-1}). Vesicles preloaded with HCO_3^-, formate and acetate showed [^{3}H]acetate uptake values at 15 s of incubation that transiently exceeded their respective equilibrium values by amounts that ranged between 80% (HCO_3^-) and 180% (acetate), suggesting that anion exchange by this mechanism can be concentrative. All exchange experiments were conducted under short-circuited conditions (in the presence of CCCP), indicating that enhanced [^{3}H]acetate uptake by trans-stimulation was not a result of electrical coupling *via* diffusion potentials.

Effects of anion specificity on [^{3}H]acetate exchange

The results shown in Fig. 2 suggested that although [^{3}H]acetate transport across BBMV of tilapia intestine was generally enhanced by anion exchange, the magnitude of acetate uptake depended upon the species of exchangeable anion. To investigate the specificity of the anion exchange mechanism, we performed a series of cis-inhibition and trans-stimulation experiments (Fig. 3). The [^{3}H]acetate exposure interval was 10 s, based on the continued linearity of uptake at that point (Fig. 2). The gluconate-loaded treatment represented the unstimulated control. In this experiment, [^{3}H]acetate influx was significantly stimulated only by internal HCO_3^- and thiocyanate (SCN^-), suggesting an apparent specific affinity of the system for these anions.

Fig. 4 illustrates the results of 4·0 mmol l^{-1} [^{3}H]acetate uptake with inorganic anions present in the reaction media, representing potential cis-inhibitors. The acetate-inside treatment incubated in gluconate and the gluconate-inside, gluconate-outside treatment were the controls for [^{3}H]acetate uptake. The results show that

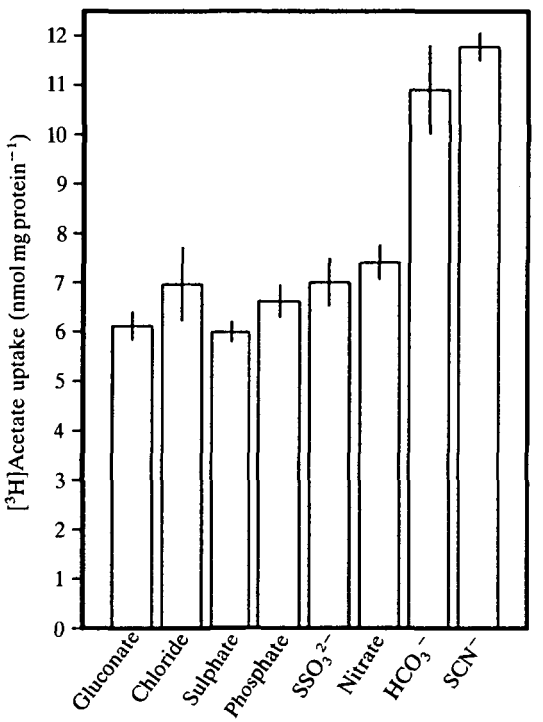

Fig. 3. Trans-stimulation of acetate uptake by various inorganic anions in BBMV of the anterior tilapia intestine. Vesicles were loaded with 80 mmol l^{-1} sodium gluconate and 25 mmol l^{-1} test anion. Incubation media contained 80 mmol l^{-1} sodium gluconate and 4·0 mmol l^{-1} [^{3}H]acetate. All solutions contained 12 mmol l^{-1} Hepes/Tris, pH 7·5 and the proton ionophore CCCP (20 μmol l^{-1}). The gluconate-loaded treatment (far left) represents the control for [^{3}H]acetate uptake. Acetate uptake is given as the mean of five 10-s uptake determinations. Error bars represent ± S.E. of the mean.

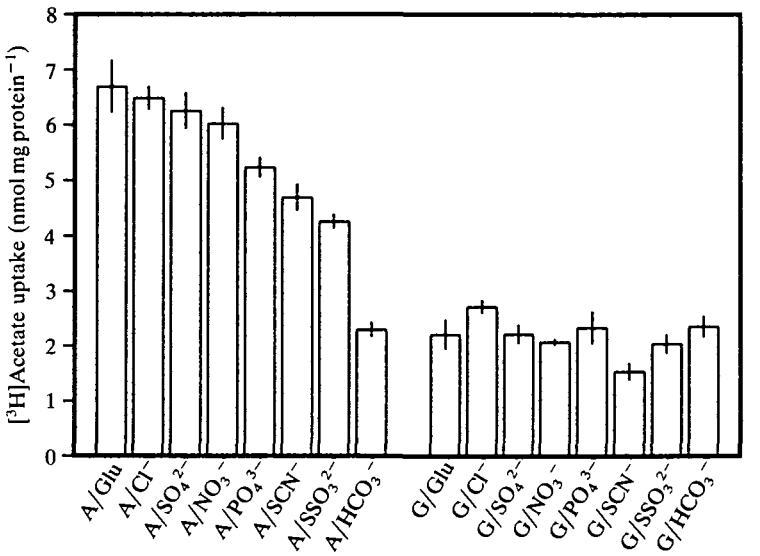

Fig. 4. Cis-inhibition of [^{3}H]acetate uptake by various inorganic anions in BBMV of tilapia anterior intestine. Vesicles were preloaded with 80 mmol l^{-1} sodium acetate (A) or 80 mmol l^{-1} sodium gluconate (G). Reaction media contained 50 mmol l^{-1} sodium gluconate, 25 mmol l^{-1} of the test anion and 4·0 mmol l^{-1} [^{3}H]acetate. All media contained 12 mmol l^{-1} Hepes/Tris, pH 7·5 and 20 μmol l^{-1} CCCP. All results are shown as the mean of five 10-s uptakes. Bars indicate ± S.E. of the mean.

HCO_3^- was the most effective inhibitor of [^{3}H]acetate uptake when vesicles were preloaded with unlabelled acetate, though most of the test anions investigated showed a limited cis-inhibitory capacity. These results, together with those shown in Fig. 3, suggest that there is an anion antiport mechanism in which acetate is preferentially exchanged with bicarbonate, but which also accepts a wide range of inorganic anions. There was no significant trend for [^{3}H]acetate uptake in vesicles preloaded with gluconate, indicating that in the absence of an internal exchangeable anion, [^{3}H]acetate influx was probably limited to diffusion.

Figs 5 and 6 illustrate the influence of organic anions on the trans-stimulation and cis-inhibition, respectively, of acetate transport. Several mono-, di- and tricarboxylates, at a concentration of 25 mmol l^{-1}, were tested for their interaction with 4·0 mmol l^{-1} [^{3}H]acetate uptake into BBMV. In the trans-stimulation experiment (Fig. 5), vesicles were preloaded with 80 mmol l^{-1} sodium gluconate and 25 mmol l^{-1} test anion. [^{3}H]Acetate uptake was markedly stimulated only into those vesicles preloaded with volatile fatty acids (VFA; acetate, propionate and butyrate) and HCO_3^-. Only partial stimulation was exhibited by other monocarboxylic acids, while di- and tricarboxylates showed very slight inhibition or no effect at all.

Results from the [^{3}H]acetate cis-inhibition experiment (Fig. 6, upper histogram) were the inverse of the trans-stimulation experiment. As in Fig. 5, vesicles were preloaded with 80 mmol l^{-1} sodium acetate and incubated in media containing

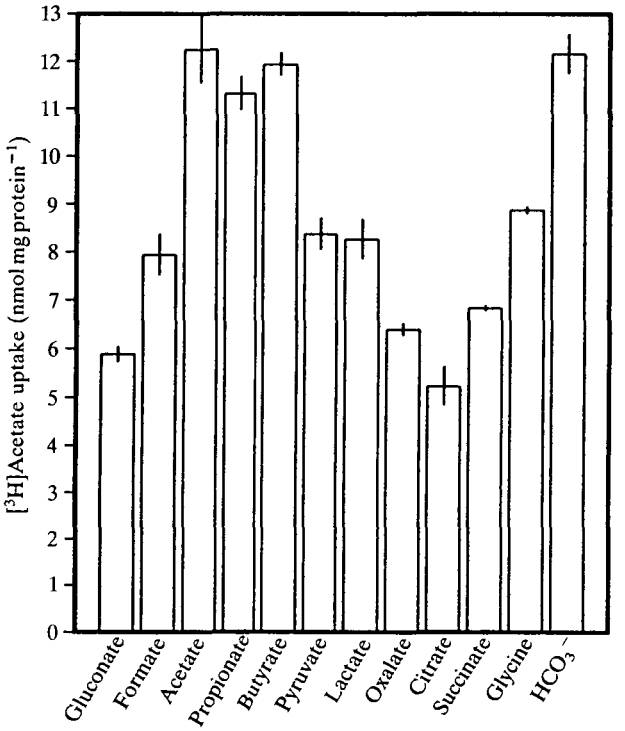

Fig. 5. Trans-stimulation of [^{3}H]acetate uptake by various organic anions. Vesicles were preloaded with 25 mmol l^{-1} of the test anion. Gluconate-loaded treatment (left) represents the control for [^{3}H]acetate uptake. Incubation media contained 1·25 mmol l^{-1} of the test anion (anion in:out = 20:1). All experimental details are identical to trans-stimulation of inorganic anions (Fig. 3).

50 mmol l^{-1} sodium gluconate, 25 mmol l^{-1} test anion and 4·0 mmol l^{-1} [^{3}H]acetate. [^{3}H]Acetate uptake was strongly cis-inhibited by the presence of VFA (acetate, propionate and butyrate) and bicarbonate. The other anions partially inhibited [^{3}H]acetate influx (4–28 %), indicating a low apparent affinity for these anions of the acetate transport system. As in Fig. 4, there was no significant trend in [^{3}H]acetate uptake when vesicles were preloaded with gluconate (Fig. 6, lower histogram). The results from Figs 5 and 6 strongly suggest that there is an anion exchange mechanism that is specific for bicarbonate and volatile fatty acids.

Fig. 6. Cis-inhibition of [^{3}H]acetate uptake by various organic anions in tilapia anterior intestine. Experimental details are identical to those for cis-inhibition by inorganic anions (Fig. 4). Upper figure shows results of 4·0 mmol l^{-1} [^{3}H]acetate uptake into acetate-loaded vesicles. A/Glu and G/Glu represent stimulated and non-stimulated controls, respectively. Lower figure shows inhibited [^{3}H]acetate uptake into gluconate-loaded vesicles, with G/Glu as control. Mannitol (Man) was added to the complement of organic test anions. Form, formate; Ac, acetate; Pro, proprionate; But, butyrate; Pyr, pyruvate; Lac, lactate; Ox, oxaloacetate; Suc, succinate; Cit, citrate; Gly, glycerate.

Kinetic characteristics of [^{3}H]acetate influx

[^{3}H]Acetate influx, as a function of external acetate concentration (0·1–35 mmol l^{-1}), was measured after 10 s of incubation to estimate the kinetic

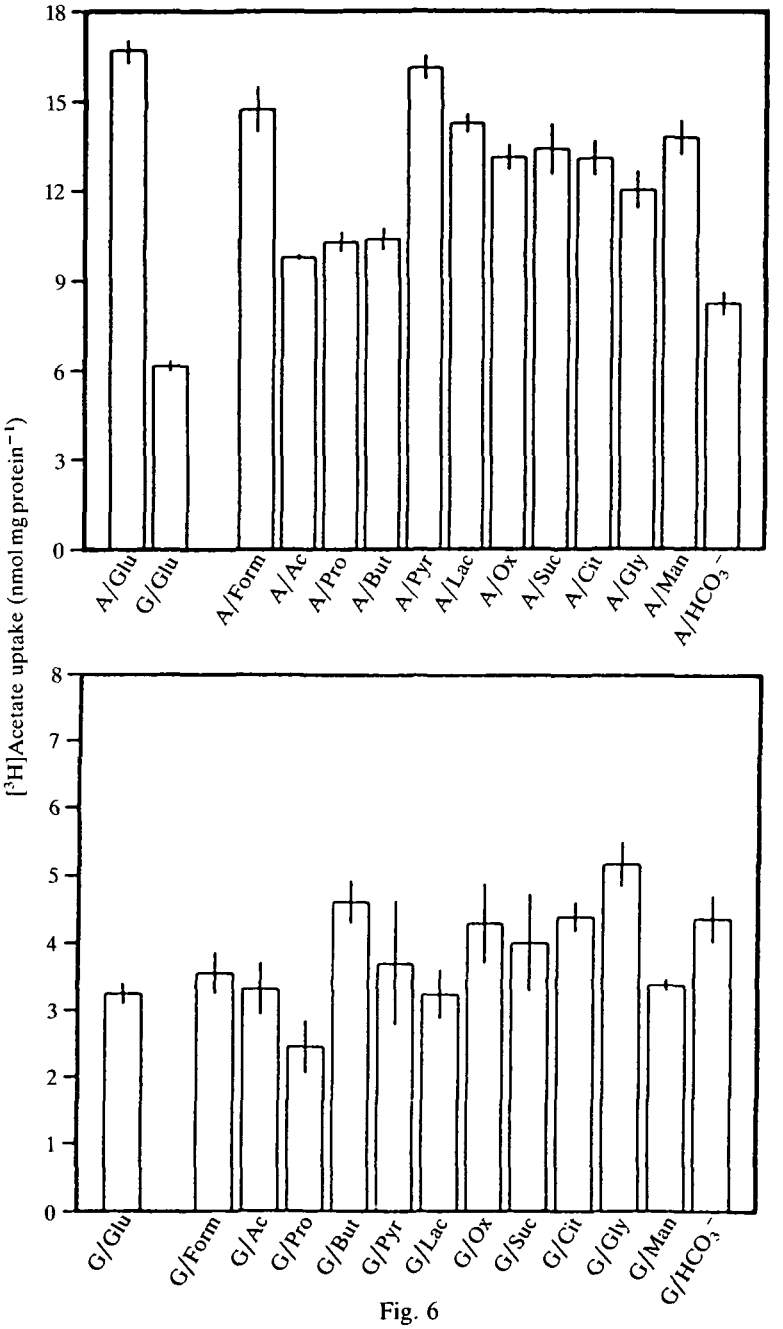

Fig. 6

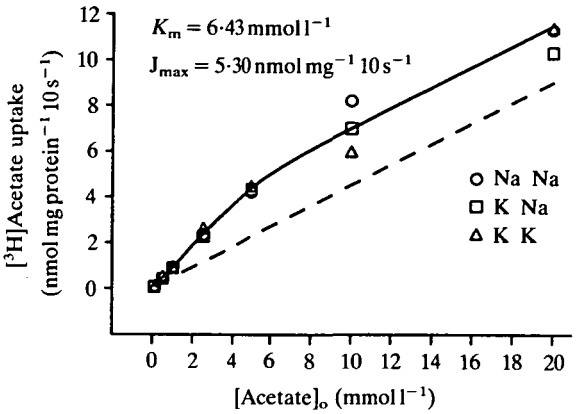

Fig. 7. Kinetic analysis of [^{3}H]acetate uptake in BBMV of tilapia upper intestine. Uptake was measured in the presence of bilateral Na^+ (Na/Na), in the presence of an inwardly directed Na^+ gradient (K/Na) and in the absence of Na^+ (K/K). Vesicles were preloaded with 25 mmol l^{-1} $NaHCO_3$, 75 mmol l^{-1} sodium gluconate (Na/Na) or 25 mmol l^{-1} $KHCO_3$, 75 mmol l^{-1} potassium gluconate (K/K, K/Na). Reaction media consisted of 100 mmol l^{-1} sodium gluconate (Na/Na, K/Na) or 100 mmol l^{-1} potassium gluconate (K/K), substituted in part by varying concentrations of the appropriate acetate salt (0·1–35 mmol l^{-1}). All media contained 12 mmol l^{-1} Hepes/Tris, pH 7·5 and 20 μmol l^{-1} CCCP. All treatment curves were not significantly from each other ($P < 0{\cdot}01$) so the best fit average curve through all points is plotted. Dashed line indicates the rate of apparent diffusive entry of acetate (i.e. total uptake minus the carrier-mediated component). The kinetic curve was fitted, and J_{max} and K_m values calculated, using a SAS curve-fitting program. All data points are the means of five 10-s uptake determinations. Error bars are obscured by the symbols.

parameters of acetate transport. Influx was observed in the presence of sodium (Na/Na), in the absence of sodium (K/K), and in the presence of an inwardly directed sodium gradient (K/Na). Fig. 7 illustrates acetate influx operating as the sum of two independent mechanisms acting concurrently; a carrier-mediated transport process which is saturable, and a non-saturable entry system with a rate proportional to the external acetate concentration. Because the influx curves of [^{3}H]acetate uptake into vesicles for the three indicated treatments were not significantly different ($P < 0{\cdot}01$), one curve was calculated to represent acetate influx into BBMV over the external concentration range selected.

Acetate binding was calculated as a percentage of total acetate uptake after 10 s of incubation for both 0·1 (Fig. 2) and 10 mmol l^{-1} (data not shown) [^{3}H]acetate by dividing the extrapolated vertical axis intercept values by total uptake values at 10 s. The averages of the two percentage values were used to estimate acetate binding at all external acetate concentrations. Kinetic constants for [^{3}H]acetate influx in the absence of non-specific binding were then determined by iterative non-linear computer fitting. This process applied to the data in Fig. 7 provided an average K_m of 6·44 ± 1·30 mmol l^{-1}, a J_{max} of 5·30 ± 0·73 nmol mg protein^{-1} 10 s^{-1}, and a rate

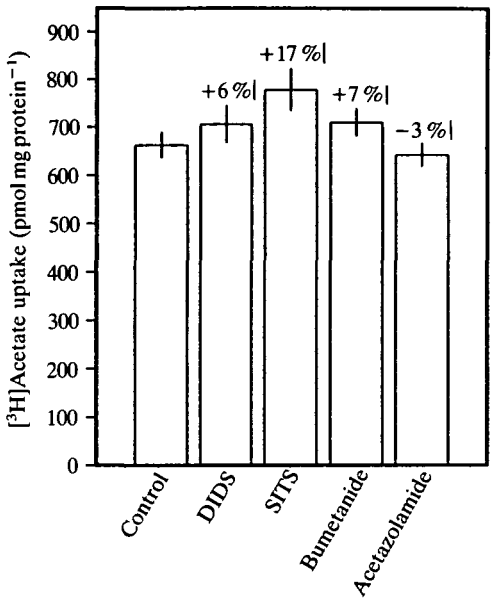

Fig. 8. Effects of anion transport inhibitory drugs on [^{3}H]acetate uptake in upper intestinal BBMV preloaded with 75 mmol l^{-1} sodium gluconate, 25 mmol l^{-1} $NaHCO_3$, 12 mmol l^{-1} Hepes/Tris (pH 7·5). Vesicles were incubated for 10 s in reaction media that consisted of 100 mmol l^{-1} sodium gluconate, 0·5 mmol l^{-1} [^{3}H]acetate, 12 mmol l^{-1} Hepes/Tris (pH 7·5) and 0·5 mmol l^{-1} of the indicated inhibitor, except for control which contained 0·5 mmol l^{-1} sodium gluconate. Histograms represent the means of five replicates per treatment, and error bars represent the standard error of the replicates. The percentage values are the deviation from the control value of [^{3}H]acetate uptake.

constant for the linear process, P, of 379 ± 15 pmol mg protein^{-1} 10 s^{-1} mmol acetate^{-1}.

Effects of drugs on [^{3}H]acetate uptake

The effects of several drugs known to inhibit anion transport in other cell types were examined for their influence on [^{3}H]acetate transport in tilapia BBMV. Vesicles were preloaded with 75 mmol l^{-1} sodium gluconate, 25 mmol l^{-1} $NaHCO_3$, 12 mmol l^{-1} Hepes/Tris (pH 7·5), and incubated for 10 s in 100 mmol l^{-1} sodium gluconate, 0·5 mmol l^{-1} [^{3}H]acetate, 12 mmol l^{-1} Hepes/Tris (pH 7·5) and 0·5 mmol l^{-1} of the following inhibitors: 4,4′-diisothiocyanostilbene-2,2′-disulphonic acid (DIDS), 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulphonic acid (SITS), bumetanide and acetazolamide, in addition to a control treatment which contained no drugs. Fig. 8 shows no apparent inhibition of [^{3}H]acetate uptake in the presence of these drugs, indicating the possible occurrence of an inhibitor-insensitive transport protein for the flux of acetate across tilapia BBMV. However, higher concentrations of these inhibitors may have more potent effects on acetate/HCO_3^- exchange than those shown in Fig. 8.

DISCUSSION

Short-chain fatty acids were found by gas and high-performance liquid chromatographic analysis to be present in biologically significant concentrations within the intestinal lumen of the herbivorous tilapia (Table 1). The characteristics of SCFA transport in tilapia brush-border membrane vesicles were studied using labelled acetate as substrate. Acetate uptake was not stimulated by an inward sodium gradient, but when vesicles were loaded with cold acetate, a five-fold increase in uptake of labelled acetate was measured. Acetate uptake into vesicles was additionally stimulated by preloading with propionate, butyrate and bicarbonate. To a lesser extent, acetate uptake was enhanced in vesicles preloaded with other biologically relevant monocarboxylates such as formate, lactate and glycine.

Sodium/SCFA transport interaction has been reported for cells from a variety of mammals and invertebrates. Most of the mammalian studies involved the observation of stimulated sodium absorption in the presence of SCFA (Engelhardt & Rechkemmer, 1983; Umesaki *et al.* 1979; Argenzio & Whipp, 1979). In addition, in rat colon and anuran lower intestine, the presence of sodium-stimulated SCFA uptake by as much as 50% (Umesaki *et al.* 1979; Hollander, Gerard & Boyd, 1986). A sodium/proton antiport mechanism has recently been demonstrated in the intestine of tilapia (Howard & Ahearn, 1988). This mechanism was investigated as a possible means of mediating SCFA uptake. The subsequent lack of stimulation of acetate uptake down sodium or potassium gradients (Fig. 1) suggests that acetate transport is not coupled, either directly or indirectly, to the sodium/proton antiporter. It also indicates that acetate is not transported by a sodium/acetate symport mechanism. Lack of sodium/acetate interaction in tilapia is different from the situation in mammals, but is similar to the observations in invertebrates, where both the cockroach hindgut and earthworm integument have been demonstrated to transport acetate independently of sodium (Richards & Arme, 1980; Hogan *et al.* 1985).

Vesicles preloaded with cold acetate displayed a transient uptake of labelled acetate five times that of gluconate-loaded vesicles. Although acetate remained the most stimulatory internal anion, formate and bicarbonate each showed significant stimulation of uptake of labelled acetate over that of gluconate-loaded vesicles (Fig. 2). In a more thorough investigation of anion specificity, involving a series of trans-stimulation and cis-inhibition experiments (Figs 3–6), the quantities of test anions used were reduced from the previous experiment to correspond with those previously reported by Low, Friedrich & Burkhardt (1984) when testing anion exchange in the rat renal basolateral membrane. Labelled acetate uptake was both markedly trans-stimulated and cis-inhibited only by bicarbonate (Figs 3, 4). No explanation can be offered for the apparent trans-stimulation by thiocyanate (SCN^-). Fig. 4 also indicates varying degrees of cis-inhibition by other inorganic anions, notably phosphate, thiocyanate and thiosulphate, although none of these anions inhibited labelled acetate uptake more than 50% of the amount of inhibition caused by bicarbonate.

Fig. 5 reveals that similar magnitudes of trans-stimulation of labelled acetate uptake is attained in vesicles preloaded with acetate, propionate and butyrate, as well as bicarbonate. Vesicles preloaded with the monocarboxylic acids – formate, pyruvate, lactate and glycine – showed partial trans-stimulation of labelled acetate uptake at nearly identical levels. The same held true for the dicarboxylates – oxalate and succinate – but at a lower magnitude. The tricarboxylate citrate did not stimulate uptake of [^{3}H]acetate. This pattern suggests an anion exchanger with a substrate affinity based in part on the valency of the substrate. The pattern for cis-inhibition of labelled acetate uptake (Fig. 6) compliments that of the trans-stimulation experiment in showing a strong affinity of the anion exchanger for short-chain fatty acids and bicarbonate.

Luminal bicarbonate secretion has been repeatedly reported in conjunction with SCFA absorption. Within the sheep rumen and the colon of the human, pig and pony, bicarbonate was secreted into the lumen as SCFA were absorbed (Stevens, Argenzio & Clemens, 1980; Argenzio & Whipp, 1979; Ruppin *et al.* 1980; Argenzio, Southworth, Lowe & Stevens, 1977). It was concluded from these studies that bicarbonate secretion was linked to the Cl^-/HCO_3^- exchange mechanism, secreted as an indirect result of H^+ absorption from undissociated SCFA uptake, or was a combination of these two mechanisms. In the perfused rat colon, a close linear relationship was also found between bicarbonate secretion and SCFA absorption (Umesaki *et al.* 1979). Acetate, propionate and butyrate were absorbed at different rates, but there was a constant ratio of SCFA absorption to bicarbonate secretion of about 4·0. The results of this study suggested that there was a brush-border carrier mechanism for bicarbonate/SCFA exchange that was distinct from the carrier-mediated chloride/bicarbonate exchange. However, no further evidence was presented to support this model. These past hypotheses and the present results clearly provide sufficient evidence for an intestinal brush-border antiport anion carrier specific for SCFA and bicarbonate exchange.

The transport of labelled acetate into tilapia brush-border membrane vesicles showed biphasic influx kinetics when plotted against external concentrations of cold acetate (Fig. 7). This demonstrates that two transfer mechanisms are operating independently: (1) a saturable Michaelis–Menten carrier component, and (2) an apparent diffusion process with a rate proportional to the external acetate concentration. The apparent half-saturation constant is characteristic of a mediated uptake system operating at substrate concentrations that are found to occur naturally in tilapia intestine. This indicates that there is a physiologically significant transfer mechanism for SCFA in herbivorous fish gastrointestinal systems. Before the current investigation, Michaelis–Menten saturation kinetics for SCFA transport had only been described in invertebrates (Richards & Arme, 1980; Hipp *et al.* 1986; Arme & Read, 1968; Hogan *et al.* 1985) and anurans (Hollander *et al.* 1986). In both the human and rat colon, SCFA uptake was reported as a linear concentration-dependent entry process (Umesaki *et al.* 1979; Ruppin *et al.* 1980).

None of the drugs tested significantly inhibited uptake of labelled acetate in tilapia brush-border membrane vesicles (Fig. 8). This suggests that SCFA transport

proteins are relatively insensitive to these drugs. It also supports the claim that the SCFA-exchange proteins are different from those for other anions, such as chloride and sulphate, in that the carriers for these anions are inhibited by bumetanide, SITS and DIDS at the concentrations used here (Haas & McManus, 1983; Hong *et al.* 1978; Koschier *et al.* 1980). In the perfused anuran intestine, DIDS ($500\,\mu mol\,l^{-1}$) was reported to decrease butyrate transport by 42% (Hollander *et al.* 1986). In contrast, the same concentration was reported to have no effect on bicarbonate secretion in the intestinal lumen of the goby (Dixon & Loretz, 1986). In addition, brush-border membrane vesicles of the human small intestine showed no inhibition of propionate uptake by DIDS until concentrations were increased to $10\,mmol\,l^{-1}$ with an incubation period of 60 min (J. Harig, personal communication). High concentrations and extended incubation periods, such as those needed for effective inhibition, further support the notion of a distinct carrier protein facilitating bicarbonate/SCFA exchange.

There have been no reports of fermentation of plant fibre in fish gut. The initial findings that SCFA occurs naturally along the gastrointestinal tract of the herbivorous tilapia is indicative of luminal microbial digestion. In conjunction with the uptake system for SCFA characterized in the current investigation, the occurrence of these substances strongly suggests that tilapia intestine is capable of plant fibre fermentation leading to the production of SCFA, which can be used as nutrient substrates.

The possible occurrence of teleost fermentation was recently investigated in the pyloric caeca of several carnivorous fish (Buddington & Diamond, 1987*a*,*b*). It was concluded that fish do not use these organs for fermentation, in contrast to mammals and birds which use their distally located caeca for fermentation purposes. It was further observed that herbivorous/omnivorous fish tend to possess longer intestinal tracts than carnivorous ones, with absent or reduced pyloric caeca (Buddington & Diamond, 1987*a*; Ferraris & Ahearn, 1983). Presumably, herbivorous and omnivorous animals rely more on SCFA assimilation as a nutrient source, suggesting that the primary location of fermentation activity in fish is the intestinal tract itself. The greater intestinal length allows for a longer retention time and therefore a more efficient fermentation.

The current investigation provides evidence of fermentation and SCFA assimilation in herbivorous teleost intestine. Further testing involving the nutritive role of SCFA in fish systems and continued investigation of fermentation/assimilation in other fish species will be required to substantiate the tentative conclusions presented here.

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