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Feed Deprivation and Re-feeding on Alterations of Proteases in Tilapia *Oreochromis mossambicus*

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Chi-Ru Chan, Der-Nan Lee, Yeong-Hsiang Cheng, Dennis Jine-Yuan Hsieh, and Ching-Feng Weng (2008) Feed deprivation and re-feeding on alterations of proteases in tilapia *Oreochromis mossambicus*. *Zoological Studies* **47**(2): 207-214. Regulation of gastrointestinal proteases becomes particularly important when fish starved. In this work, tilapia (*Oreochromis mossambicus*) were allotted to various treatments including starvation times (3, 5, and 7 d) and 1 d of re-feeding after various starvation times to test whether food deprivation causes changes in protease activities in the gastrointestinal tract. No significant differences in body weights were found among all groups. The relative stomach weights of the starved groups (3 and 5 d) were significantly higher than those of the control group. The relative intestinal weight of the starved group after 7 d of starvation was significantly higher than that of the control group. Additionally, re-feeding resulted in relative stomach and intestinal weight increases after 5 and 7 d of starvation, respectively. The activity of trypsin (T) in fish subjected to a short starvation period was lower than that in fish fed normally, whereas chymotrypsin (C) activity increased. The T/C ratio decreased with starvation time, indicating the possibility that the growth of tilapia is inhibited by starvation. Furthermore, re-feeding after starvation resulted in increased trypsin and chymotrypsin activities, and a decrease in the T/C ratio compared to before feeding. These data suggest that short-term starvation dampens trypsin activity and the T/C ratio in relation to the growth of tilapia, and that re-feeding consequently stimulates the activities of intestinal proteolytic enzymes in tilapia.
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Key words: Pepsin, Trypsin, Chymotrypsin, Starvation, *Oreochromis mossambicus*.

Digestive enzymes have been studied in various fish species (Soengas et al. 1996, Jonsson et al. 1997, Hidalgo et al. 1999, Lo and Weng 2006). Studies of proteolytic enzymes in fish have provided knowledge for improving protein utilization and established the importance of trypsin as a key enzyme for feed utilization and growth through its role in the processes of protein digestion (Rungruangsak-Torrissen et al. 2006). Trypsin activity has been reported to be related to the growth rate of fish larvae fed high-quality diets, such as in goldfish (*Carassius auratus*, Abiayad and Kestemont 1994), sea bass (*Dicentrarchus labrax*, Cahu et al. 1998, Nolting et al. 1999), and red drum (*Sciaenops ocellatus*, Lazo et al. 2000). Atlantic salmon (*Salmo salar*) grows fastest in June and July, when trypsin activity also reaches a peak (Jonsson et al. 1997). In rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon, a linear relationship between trypsin activity and protein digestibility is found (Krogdahl et al. 1994, Rungruangsak-Torrissen et al. 2006). Trypsin is a key protease which activates other pancreatic proteases including chymotrypsin in mammals and fish (Sunde et al. 2001). Trypsin-specific activity and the protease ratio of trypsin

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to chymotrypsin (T/C ratio) increase as growth is promoted. Moreover, increments of chymotrypsin-specific activity lead to a reduction in the growth rate, whereas fish with lower growth have a higher specific activity of chymotrypsin resulting in a lower T/C ratio (Rungruangsak-Torrissen et al. 2006). Many factors including species, age, habitat, temperature, and intestinal pH affect proteolytic enzyme activities (Hidalgo et al. 1999). In Nile tilapia (*Oreochromis niloticus*) kept at different seawater salinities, trypsin activity reaches the highest levels in fish with the highest growth rate and serum thyroxine levels (Woo et al. 1997).

Many studies have demonstrated that short-term food deprivation (Rueda et al. 1998, Tian and Qin 2003) or multiple periods of food deprivation and re-feeding increase feed efficiency and growth in various fish species (Hayward et al. 1997, Wu et al. 2002, Zhu et al. 2004). Changes in digestive enzyme activities in response to periods of starvation reveal the most critical nutrient and energy reserves, and those metabolized or conserved in the face of increasing food deprivation (Harms et al. 1991, Johnston et al. 2004). Recent data have demonstrated that starvation or a shortage of food may lead to increases in enzyme activities in different sections of the intestines (Harpaz et al. 2005, Krogdahl and Bakke-McKellep 2005). Long-term fasting in Atlantic cod (*Gadus morhua*), however, led to a drop in digestive enzyme activity levels in the pyloric caecum and intestine, as well as trypsin activity in pyloric caecum homogenate, which are all mostly restored upon re-feeding (Bélanger et al. 2002). Previously, the activity of digestive proteases is not significantly reduced with increasing starvation periods in Atlantic cod subjected to extended starvation for up to 25 d (Gildberg 2004). Asian sea bass subjected to restricted feeding regimes (50% less than the control) shows higher levels of overall proteolytic enzyme activities (leucine amino peptidase) (Harpaz et al. 2005). Digestive enzyme activities in the gastrointestinal tract of Nile tilapia are found to increase following short-term (5 d) fasting (Mommsen et al. 2003). However, alterations of digestive enzymes in tilapia (*O. mossambicus*) particularly during different periods of a single phase of food deprivation and re-feeding are very interesting. This work investigated the effects of various lengths of starvation and re-feeding after various lengths of starvation on digestive enzyme activities in tilapia, and compared changes in pepsin, trypsin, and chymotrypsin before and after re-feeding.

MATERIALS AND METHODS

Sample and experimental design

Ninety-six tilapia (*O. mossambicus*) were originally obtained from the Mariculture Research Center of the Fisheries Research Institute, Council of Agriculture, Executive Yuan (Tainan, Taiwan). Tilapia were reared in circulating fresh water at 25-28°C under a photoperiod of 12 h light: 12 h dark, and were fed a daily experimental dry diet at the Institute of Biotechnology, National Dong Hwa University (Hualien, Taiwan). The animal experiments were performed according to the *Guide for the Care and Use of Laboratory Animals* of National Dong-Hwa University. Both gender tilapia of 25-35 g in body weight were chosen for the experiment.

At the start of the experiment, fish were starved for 1d to empty the gut. The fish were randomly divided into tanks (4 fish/tank, 6 tanks/treatment) containing the normal feeding group, groups subjected to 3, 5, and 7 d of starvation, and groups allowed 1d of re-feeding after 3, 5, and 7 d of starvation. The formulation of the experimental diet is shown in table 1. The fish were then killed by ice anesthetic, and the intestines and stomach were collected. For the 1d of re-feeding after fasting, fish was given free access to food for 24 h. After weighing, one fish was randomly caught from every tank of the various treatments, and its tissues were removed, immediately frozen, and stored at -80°C until being assayed.

Protein isolation

The frozen stomach and intestines were separately homogenized in saline with 0.1% triton X-100 (5 fold volume of tissue), and the tissues were then homogenized in a polytron homogenizer. After centrifugation (12,092 x*g* for 15 min at 4°C), the supernatant was maintained at -80°C until assays. The total protein concentration for each sample was obtained using a Bradford protein assay kit (Sigma, St. Louis, MO, USA) with bovine serum albumin as a standard expressed in micrograms of protein per microliter.

Measurement of pepsin enzyme activity

Gastric pepsin activity (EC3.4.23.1) was

determined by measuring the hydrolysis rate of hemoglobin (Rick and Fritsch 1974). Before the pepsin activity assay, the pepsinogen in the homogenate was activated by incubation at 4°C for 10 min with 0.1 M HCl at pH 2.0. In total, 50 μg/100 μL of previously isolated stomach protein was mixed with 0.5 mL of 0.5% bovine hemoglobin (Sigma) in 0.1 M citrate acid (pH 2.0), and reacted at 37°C for 2 h. The reaction was terminated by adding 375 μL of 0.5 M trichloroacetic acid (TCA) to the mixture, which was then centrifuged at 12,092 x*g* for 20 min at 4°C. One unit of enzymatic activity was defined as the amount of protein that catalyzed an increase of 1.0 in the absorbance at 280 nm/min using a spectrophotometer (DU640i, Beckman, Fullerton, CA, USA) under the assay conditions. Pepsin activity was expressed as specific activity (U/mg protein) with 1 U representing 1 mL equivalent of tyrosine liberated per minute per milligram of protein.

Measurement of trypsin enzyme activity

Table 1. Formulation of the experimental diet

Ingredients (g/kg diet)	
Casein (vitamin free)	380
Maize starch	380
Maize oil	70
Fish oil	40
Vitamin mixture[a]	20
Mineral mixture[b]	40
Carboxymethylcellulose	20
α-Cellulose	50
Calculated nutrient proximate composition	
Crude protein	308
Diethyl ether extract	110
Crude fiber	37
Ash	35

[a]Vitamin mixture (mg/g mixture): thiamin hydrochloride, 5; riboflavin, 5; calcium pantothenate, 10; nicotinic acid, 6.05; d-biotin, 0.003; pyridoxine hydrochloride, 0.825; folic acid, 0.041; inositol, 200; L-ascorbyl-2-monophosphate-Mg, 2.025; choline chloride, 44; menadione, 4; α-tocopheryl acetate, 3.35; retinyl acetate, 0.4; para-aminobenzoic acid, 5; and cholecaliferol, 0.0004685. All ingredients were diluted with α-cellulose to 1 g. [b]Mineral mixture (mg/g mixture): $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $CuSO_4$, 0.1; $MnSO_4 \cdot H_2O$, 0.8; $CoCl_2 \cdot 6H_2O$, 1; $ZnSO_4 \cdot 7H_2O$, 3; Ca $(H_2PO_4)2 \cdot H_2O$, 135.8; Ca $(CH_3CHOHCOO)_2 \cdot 5H_2O$, 327; $FeSO_4 \cdot 6H_2O$, 2.125; $MgSO_4 \cdot 7H_2O$, 137; KCl, 75; and NaCl, 43.5. All ingredients were diluted with α-cellulose to 1 g.

The pancreatic trypsin (EC3.4.21.4) and chymotrypsin (EC3.4.21.1) activities were measured following the methods outlined by Geiger (1984) and Geiger and Fritz (1984), respectively. Trypsinogen and chymotrypsinogen in the homogenates were activated by the addition of enterokinase at a concentration of 4 mg/mL and incubated at 4°C for 24 h. Totally 50 μg/100 μL of previously isolated intestinal protein was mixed with 0.5 mL of 10 mM p-toluene sulphonyl-L-arginine methyl ester (TAME, Sigma) in 0.1 M Tris-HCl (pH 8.0), and reacted in a water bath at 37°C for 10 min. The absorbance was then measured at OD_{247} using a spectrophotometer (Beckman DU640i) to quantify the digestive enzyme activity of trypsin. Trypsin activity was expressed as units/mg protein.

Measurement of chymotrypsin enzyme activity

Totally 50 μg/100 μL of previously isolated intestinal protein was mixed with 0.5 mL of 1 mM N-benzoyl-L-arginine ethyl ester (BAEE, Calbiochem, Darmstadt, Germany) in 0.1 M Tris-HCl (pH 7.8), and reacted in a water bath at 37°C for 10 min. The absorbance was then measured at OD_{256} using a spectrophotometer (Beckman DU640i) to quantity the digestive enzyme activity of chymotrypsin. Chymotrypsin activity was expressed as units/mg protein.

Statistical analysis

Experimental data are presented as the mean ± standard error (n = 6). The data were analyzed as a split-plot design using the GLM procedures of SAS (1999). When a significant F-value for treatment means ($p < 0.05$) was observed by analysis of variance (ANOVA), treatment means were compared with Duncan's multiple-range test (Duncan 1955).

RESULTS

Changes in gastrointestinal (GI) tract weight and final body weight

During the experimental period, no fish died in any group. No significant differences in body weights were found among the groups. The relative stomach weights of starved groups (for 3 and 5 d) were significantly higher than that of

the control group. Additionally, 1 d of re-feeding after 7 d of starvation resulted in an increase in the relative stomach weight (Table 2). The relative intestinal weight of the starved group after 7 d of fasting was significantly higher than that of the control group. Moreover, 1 d of re-feeding after 3 and 5 d of starvation resulted in a relative recovery of intestinal weight (Table 2).

Changes in the activities of pepsin, trypsin, and chymotrypsin during starvation and re-feeding after starvation

The effects of fasting on the expressions of pepsin in the stomach and of trypsin and chymotrypsin in the intestines are shown in table 3. The gastric pepsin activity of tilapia fell after 3 d of starvation compared to the control group, but no significant differences were exhibited among the tested groups at 5 and 7 d of starvation and the control group. The stomach pepsin activities after re-feeding showed no significant differences compared with that after starvation. In the group re-fed after 3 d of starvation, the stomach pepsin activity was lower than that of the control group (Table 3). The intestinal trypsin activity after 3 d of starvation decreased, and the intestinal trypsin activity exhibited the lowest level among all tested groups after 5 d of starvation. The intestinal trypsin activity increased with re-feeding after 3, 5, and 7 d of starvation, and the levels in the re-fed groups were higher than that of the control (Table 3). The intestinal chymotrypsin activity showed no significant differences after 3 and 5 d of starvation compared to the control group, but the intestinal chymotrypsin activity significantly increased after 7 d of starvation. This resulted in a significantly reduced activity ratio of trypsin to chymotrypsin (the T/C ratio) with increasing time from 3 to 7 d of starvation (Table 3). Re-feeding caused the intestinal chymotrypsin activity to increase after 3, 5, and 7 d of starvation, and levels were higher than those of the control group. The intestinal chymotrypsin activity was higher in the re-fed group after 7 d of starvation than those of the other groups. In addition, the intestinal chymotrypsin activity after re-feeding was also higher than that of the control. The T/C ratios with re-feeding after 5 and 7 d of starvation were lower than those of the control group. The decreasing tendency was greater with re-feeding after 7 d of starvation. In contrast, the T/C ratio of the re-fed group did not significantly differ from the fasting group. The results of re-feeding were compared with the control, there were significant decreases in both re-fed after 5 and 7 d of starvation groups (Table 3).

DISCUSSION

Recent studies have demonstrated that variable food intake and re-feeding lead to increases in tissue mass, protein content, and enzyme activities synchronous with the response to fasting, possibly due to a combination of increased cellular proliferation (Secor et al. 2000, Krogdahl and Bakke-McKellep 2005), higher rates of protein synthesis, and lower levels of protein degradation (Houlihan et al. 1988, Krogdahl and Bakke-McKellep 2005). The body weights of starved tilapia showed no significant changes. The relative stomach weights of the starved groups

Table 2. Alterations in body weight, stomach weight, and intestinal weight of tilapia after various periods of starvation and re-feeding after various period of starvation

	Control	Starvation periods (d)						SE	*p* value		
		3		5		7			Starvation period (S)	Feeding (F)	S*F
		Starved	Re-fed	Starved	Re-fed	Starved	Re-fed				
Body weight (g)	29.38	28.59	28.76	28.15	28.63	27.55	27.86	0.79	0.35	0.14	0.08
Stomach weight (g)	0.13^{c}	0.16^{bc}	0.14^{bc}	0.18^{a}	0.15^{bc}	0.13^{c}	0.18^{ab}	0.01	0.01	0.62	0.01
Intestinal weight (g)	0.29^{c}	0.30^{c}	0.29^{c}	0.29^{c}	0.33^{bc}	0.38^{ab}	0.40^{a}	0.02	0.01	0.39	0.39
Relative stomach weight (g/g body weight)	4.43^{c}	5.60^{b}	4.87^{c}	6.39^{a}	5.24^{bc}	4.72^{bc}	6.46^{ab}	0.42	0.01	0.20	0.05
Relative intestine weight (g/g body weight)	9.87^{bc}	10.49^{bc}	10.08^{c}	10.30^{bc}	11.53^{ab}	13.79^{a}	14.36^{a}	0.69	0.01	0.78	0.05

$^{a, b, c}$Different superscript letters indicate a significant difference between values on a given row ($p < 0.05$).

were significantly higher than those of the control group. Furthermore, the relative intestinal weight of the starved group after 7 d of starvation was significantly higher than that of the control group. Moreover, re-feeding resulted in the relative stomach weight recovering after 7 d of starvation and the relative intestinal weight recovering after 5 d of starvation (Table 2). It is recently reported that fasting caused extensively rapid decreases in the tissue mass, protein, and enzyme capacities of Atlantic salmon (*S. salar*) within 2 d (Krogdahl and Bakke-McKellep 2005). However, intestinal wasting slows down during long-term starvation periods. Protein degradation in other tissues, particularly white muscle, apparently levels up at such a time to provide more amino acids for vital body functions (reviewed by Navarro and Gutierrez 1995). Previous studies have revealed that starving fish for periods of 1-2 mo have little effect on body mass or length (Foster and Moon 1991, Navarro et al. 1992, Bélanger et al. 2002). Intestinal tissue proteins degrade more intensely than other tissue proteins in well-nourished fish during the early phases of starvation (Weatherley and Gill 1981, Houlihan et al. 1988, Krogdahl and Bakke-McKellep 2005). In rainbow trout (*O. mykiss*) fed a meal after a 6 d fasting period, fractional rates of protein synthesis in the intestines are higher than that 3 h after feeding, and these were brought about by an increase in protein synthesis per unit of RNA (McMillan and Houlihan 1989, Krogdahl and Bakke-McKellep 2005). Our study found that the stomach and intestinal weight to body weight percentages increased after short-term starvation. It is possible that a greater decrease in body weight occurred than for the GI tract.

Pepsin, a stomach proteolytic enzyme, digests ingested proteins by preferentially cleaving carboxylic groups of aromatic amino acids such as phenylalanine and tyrosine. The digestive enzymes express strong activity at an optimal pH, which is previously reported to be pH 2.0 for pepsin in fish (Clark et al. 1985). The structure of fish pepsins is quite similar to that of mammalian pepsins; nevertheless, they are more active at low temperatures and weakly acidic conditions and more easily sensible heating (Martinez and Olsen 1989). In general, tilapia (*O. mossambicus*) can survive in a pH ranging from 5 to 10 but do best in a pH range of 6 to 9. The optimal temperature for tilapia ranges from 20 to 38°C (Ndong et al. 2006). The involvement of the activities of pepsin in stomach changes during starvation and re-feeding are inadequately understood in fish, and have seldom been explored in tilapia. The results indicated that there was no influence on the activity of pepsin in tilapia after 5 and 7 d of starvation, but a decrease was observed after only 3 d of starvation (Table 3). A similar result is previously reported of starvation periods of 10 to 25 d producing no significant reductions in pepsin activities (Gildberg 2004). Furthermore, the activity of pepsin did not change before or after re-feeding in this work. However, no data are reported in relation to alterations in pepsin activities after re-feeding, and more work is required to explore this.

Trypsin cleaves proteins on the carboxyl side of the basic amino acids, lysine and arginine, which show higher digestibility than other amino acids (Espe et al. 1993, Skrede et al. 1998). Previously, relationships between the food conversion efficiency (and/or specific growth rate) and trypsin-specific activity (Lemieux et al. 1999, Zabielski et al. 1999, Sunde et al. 2001) are observed in different fish species. Trypsin

Table 3. Activities of pepsin, trypsin, and chymotrypsin, and the ratio of trypsin to chymotrypsin (T/C) in tilapia after various periods of starvation and re-feeding after various periods of starvation

	Control	Starvation periods (d)						SE	*p* value		
		3		5		7			Starvation period (S)	Feeding (F)	S*F
		Starved	Re-fed	Starved	Re-fed	Starved	Re-fed				
Pepsin[x]	1.23^{bc}	0.96^{d}	0.98^{d}	1.27^{abc}	1.14^{c}	1.32^{ab}	1.39^{a}	0.05	0.01	0.64	0.17
Trypsin (T)[x]	0.17^{c}	0.14^{d}	0.24^{a}	0.12^{d}	0.21^{b}	0.14^{d}	0.21^{b}	0.01	0.01	0.01	0.13
Chymotrypsin (C)[x]	0.58^{c}	0.57^{c}	0.94^{b}	0.61^{c}	0.98^{b}	1.07^{b}	1.82^{a}	0.06	0.01	0.01	0.01
T/C[y]	0.30^{a}	0.29^{a}	0.26^{ab}	0.20^{bc}	0.21^{b}	0.13^{cd}	0.12^{d}	0.03	0.01	0.59	0.72

[x]Pepsin, trypsin, and chymotrypsin activities are expressed as specific activities (U/mg protein). [y]The T/C ratio value was calculated as the ratio of trypsin activity to chymotrypsin activity. [a, b, c, d]Different superscript letters indicate a significant difference between values on a given raw ($p < 0.05$).

is a sensitive key protease under conditions favoring growth (Rungruangsak-Torrissen et al. 2006). Our results showed that the activity of trypsin decreased with starvation time (3 and 5 d) (Table 3). It is possible that starvation inhibits the growth of tilapia according to the relationship between specific growth rates and trypsin-specific activity. Chymotrypsin cleaves proteins at the carboxyl side of the aromatic amino acids, phenylalanine, tyrosine, and tryptophan, as well as of large hydrophobic residues such as methionine. Higher chymotrypsin-specific activity with limited or depressed growth can result from starvation or food deprivation (Rungruangsak-Torrissen et al. 2006). By contrast to the decreased trypsin activity, the activity of chymotrypsin increased with starvation time (5 and 7 d) (Table 3). The protease activity ratio of trypsin to chymotrypsin (T/C ratio) is higher during rapid growth and lower during slower growth periods (Rungruangsak-Torrissen et al. 2006). In different fish species, relationships between the food conversion efficiency (and/or the specific growth rate) and the T/C ratio (Sunde et al. 2001) have been observed. The T/C ratio decreased with starvation time (5 and 7 d) in the present study (Table 3). The T/C ratio seems to be more sensitive and representative than trypsin-specific activity for comparing between fish with potentially different growth rates (Sunde et al. 2001). According to the relationships of growth and the T/C ratio, the growth of tilapia was inhibited with short fasting periods based on a reduction in the T/C ratio. However, no growth inhibition was found in this work. More work is required to investigative the effects of starvation on the growth of tilapia.

Methods for increasing growth have been explored for years in many studies. One of these methods, food restriction and re-feeding, results in increased various extents of growth (Johansen and Overturf 2006). The activities of trypsin and chymotrypsin increased after re-feeding (Table 3). The results indicated that re-feeding caused a rise in the proteolytic activity in tilapia with a short starvation period. In previous studies exposing gibel carp (*Carassius auratus gibelio*) to a single period of food deprivation and re-feeding, the deprived fish exhibit similar growth rates to control fish during the re-feeding period (Qian et al. 2000, Xie et al. 2001). Long-term fasting in Atlantic cod (*G. morhua*) led to a fall in metabolic enzyme activities in the pyloric caecum and intestine, as well as trypsin activity, which are all largely restored upon re-feeding (Bélanger et al. 2002). In the channel catfish (*Ictalurus punctatus*), overcompensation has been reported for deprivation periods of 1, 2, and 3 d (Chatakondi and Yant 2001). Overcompensation of various parameters upon re-feeding is noted in rainbow trout (*Salmo gairdneri*, Weatherley and Gill 1981), Atlantic salmon (Krogdahl and Bakke-McKellep 2005), and Atlantic cod (Bélanger et al. 2002) and has been proposed as a mechanism for compensatory growth. The gross growth efficiency of deprived fish is marginally higher than normally fed Chinese longsnout catfish (*Leiocassis longrostris*, Zhu et al. 2004) and barramundi (*Lates calcarifer*, Tian and Qin 2003). Re-feeding after deprivation has been reported to improve the feeding efficiency and induce growth in various fish species (Hayward et al. 1997, Rueda et al. 1998, Wu et al. 2002, Tian and Qin 2003, Zhu et al. 2004, Johansen and Overturf 2006). The T/C ratio was lower in both starved and re-fed groups, and the T/C ratio after 1 d of re-feeding was much lower than that of the starved groups. However, the contributions of an extending period of re-feeding to the recovery and activation of growth rate in tilapia are interesting and need to be investigated in the future.

In conclusion, the activity of trypsin in fish subjected to short starvation periods was lower, but chymotrypsin activity increased. The T/C ratio decreased with starvation time, revealing the potential for inhibited growth of tilapia. Furthermore, re-feeding after starvation increased the activities of trypsin and chymotrypsin, and decreased the T/C ratio compared to those before and after re-feeding. These results reveal that 1 d of re-feeding after starvation is insufficient to lead to the recovery and activation of growth in tilapia.

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REFERENCES

Abiayad A, P Kestemont. 1994. Comparison of the nutritional status of goldfish (*Carassius auratus*) larvae fed with live, mixed or dry diet. Aquaculture **128:** 163-176.

Bélanger F, PU Blier, JD Dutil. 2002. Digestive capacity and compensatory growth in Atlantic cod (*Gadus morhua*). J. Fish Biol. **26:** 121-128.

Cahu CL, JLZ Infante, A Peres, P Quazuguel, MM Le Gall. 1998. Algal addition in sea bass (*Dicentrarchus labrax*) larvae rearing: effect on digestive enzymes. Aquaculture

161: 479-489.

Chatakondi NG, RD Yant. 2001. Application of compensatory growth to enhance production in channel catfish *Ictalurus punctatus*. J. World Aquacult. Soc. **32:** 278-285.

Clark J, NL McDonald, JR Stark. 1985. Metabolism in marine flatfish: II. Protein digestion in Dover sole (*Solea solea* L). Comp. Biochem. Physol. B **81:** 217-222.

Duncan DB. 1955. Multiple range and multiple *F*-test. Biometrics **11:** 1-42.

Espe M, E Lied, KR Torrissen. 1993. *In vitro* protein synthesis in muscle of Atlantic salmon (*Salmo salar*) as affected by the degree of proteolysis in feeds. J. Anim. Physiol. Nutr. **69:** 260-266.

Foster GD, TW Moon. 1991. Hypometabolism with fasting in the yellow perch (*Perca flavescens*): a study of enzymes, hepatocyte metabolism, and tissue size. Physiol. Zool. **64:** 259-275.

Geiger R. 1984. Chymotrypsin. *In* HU Bergmeyer, ed. Methods of enzymatic analysis. Vol. 2. New York: Academic Press, pp. 99-118.

Geiger R, H Fritz. 1984. Trypsin. *In* HU Bergmeyer, ed. Methods of enzymatic analysis. Vol. 2. New York: Academic Press, pp. 119-129.

Gildberg A. 2004. Digestive enzyme activities in starved pre-slaughter farmed and wild-captured, Atlantic cod (*Gadus morhua*). Aquaculture **238:** 343-353.

Harms J, K Anger, S Klaus, B Seeger. 1991. Nutritional effects on ingestion rate, digestive enzyme activity, growth and biochemical composition of *Hyas araneus* L. (Decapoda: Majidae) larvae. J. Exp. Mar. Biol. Ecol. **145:** 233-265.

Harpaz S, Y Hakim, T Slosman, A Barki, I Karplus, OT Eroldoğan. 2005. Effects of different feeding levels during day and/or night on growth and brush border enzyme activity in juvenile *Lates calcarifer* fish reared in freshwater re-circulating tanks. Aquaculture **248:** 325-335.

Hayward RS, DB Noltie, N Wang. 1997. Use of compensatory growth to double hybrid sunfish growth rates. Trans. Am. Fish Soc. **126:** 316-322.

Hidalgo MC, E Urea, A Sanz. 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. Aquaculture **170:** 267-283.

Houlihan DF, SJ Hall, C Gray, BS Noble. 1988. Growth rate and protein turnover in Atlantic cod, *Gadus morhua*. Can. J. Fish Aquat. Sci. **45:** 951-964.

Johansen K, K Overturf. 2006. Alterations in expression of genes associated with muscle metabolism and growth during nutritional restriction and re-feeding in rainbow trout. Comp. Biochem. Physol. **144:** 119-127.

Johnston DJ, AJ Ritar, CW Thomas. 2004. Digestive enzyme profiles reveal digestive capacity and potential energy sources in fed and starved spiny lobster (*Jasus edwardsii*) phyllosoma larvae. Comp. Biochem. Physol. B **138:** 137-144.

Jonsson AC, S Einarsson, PS Davies. 1997. Seasonal variation in trypsin activity in juvenile Atlantic salmon upper and lower modal groups. J. Fish Biol. **51:** 1209-1218.

Krogdahl A, AM Bakke-McKellep. 2005. Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (*Salmo salar* L.). Comp. Biochem. Physol. A **141:** 450-460.

Krogdahl A, TB Lea, JL Olli. 1994. Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physol. A **107:** 215-219.

Lazo JP, MT Dinis, GJ Holt, C Faulk, CR Arnold. 2000. Co-feeding microparticulate diets with algae: toward eliminating the need of zooplankton at first feeding in larval red drum (*Sciaenops ocellatus*). Aquaculture **188:** 339-351.

Lemieux H, P Blier, JD Dutil. 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadus morhua*)? J. Fish Biol. **20:** 293-303.

Lo MJ, CF Weng. 2006. Developmental regulation of gastric pepsin and pancreatic serine protease in larvae of the euryhaline teleost, *Oreochromis mossambicus*. Aquaculture **261:** 1403–1412.

Martinez A, RL Olsen. 1989. Characterization of pepsins from cod. US Biochem. **16:** 22-23.

McMillan DN, DF Houlihan. 1989. Short-term responses of protein synthesis to re-feeding in rainbow-trout. Aquaculture **79:** 37-46.

Mommsen TP, HL Osachoff, ME Elliott. 2003. Metabolic zonation in teleost gastrointestinal tract. Effects of fasting and cortisol in tilapia. J. Comp. Physol. B **173:** 409-418.

Navarro I, J Gutiérrez. 1995. Fasting and starvation. *In* PW Hochachka, TP Mommsen, eds. Biochemistry and molecular biology of fishes. Amsterdam: Elsevier, pp. 393-434.

Navarro I, J Gutiérrez, J Planas. 1992. Changes in plasma glucagon, insulin and tissue metabolites associated with prolonged fasting in brown trout (Salmo trutta fario) during two different seasons of the year. Comp. Biochem. Physiol. Comp. Physiol. **102:** 401-407.

Ndong D, YY Chen, YH Lin, B Vaseeharan, JC Chen. 2006. The immune response of tilapia *Oreochromis mossambicus* and its susceptibility to *Streptococcus* iniae under stress in low and high temperatures. Fish Shellfish Immunol. **22:** 1-9.

Nolting M, B Ueberschar, H Rosenthal. 1999. Trypsin activity and physiological aspects in larval rearing of European sea bass (*Dicentrarchus labrax*) using live prey and compound diets. J. Appl. Ichthyol. **15:** 138-142.

Qian X, Y Cui, B Xiong, Y Yang. 2000. Compensatory growth, feed utilization and activity in gibel carp, following feed deprivation. J. Fish Biol. **56:** 228-232.

Rick W, WP Fritsch. 1974. Chymotrypsin, trypsin and pepsin. *In* HU Bergmeyer, ed. Methods of enzymatic analysis. Vol. 2. 2nd ed. New York: Academic Press, pp. 1006-1057.

Rueda FM, FJ Martinez, S Zamora, M Kentouri, P Divanach. 1998. Effect of fasting and refeeding on growth and body composition of red porgy, *Pagrus pagrus* L. Aquac. Res. **29:** 447-452.

Rungruangsak-Torrissen K, R Moss, LH Andresen, A Berg, R Waag. 2006. Different expressions of trypsin and chymotrypsin in relation to growth in Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. **32:** 7-23.

SAS. 1999. SAS/STAT user's guide (Release 6.12). Cary, NC: SAS Institute.

Secor SM, EE Whang, JS Lane, SW Ashley, J Diamond. 2000. Luminal and systemic signals trigger intestinal adaptation in the Burmese python (*Python molurus*). Am. J. Physiol. **279:** 1177-1187.

Skrede A, GM Berge, T Storebakken, O Herstad, KG Aarstad,

F Sundstol. 1998. Digestibility of bacterial protein grown on natural gas in mink, pigs, chicken and Atlantic salmon. Anim. Feed Sci. Technol. **76:** 103-116.

Soengas JL, EF Strong, J Fuentes, JAR Veira, MD Andres. 1996. Food deprivation and refeeding in Atlantic salmon, *Salmo salar*: effects on brain and liver carbohydrate and ketone bodies metabolism. J. Fish Biol. **15:** 491-511.

Sunde J, GL Taranger, K Rungruangsak-Torrissen. 2001. Digestive protease activities and free amino acids in white muscle as indicators for feed conversion efficiency and growth rate in Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. **25:** 335-345.

Tian X, JG Qin. 2003. A single phase of food deprivation provoked compensatory growth in barramundi *Lates calcarifer*. Aquaculture **224:** 169-179.

Weatherley AH, HS Gill. 1981. Recovery growth following periods of restricted rations and starvation in rainbow trout *Salmo gairdneri* Richardson. J. Fish Biol. **18:** 195-208.

Woo NYS, TB Ng, TC Leung, CY Chow. 1997. Enhancement of growth of tilapia *Oreochromis niloticus* in iso-osmotic medium. J. Appl. Ichthyol. **13:** 67-71.

Wu L, S Xie, X Zhu, Y Cui, RJ Wootton. 2002. Feeding dynamics in fish experiencing cycles of feed deprivation: a comparison of four species. Aquac. Res. **33:** 481-489.

Xie S, X Zhu, Y Cui, RJ Wootton, W Lei, Y Yang. 2001. Compensatory growth in the gibel carp following feed deprivation: temporal patterns in growth, nutrient deposition, feed intake and body composition. J. Fish Biol. **58:** 999-1009.

Zabielski R, IL Huerou-Luron, P Guilloteau. 1999. Development of gastrointestinal and pancreatic functions in mammalians (mainly bovine and porcine species): influence of age and ingested food. Rep. Nutr. Dev. **39:** 5-26.

Zhu X, S Xie, Z Zou, W Lei, Y Cui, Y Yang, RJ Wootton. 2004. Compensatory growth and food 14 consumption in gibel carp, *Carassius auratus gibelio*, and Chinese longsnout catfish, *Leiocassis longrostris*, experiencing cycles of feed deprivation and re-feeding. Aquaculture **241:** 235-247.