RESPONSE OF COMMON CARP AND TILAPIA TO DIETS BASED ON PLANT PROTEIN SUPPLEMENTED WITH ESSENTIAL AMINO ACIDS

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ODGOVOR ŠARANA I TILAPIJE NA ISHRANU ZASNOVANU NA PROTEINIMA BILJNOG POREKLA DOPUNJENIM ESENCIJALNIM AMINO KISELINAMA

Prošireni abstrakt

Usled svog odličnog sastava mikro i makro nutrijenata i velike palatabilnosti riblje brašno je bilo osnovni sastojak hrane za vodene životinje. Danas se više od 65% svetske proizvodnje ribljeg brašna koristi za hranu za vodene organizme, mada su rastuće cene na tržištu dovele do značajnog povećanja upotrebe proteina biljnog porekla. Biljno brašno, koncentrati i izolati biljnog porekla deficitarni su bar u jednoj ili više od 10 za ribe esencijalnih amino kiselina (essential amino acids (EAA), pa je i integrisanje u hranu ograničeno. Čak i kada se nedostaci nadoknade kristalnim amino kiselinama javlja se problem nesposobnosti nekih riba da iskorišćavaju biljne proteine. Na primer, pastrmke mogu dobro da iskorišćavaju biljne proteine obogaćene kristalnim amino kiselinama dok je kod šarana različito. Jedan razlog može biti nedostatak želuca kod šarana a tako i nedostatak pepsina i kisele faze varenja. Kristalne amino kiseline mogu da dovedu do visokog sadržaja jedne amino kiseline u krvnoj plazmi šarana koja tada ne može da se iskoristi za nastanak novog tkiva, na primer mišića, već se koristi kao izvor energije.

Da bi se ispitale moguće razlike u iskorišćavanju biljnih proteina kod šarana i tilapije, izvedena su dva odvojena randomizovana hranidbena ogleda sa šaranom (*Cyprinus carpio*, starosti 12 nedelja) i tilapijom (*Oreochromis niloticus*, starosti 9 nedelja). Eksperimenti su izvedeni u postrojenju za akvakulturu na vTI-Institutu, Institut za ribarstvenu ekologiju u Ahrensburgu, Nemačka. Za oba eksperimenta 12 akvarijuma su nasađeni sa 7 šarana ili 10 tilapija. Pre početka eksperimenta pripremljena su 3 izo-azotna, izo-lipidna i izo-energetska test obroka sastavljena na osnovu potreba šarana: obrok FM sastojao se od 100% protein iz ribljeg brašna, obrok WG od 100% protein pšeničnog glutena i obrok WG+AA od proteina pšeničnog glutena + EAA. Da bi se ispitali efekti

dodavanja EAA testirane ribe su dobijale umanjenu količinu hrane u odnosu na njihovu metaboličku telesnu masu.

Uprkos sličnim nutricionim potrebama šarana i tilapije, utvrđene su jasne razlike u rastu i parametrima iskoristljivosti hrane, kao i u telesnom sastavu. Dok je najveće povećanje telesne mase dobijeno sa obrokom WG+AA kod šarana, tilapija je imala najveće povećanje telesne mase sa obrokom FM. I šaran i tilapija su mogli da koriste dodate EAA. Kod šarana je dodavanje EAA dovelo do povećanog sadržaja sirovih protein u poređenju sa WG obrokom, ali je to bilo niže u poređenju sa FM obrokom. Šaran hranjen WG obrokom je pokazivao jasne znake adipoze koje se pojavljuju u obrocima sa deficitom proteina. Međutim, sirovi pepeo je bio najviši u šarana hranjenog WG+AA obrokom što je znak ograničenosti sadržaja ili kvaliteta proteina. Kod tilapije je dodatak EAA rezultirao u nižem sadržaju sirovih proteina, kao i povećanom sadržaju sirovih lipida. I šaran i tilapija su iskorišćavali značajne količine dodatih EAA za prikupljanje rezervi lipida.

Potrebna su dalja istraživanja da bi se ispitalo ugrađivanje dodatih amino kiselina u tkivo riba. Ovo se može ostvariti tečnom hromatografijom/izotop masenom spektrometrijom sa $\delta^{13}C$ obeleženim amino kiselinama koja bi u budućim ispitivanjima mogla da dovede do boljeg razumevanja upotrebe protein biljnog porekla među različitim vrstama riba.

***Ključne reči**:riblje brašno, biljni protein, ishrana, šaran, tilapija*

INTRODUCTION

Fish meal replacement in diets for various fish species has been one of the key topics in fish nutrition for the past few years. Due to its excellent composition in micro and macro nutrients and its high palatability, fish meal has been the ingredient of choice in aquafeeds. Between 1983 and 2005 international market prices for fish meal ranged between US$ 200 and 800 per metric ton (Tacon & Metian 2008). Ever since the El Niño year 2006, fish meal prices have been on a steady rise, up to almost US$ 2000 per metric ton during the first quarter of 2011. Today, up to 65% of the world's fish meal production is utilized in aquafeeds (Hardy 2010), of which roughly 80% are utilized in diets for carnivorous fish species and crustaceans. However, the remaining 20% are utilized in starter and fingerling diets for omnivorous species, such as carp, tilapia and catfish (Tacon & Metian 2008). Therefore, it is of major importance to increase the use of plant proteins as a fish meal substitute, to satisfy the increasing demand for aquafeeds from aquaculture farming practices. Nevertheless, most plant meals, concentrates or isolates are deficient in at least one or more of the ten essential amino acids (EAA) in fish. But even when these deficiencies are supplemented by crystalline amino acids, there are differences among fish species in the ability to utilize plant proteins. For instance, trout are able to utilize plant proteins supplemented with crystalline amino acids well (Rodehutscord et al. 1995, 1997; Dabrowski and Dabrowska 1981) while the utilization differs in carp. One reason may be the absence of a stomach in carp and hence their lack of acid and pepsin digestion.

Therefore, crystalline amino acids seem to be absorbed more quickly by the intestine, while larger proteins are broken down to di- and tripeptides and absorbed slower over the length of the intestine. This may cause peak situations of single amino acids in the blood plasma of common carp (Plakas et al. 1980), which cannot be sufficiently

utilized for the assembly of new tissues, e.g. muscle, and are therefore utilized as an energy source by carp.

The purpose of this study is to compare carp and tilapia, two internationally very important aquaculture species with similar nutritional requirements but different anatomy and digestive physiology, in their ability to utilize plant proteins supplemented with EAA.

MATERIALS AND METHODS

Subsequently, two separate fully randomized feeding experiments with common carp and tilapia were conducted in the aquaculture facilities at the vTI-Institut, Institute of Fisheries Ecology, in Ahrensburg, Germany.

Prior to the feeding experiments, three iso-nitrogenous, iso-lipidic and iso-energetic (on the basis of digestible energy, DE) diets were composed for the two separate feeding experiments. EAA deficiencies (lysine, arginine and threonine) of the plant protein carrier wheat gluten (WG) were supplemented with crystalline amino acids to meet the requirements of common carp (NRC 1993). Table 1 shows the composition of the experimental diets: the diets were formulated to provide 5.6% nitrogen (= 35% crude protein), 10% crude lipids, and 11.1% crude ash. Nitrogen-free-extract was calculated to be 31% in diet FM (100% fish meal protein) and 34% in diets WG (100% wheat gluten protein) and WG+AA (wheat gluten + EAA) to adjust digestible energy.

The carp experiment was carried out in twelve 40 L-tanks, four tanks per diet, in a flow-through system supplied with vented well water for twelve weeks. Water temperature was held at 23°C ± 0.5°C by a heating element. Lighting regime was 12 h light: 12 h dark and the fish were hand-fed three times a day at 8:00, 13:00 and 17:00 hours. The tanks were stocked with seven carp with an initial body mass of 10.3 g ± 0.2 each. To test the effect of EAA supplementation all fish received the same amount of feed. Because feed intake of diet WG was lowest, all fish were fed accordingly with three times the maintenance requirement for common carp per day of 3.2 g $kg^{-0.8}$ (Becker et al. 1993), therefore, carp received a total of 9.6 g $kg^{-0.8}$ per fish per day.

The tilapia experiment was carried out in accordance to the carp experiment in a recirculating system. Twelve tanks were stocked with ten fish per tank with an initial body mass of 12.2 g ± 0.8. Water temperature was 24.8°C ± 1.0°C over the experimental period of 9 weeks. Since feed intake of the wheat gluten diets was very low, 20% of diet FM were added to 80% of diets WG and WG+AA (= WG_T and $WG+AA_T$) so a total amount of 10% fish meal was added to the wheat gluten diets (see table 1). Tilapia were fed a total amount of 10 g $kg^{-0.8}$ per day, supplied via three feedings by hand at 8:00, 13:00 and 17:00 hours.

At the end of both experiments all fish were sacrificed, after being starved for 24 hrs to evacuate guts from feed residues, with 8 g of 2-Phenoxyethanol (807291, Merck KGaA, Germany) per 10 L of water and stored at -20°C until proximate composition analysis. All fish from one tank were thoroughly homogenized using a Retsch Grindomix (GM 200, Retsch GmbH, Germany) at 3000 rpm for 3 minutes. Moisture content of the homogenized fish was determined in duplicates by mixing 5 g of the fresh material with approximately 15 g of sea sand (7712, Merck KGaA, Germany) in a crucible and drying to constant weight at 105°C in a drying oven. Afterwards, homogenized fish samples were freeze-dried for proximate composition analyses. Proximate composition analyses of fish and feeds were conducted according to AOAC (2000), all analyses

were carried out in duplicates: dry matter was determined by drying sample material to constant weight at 105°C in a drying oven. Subsequently, ash content of samples was determined by incineration in a muffle furnace at 550°C for 5 hours. Nitrogen content was determined by varioMAX CN (Elementar Analysesysteme GmbH, Germany) and multiplied with the nitrogen conversion factor of 6.25 to calculate the protein content of fish samples. Lipid content was determined according to Smedes (1999, Koch et al. 2011). Differences between groups (FM/ WG, FM/WG+$AA_{(T)}$, WG/WG+$AA_{(T)}$) were analyzed by T-test for independent samples ($P < 0.05$). All statistical analyses were performed using Statistica Version 8 (StatSoft Inc., Tulsa, OK, USA). Values are expressed as means ± standard deviation.

Table 1 Composition of the experimental diets (g kg^{-1} diet) for common carp (*Cyprinus carpio*; FM, WG, Wg+EAA) and Tilapia (*Oreochromis niloticus*, FM, WG_T, WG+EAA_T).

Ingredients	FM	WG	WG+AA	WG_T	WG+AA_T
Wheat gluten	-	438.2	414.3	350.5	331.5
Fish meal	536.7	-	-	107.3	107.3
Fish oil	22.3	74.6	74.9	64.2	64.4
Sunflower oil	20.0	20.0	20.0	20.0	20.0
Wheat starch	329.8	269.5	274.5	281.5	285.5
Cellulose	103.7	106.7	107.1	106.1	106.4
Vitamin premix[1]	20.0	20.0	20.0	20.0	20.0
Mineral premix[2]	10.0	20.0	20.0	18.0	18.0
Additional minerals[3]	-	89.1	89.1	71.3	71.3
TiO_2	10.0	10.0	10.0	10.0	10.0
Lysine	-	-	11.6	-	9.2
Threonine	-	-	3.5	-	2.8
Arginine	-	-	2.0	-	1.6
Sum	1052.5	1048.1	1047.0	1049.0	1046.5
Sum DM	1000.0	1000.0	1000.0	1000.0	1000.0
Digestible Energy (MJ kg^{-1})	14.1	13.9	13.9	13.9	13.9

[1] Vitamin premix (per kg premix): 500000 I.U. retinol, 50000 I.U. cholecalciferol, 2500 mg tocopherol, 1000 mg menadione, 5000 mg thiamine, 5000 mg riboflavin, 5000 mg pyridoxine, 5000 µg cyanocobalamine, 25000 mg myo-inositol, 10000 mg pantothenic acid, 100000 mg choline chloride, 25000 mg niacin, 1000 mg folic acid, 250 mg biotin, 10000 mg ascorbic acid

[2] Mineral premix (per kg premix): 314.0 g $CaCO_3$, 469.3 g KH_2PO_4, 147.4 g $MgSO_4$ x $7H_2O$, 49.8 g NaCl, 10.9 g Fe(II)gluconat, 3.120 g $MnSO_4$ x H_2O, 4.670 g $ZnSO_4$ x $7H_2O$, 0.620 g $CuSO_4$ x $5H_2O$, 0.160 g KJ, 0.080 g $CoCl_2$ x $6H_2O$, 0.060 g NH_4molybdat, 0.020 g $NaSeO_3$

[3] Additional minerals (kg^{-1} mix): 337.4 g $CaCO_3$, 504.2 g KH_2PO_4, 158.4 g $MgSO_4$ x $7H_2O$

RESULTS

Table 2 shows the proximate composition of the experimental diets. Results for body mass gain and growth parameters are shown in table 3, while body composition of the experimental fish is shown in table 4.

Table 2 Proximate composition of experimental diets based on fish meal and wheat gluten.

Carp	Experiment	FM	WG	WG+AA
DM	(%)	95.6	92.2	93.7
N	(% DM)*	5.6	5.7	5.7
CL	(% DM)	10.1	10.3	10.6
CA	(% DM)	12.4	10.6	10.2
Tilapia	Experiment	FM	WG_T	$WG+AA_T$
DM	(%)	94.5	92.9	93.1
N	(% DM)*	5.6	5.6	5.6
CL	(% DM)	10.3	10.2	10.1
CA	(% DM)	12.7	11.2	11.9

DM = dry matter; N = nitrogen; CL = crude lipids; CA = crude ash; NFE = nitrogen-free extract

* Due to differences in N:P conversion factors of proteins from animal and plant origin (Sriperm 2011) comparisons are done on the basis of N for experimental diets

Final body mass, body mass gain, specific growth rate and metabolic growth rate are highest in carp fed diet WG+AA and accordingly feed conversion ratio is lowest. These parameters are significantly different ($P < 0.05$) from carp fed diets FM and WG, except for feed conversion ratio, where no significant difference was identified between WG and WG+AA. No significant differences for these parameters were identified between carp fed diets FM or WG. In tilapia the parameters final body mass, body mass gain, specific growth rate and metabolic growth rate are lowest in fish fed diet WG_T while they are highest in tilapia fed diet FM. All parameters shown in table 3 are significantly different ($P < 0.05$) between the three diets.

Table 3 Body mass gain, feed conversion, specific and metabolic growth rate of experimental carp and tilapia fed different diets based on fish meal and wheat gluten.

Carp	FM	WG	WG+AA	*P* FM/ WG	*P* FM/ WG+AA	*P* WG/ WG+AA
$BM_{Initial}$ (g)	10.4 ± 0.3	10.2 ± 0.2	10.3 ± 0.2			
BM_{Final} (g)	19.7 ± 1.2	19.7 ± 1.8	25.3 ± 3.8		*	*
BMG (g)	9.4 ± 1.3	9.5 ± 1.8	15.1 ± 3.7		*	*
FCR	2.4 ± 0.3	2.4 ± 0.5	1.7 ± 0.3		*	
SGR (% d^{-1})	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.2		*	*
MGR (g $kg^{0.8}$ d^{-1})	3.2 ± 0.4	3.3 ± 0.5	4.5 ± 0.7		*	*
Tilapia	FM	WG_T	$WG+AA_T$	*P* FM/ WG_T	*P* FM/ $WG+AA_T$	*P* WG/ $WG+AA_T$
$BM_{Initial}$ (g)	12.5 ± 0.5	11.5 ± 1.4	12.6 ± 0.4			
BM_{Final} (g)	25.1 ± 1.5	16.1 ± 0.9	22.6 ± 1.3	*	*	*
BMG (g)	12.6 ± 1.2	4.6 ± 0.8	10.1 ± 1.0	*	*	*
FCR	1.5 ± 0.1	3.3 ± 0.7	1.8 ± 0.2	*	*	*
SGR (% d^{-1})	1.1 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	*	*	*
MGR (g $kg^{0.8}$ d^{-1})	3.6 ± 0.2	1.7 ±0.4	3.1 ± 0.3	*	*	*

BM (g) = body mass; BMG (g) = body mass gain = BM_{Final} - $BM_{Initial}$; FCR = feed conversion ratio = feed fed (g)/ BMG; SGR (% d^{-1}) = specific growth rate = [(ln BM_{Final} - ln $BM_{Initial}$)/ experimental days] x 100; MGR (g $kg^{0.8}$ d^{-1}) = metabolic growth rate = BMG/ [{($BM_{Initial}$/ 1000)$^{0.8}$ + (BM_{Final}/ 1000)$^{0.8}$/ 2]/ experimental days

* Significantly different ($P < 0.05$), T-test for independent samples, (n = 4; carp 7 fish per replicate, tilapia 10 fish per replicate)

Table 4 shows a significant decrease ($P < 0.05$) in moisture and crude protein content in carp fed diet WG compared to diets FM or WG+AA, as well as a significant increase ($P < 0.05$) in crude lipids. Carp fed diets WG and WG+AA also show a significant increase in nitrogen-free extract when compared to diet FM. Tilapia fed diet $WG+AA_T$ show a significant decrease ($P < 0.05$) in moisture and crude protein. Crude lipids are highest in tilapia fed diet $WG+AA_T$ and lowest when fed diet WG_T, while crude ash is vice versa.

Table 4 Body composition of experimental carp and tilapia fed different diets based on fish meal and wheat gluten.

Carp	FM	WG	WG+AA	*P* FM/ WG	*P* FM/ WG+AA	*P* WG/ WG+AA
Moisture (%)	76.8 ± 1.1	74.2 ± 0.5	75.3 ± 0.8	*		
CP (% DM)	59.8 ± 3.9	43.9 ± 1.8	48.0 ± 1.6	*	*	*
CL (% DM)	28.8 ± 4.4	41.0 ± 1.7	37.0 ± 2.2	*	*	*
CA (% DM)	9.2 ± 1.0	10.2 ± 0.4	11.3 ± 1.0		*	
NFE (% DM)	2.2 ± 1.0	4.8 ± 1.6	3.8 ± 0.5	*	*	
GE (kJ)	114.5 ±13.3	130.7 ± 13.2	157.0 ± 17.6		*	
Tilapia	**FM**	**WG_T**	**$WG+AA_T$**	***P* FM/ WG_T**	***P* FM/ $WG+AA_T$**	***P* WG/ $WG+AA_T$**
Moisture (%)	74.5 ± 0.2	75.6 ± 1.1	73.7 ± 0.7			*
CP (% DM)	56.8 ± 1.0	56.9 ± 1.5	54.8 ± 1.1		*	
CL (% DM)	15.5 ± 0.9	11.9 ± 2.6	18.8 ± 0.4	*	*	*
CA (% DM)	23.2 ± 0.7	26.1 ± 2.2	21.1 ± 0.6	*	*	*
NFE (% DM)	4.5 ± 0.4	5.1 ± 1.2	5.4 ± 0.7			

DM (%) = dry matter; CP (% DM) = crude protein; CL (% DM) = crude lipids; CA (% DM) = crude ash; NFE (% DM) = nitrogen-free extract; GE (kJ) = gross energy = [0.2431 x CP (% DM) + 0.3884 x CL (% DM)] x DM of fish (g) (Focken and Becker 1993)

* Significantly different ($P < 0.05$), T-test for independent samples, (n = 4; carp 7 fish per replicate, tilapia 10 fish per replicate)

DISCUSSION

Even though carp and tilapia have similar nutritional requirements, both species show a clearly different response to diets based on fish meal or wheat gluten. While highest body mass gain was obtained with diet WG+AA in carp, tilapia observed highest body mass gain with diet FM. Both carp and tilapia were able to utilize supplemented EAA: in carp, EAA supplementation resulted in higher crude protein content compared to diet WG, but it was lower in comparison to diet FM. Carp fed diet WG show clear

signs of adiposis which is only likely to occur in highly protein deficient diets (Focken and Becker 1993). Nevertheless, crude ash is highest in carp fed diet WG+AA which is a sign of either protein limitation or poor protein quality (Focken and Becker 1993). In tilapia, EAA supplementation resulted in lower crude protein content, as well as higher crude lipid content. Both carp and tilapia seem to have utilized considerable amounts of the supplemented EAA for the assembly of lipid reserves. Further studies have to be performed to verify integration of supplemented amino acids into the fish tissue. This can be achieved by liquid chromatography/ isotope ratio mass spectrometry of $\delta^{13}C$ labeled amino acids (McCullagh et al. 2008, Gaye-Siesegger et al. 2011) and should be considered for further studies.

CONCLUSION

This study shows clear differences in plant protein utilization among carp and tilapia in spite of their similar nutritional requirements. Further studies have to be performed to come to a broader understanding of plant protein utilization among various fish species, why these differences occur and how they can possibly be overcome to increase the use of plant proteins in aquafeeds for omnivorous fish species.

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