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Benfotiamine in high carbohydrate diet enhances growth and reproductive performance of the Asian native catfish *Clarias macrocephalus* subadults

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Abstract

Clarias macrocephalus has been extirpated in most regions in the Philippines and as a preliminary study towards its reintroduction, we aimed at enhancing the growth and reproductive performance of the broodstock. Several studies have identified thiamine deficiency as a factor in the extirpation of some important fish species in the wild. The present study aimed to determine the effects of elevated dietary carbohydrates and benfotiamine, a more bioavailable analog of thiamine (vitamin B_1). Three diets were used, namely, a commercial diet specific for catfish was used as the control diet (Com), a high carbohydrate (HC) diet, and a benfotiamine-supplemented HC diet (HCB). Results show that survival of the subadult catfish in captivity for 10 weeks was remarkably high, exhibiting an average of 99% for all three treatments (P>0.05). HCB diet resulted in significantly higher final average body weight (FABW), weight gain (WG), specific growth rate (SGR), and significantly best food conversion ratio (FCR) (P<0.05) than the values for both Com and HC groups (P>0.05). For the reproductive performance, female Asian catfish fed the HCB diet exhibited significantly heavier ovary and liver (P<0.05) than those female catfish fed either the Com or HC diet (P>0.05). GSI of the female catfish and fecundity were significantly higher in the HCB group (P<0.05) than in the Com or HC group (P>0.05). In the male catfish, testes were significantly heavier in the HCB group (P<0.05) than in the Com or HC diet (P>0.05). HSI, male LW, and LL were significantly higher in the HC and HCB groups (P>0.05) than in the Com group (P<0.05). GSI values of the male catfish were significantly highest in the HCB (P<0.05), followed by the HC group, and significantly lowest in the

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Com group (P<0.05). The larval survival rate for one week was significantly higher (P<0.05) in the Asian catfish fed the HCB diet than in those fed the Com or HC diet (P>0.05). In conclusion, elevating dietary carbohydrates in the diet from 7.70% to 22.73% (i.e., Com to HC diet, respectively) did not negatively affect the growth and reproductive performance of the Asian catfish. Further supplementation of benfotiamine at 0.02% to the HC diet enhanced significantly both the growth and reproductive performance of the Asian catfish.

Introduction

The Asian catfish, *Clarias macrocephalus* is one of the most important but declining fishes in the Philippines (Coniza et al., 2003), which it has been extirpated from most regions of the country. Though native catfish (*C. macrocephalus*) has been overtaken by the African catfish (*C. gariepinus*) as a preferred catfish species in aquaculture, there is still high demand for native catfish because of the better consumer acceptability of its meat and flavor (Coniza et al., 2008). The species was once described by Conlu (1986) as widely distributed in the Philippines, but its availability has now been confined to three separate areas. This is mainly because of anthropogenic factors such as water pollution, conversion of suitable habitats and introduction of larger-sized non-native species (NaNakorn et al., 2004; Tan et al., 2016). Furthermore, very low haplotype and nucleotide diversity are exhibited by the remaining viable populations of *C. macrocephalus* in the wild and are clearly suffering from a genetic bottleneck (Tan et al., 2016). Santos et al. (2015) have claimed that the remaining populations could only be found in two regions in the Philippines, namely Agusan del Sur in the southern and Cagayan Valley in the northern Philippines; however, we have recently confirmed that a third source of *Clarias macrocephalus* exists in Palawan, located at western central Philippines.

Arinez et al. (2020) compared the two populations from Agusan del Sur and those from Cagayan valley through transcriptomics as a preliminary assessment of a possible source of population for reintroduction. They observed that those from Agusan del Sur were better than those in the Cagayan Valley in terms of differentially expressed growth-, immune- and reproduction-related genes. However, our happenstance of the existence of the population in Palawan came a little later but based on our observations on the Asian catfish in the area, the population appeared to be better than those from Agusan del Sur in terms of size of the breeders, quality of eggs and testes. From then on, we have focused our attention on the Palawan extant population.

In some natural bodies of water, some fish populations have been showing negative trends for health biomarkers and recruitment, the cause of which is unknown. Examples of these are the Atlantic salmon *Salmo salar* and the lake trout *Salvelinus namaycus* (Mills et al., 1993). There are many factors that reduce the reproductive success of fish in the wild but the more notable ones involve the natural diet. Fish meat has high activity of the enzyme thiaminase, an enzyme that breaks down thiamine. Fisher et al. (1996) measured tissue concentrations of thiamine hydrochloride and found that they were greatly reduced in these salmonid fish relative to unaffected control stocks. Through yolk-sac injection of or bath immersion in thiamine, complete recovery from the early mortality syndrome was observed. The population of European perch (*Perca fluviatilis*) also suffered from reproductive failure in their natural habitat which include poor recruitment (Bryhn et al., 2020). Deficiency of thiamine (vitamin B_1) has been demonstrated in several species to be one cause for the declining populations (Gustaffson et al., 2021). It could be the case that the Asian catfish population is experiencing a similar situation in most areas in the Philippines.

Thiamine (vitamin B_1) is a vitamin that is present in cells mainly as non-phosphorylated thiamine (T), thiamine monophosphate (TMP), and thiamine diphosphate (TDP)(Nelson &

Cox, 2017). Among these, TDP is a biologically active form and functions as a cofactor for several enzymes. These enzymes are responsible for key pathways involved in converting nutrients into energy and synthesizing building blocks in the cell e.g. transketolase in the pentose phosphate shunt, pyruvate dehydrogenase in glycolysis, alpha-ketoglutarate dehydrogenase in the citric acid cycle (Nelson & Cox, 2017). The primary source of thiamine for fish is through their diet (Harder et al., 2018). In contrast to thiamine, benfotiamine (*S*-benzoylthiamine *O*-monophosphate) is a synthetic S-acyl derivative of vitamin B_1 with much higher bioavailability. Its unique open thiazole ring makes it highly lipid-soluble and enables it to enter directly through the cell membrane resulting in higher bioabsorption and bioavailability. Oral administration of this vitamin has led to increases of T, TMP, and TDP or thiamine pyrophosphate (TPP) (Balakumar et al., 2010). Since benfotiamine is more bioavailable than thiamine itself, we hypothesize in the present study that supplementing a high carbohydrate diet with benfotiamine would result in a more sufficient level of active thiamine in the body of the fish. Simultaneously, it would increase carbohydrate utilization which would lead to a faster growth rate and faster attainment of sexual maturity than it would if a low carbohydrate diet containing a normal amount of dietary thiamine.

Materials and Methods

Experimental fish

Wild native catfish (*Clarias macrocephalus*) were purchased from local fisherfolk some 60 km south of Puerto Princesa City in Palawan, Philippines. Live fish samples were put in a plastic bag with freshwater inside a Styrofoam box and transported to the experimental site in Salem Aquafarm in Puerto Princesa City, Palawan, where the growth trial was conducted. Fish were acclimatized to controlled conditions and fed with commercial diets prior to the start of the feeding trial. Ninety (90) subadult native catfish were divided into 9 rectangular tanks (61 cm x 122 cm x 61 cm) filled with shallow water (about 250 L) at 10 fish·tank^{-1} consisting of 5 males and 5 females in static culture and well-aerated system. Partial siphoning off of wastes was done daily and water was replaced 3 times a week.

Experimental diets and feeding

A total of three experimental diets were used in the study, namely: (1) commercial diet (Com), (2) high carbohydrate diet (about 27 %, HC), and (3) the HC diet supplemented with 0.02 % dietary benfotiamine (HCB). The composition of the experimental diets is presented in **Table 1** which included similar amounts of vitamin and mineral premixes. Triplicate groups of catfish were given the dietary treatments in a completely randomized design for 10 weeks. Fish were fed to satiation twice daily (0500 and 2000 h). Sampling by bulk weighing was done on the first day of the experiment and periodic sampling every 15 days thereafter. Water quality parameters such as dissolved oxygen, temperature, and pH were measured 3 times a week while nitrite, nitrate, and ammonia were monitored once a week using commercially available kits. At the termination of the feeding trial, induced spawning was done followed by artificial fertilization as described below. Liver and gonads (i.e., ovary and testis) were excised and weighed, and liver length was measured.

Induced spawning, artificial fertilization

To measure the spawning performance, females were injected intramuscularly with a commercial solution of gonadotropin and dopamine inhibitor (0.5 ml/kg) following manufacturers' instructions (Ovaprim, Syndel Lab. Ltd., USA) into the dorsal muscle above the lateral line and below the anterior part of the dorsal fin. Stripping was conducted after ovulation the following morning. The dry method of artificial fertilization was carried out. Briefly, male Asian catfish were sacrificed, testis excised, cut into small pieces, and placed onto a small piece of a white mosquito net (20 cm x 20 cm). The cut pieces were squeezed into the stripped eggs contained in a small bowl; the remaining portion of the testis was washed with a small amount of distilled water. The mixture of testis and ovulated eggs was then gently stirred with disinfected chicken feathers for about 5 min and incubation was immediately carried out by spreading the fertilized eggs onto previously disinfected egg mats (~30 cm x 30 cm white mosquito net set on an improvised PVC tube frame) floating on well-aerated tanks.

Table 1 Composition of the experimental diets.

Ingredient	*Experimental Diet*		
	Com	*HC*	*HCB*
Fish meal	-	48.59	48.59
Soybean meal	-	14.69	14.69
Rice bran	-	10.37	10.37
Corn starch	-	20.00	20.00
Soybean oil	-	1.00	1.00
Vit.[a] & Min.[b]	-	2.00	2.00
CMC	-	3.35	3.33
Benfotiamine	0.00	0.00	0.02
Total			
Proximate analyses			
Crude protein	52.67	50.82	52.02
Crude fat	16.31	10.72	10.04
Crude fiber	6.80	1.90	1.90
Ash	13.59	10.05	10.51
Moisture	2.93	3.78	4.45
NFE	7.70	22.73	21.08

[a]*Vitamin mix: (composition expressed as per kg of the vitamin mixture and not of the formulated diet): Vitamin A, 1 200 000 IU·kg^{-1}; Vitamin D3, 200 000 IU·kg^{-1}; Vitamin E, 20 000 IU·kg^{-1}; Vitamin B_1, 8 000 mg·kg^{-1}; Vitamin B_2, 8 000 mg·kg^{-1}; Vitamin B_6, 5 000 mg·kg^{-1}; Vitamin B_{12}, 2000 mcg·kg^{-1}; Niacin, 40 000 mg·kg^{-1}; Calcium Pantothenate, 20 000 mg·kg^{-1}; Biotin, 40 mg·kg^{-1}; Folic Acid, 1 800 mg·kg^{-1}; Ethoxyquin, 500 mg·kg^{-1}.*

[b]*Mineral mix: Fe, 40 000 mg·kg^{-1}; Mn, 10 000 mg·kg^{-1}; Zn, 40 000 mg·kg^{-1}; Cu, 4 000 mg·kg^{-1}; Cu, 4 000 mg·kg^{-1}; I, 1 800 mg·kg^{-1}, Co, 20 mg·kg^{-1}; Se, 200 mg·kg^{-1}.*

Growth parameters

During the experiment, the weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), and survival rate (SR) were computed using the following formula:

Weight Gain (WG, g) = Final Average Body Weight (FABW) – Initial Average Body Weight (IABW)

Specific Growth Rate (SGR, % day^{-1}) = [Ln (Final Average Body Weight in g) – Ln (Initial Average Body Weight in g)] / (No. of days) x 100

Feed Intake (FI, g) = sum of daily feed offered for the whole culture period (g)

Feed Conversion ratio (FCR) = Total Feed Intake (g) / Weight Gain (g)

Survival Rate (%) = (Total no. of fish survived) / (Total no. of fish stocked) x 100

Reproductive Performance

Batch fecundity was estimated for each fish i.e., three pieces of ~0.50 g each were cut from one lobe of the ovary, weighed, and the ripe eggs counted. This procedure assumes no significant differences in the number of eggs per unit weight between the left and the right ovaries. The mean number of eggs per g was then extrapolated for the weight of the ovary. The ovulation rate was calculated by counting the number of ovulated females divided by the total number of injected female catfish multiplied by 100. The fertilization rate was calculated by counting the number of fertilized eggs (i.e., the total number of eggs minus the number of nonviable eggs which appeared opaque and whitish) divided by the total number of eggs multiplied by 100. Counting of fertilized eggs was done at least 12 h from the time the eggs were spread onto the floating mosquito net. The hatching rate was calculated by subtracting the remaining nonviable eggs on the mosquito net from the total number of fertilized eggs multiplied by 100. Survival of offspring was assessed by the hatching rate calculated according to the percentage of eggs that produced live larvae and also by the larval survival rate.

The formulae for the indices described above are given as follows:

Gonadosomatic Index (GSI) (%) = Gonad weight (g) / Body weight (g) x 100

Fecundity, F = (No. of eggs in the sample / Sample weight) x Gonad weight

Ovulation (%) = (No. of fish ovulated / Total no. of fish injected) x 100

Fertilization Rate (%) = (No. of fertilized eggs / Total no. of eggs in a batch) x 100

Hatching Rate (%) = (No. of eggs hatched / Total no. of fertilized eggs) x 100

Survival Rate (%) = (Total no. of survived larvae until day 7 / Total no. of larvae at day 1) x 100

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 24 software was used to perform statistical analysis. Data from the study were presented as mean ± standard error of the mean (SEM) and were tested for normality using the Shapiro-Wilk test and variance homogeneity using Levene's test. The one-way analysis of variance (ANOVA) was used on data that passed the tests while those that did not were subjected to transformation until they passed the tests, after which one-way ANOVA tests were used at $\alpha=0.05$. Post hoc analyses were done using Duncan's Multiple Range Test (DMRT) to identify significant differences among the means and rank them accordingly.

Results

Growth Parameters and Feed Utilization

Survival of the catfish in captivity for 10 weeks was remarkably high, exhibiting an average of 99% for all three treatments ($P>0.05$) (**Table 2**). The HC and HCB groups exhibited higher FABW and better FCR than the Com group (P<0.05). FI, WG, and SGR were significantly highest in the HCB group ($P<0.05$) than did the Com and HC groups which were statistically similar ($P>0.05$). The significantly highest FI in the HCB group indicated a superior attractiveness of the diet.

Table 2 Growth parameters, feed utilization, and survival of native catfish fed the experimental diets for 75 days.

Diet	*IABW (g)*	*FABW (g)*	*WG (g)*	*FI (g)*	*SGR (%·day^{-1})*	*FCR*	*Survival (%)*
COM	130.9 ± 3.6^{a}	148.2 ± 4.7^{b}	17.36 ± 1.07^{b}	78.5 ± 1.8^{b}	0.16 ± 0.01^{b}	4.54 ± 0.17^{b}	100.0 ± 0.0^{a}
HC	134.1 ± 2.8^{a}	157.0 ± 3.6^{ab}	22.93 ± 1.76^{b}	83.3 ± 3.2^{b}	0.21 ± 0.02^{b}	3.70 ± 0.39^{ab}	96.7 ± 3.3^{a}
HCB	132.6 ± 4.9^{a}	166.6 ± 3.5^{a}	33.93 ± 1.60^{a}	106.3 ± 0.7^{a}	0.31 ± 0.02^{a}	3.15 ± 0.12^{a}	100.0 ± 0.0^{a}

Values in the same column with different superscript letters are significantly different (p<0.05). Values were expressed as mean ± SEM; IABW – initial average body weight; FABW – final average body weight; WG – weight gain; FI – feed intake; SGR – specific growth rate; FCR – feed conversion ratio.

Reproductive performance

Asian catfish females fed the HCB diet exhibited significantly heavier ovary and liver ($P<0.05$) than did female catfish fed either the Com or HC diet ($P>0.05$)(**Table 3**) indicating a more active reproductive activity. The liver of HC and HCB female groups were significantly longer in length than that of the Com female group, probably an indication of the readiness of the liver to supply the necessary intermediate metabolites for egg development. GSI of the female catfish and fecundity was significantly higher in the HCB group than in either the Com or HC group, which was clearly associated with the body weight of the female. In contrast, egg diameter, ovulation rate, and HSI did not exhibit any significant differences among the dietary groups ($P>0.05$) despite the that the values were numerically higher in the HCB group than either the Com or HC group. Female liver weight (LW) was significantly higher in the HCB group than in the Com or HC group but female liver length (LL) was statistically similar in both HC and HCB significantly longer than that of the liver of the female in the Com group. In the case of male catfish, testes were significantly heavier in the HCB group ($P<0.05$) than those in the male catfish of the Com or HC group ($P>0.05$). HSI, male LW, and LL were statistically similar in the HC and HCB groups, the values of which were significantly higher than that of the Com group ($P<0.05$)(**Table 4**). GSI values of the male catfish were significantly highest in the HCB, followed by the HC group, and significantly lowest in the Com group. Fertilization and hatching rates did not differ significantly in all treatments but were numerically highest in the HCB group; however, the larval survival rate was significantly higher ($P<0.05$) in the HCB group than in those in the Com or HC groups ($P>0.05$)(**Table 5**).

Table 3 Effects of high carbohydrate (HC) and high carbohydrate + benfotiamine (HCB) diets on the reproductive performance and liver condition of female Asian catfish *Clarias macrocephalus* subadults.

Diet	*Ovary wt (g)*	*GSI_Female*	*Fecundity ($x10^3$)*	*Egg Diameter (mm)*	*Ovulation rate (%)*	*HSI(female)*	*Female LW (g)*	*Female LL (cm)*
Com	7.03 ± 0.60^{b}	5.39 ± 0.41^{b}	3.35 ± 0.36^{b}	1.00 ± 0.06^{a}	77.78 ± 11.11^{a}	0.59 ± 0.02^{a}	0.77 ± 0.03^{b}	1.20 ± 0.00^{b}
HC	8.30 ± 0.44^{b}	5.56 ± 0.24^{b}	3.95 ± 0.24^{b}	1.07 ± 0.07^{a}	88.89 ± 11.11^{a}	0.56 ± 0.11^{a}	0.83 ± 0.09^{b}	1.47 ± 0.03^{a}
HCB	10.80 ± 0.64^{a}	7.00 ± 0.25^{a}	5.07 ± 0.28^{a}	1.13 ± 0.12^{a}	100.00 ± 0.00^{a}	0.67 ± 0.02^{a}	1.03 ± 0.03^{a}	1.53 ± 0.03^{a}

Values in the same column with different superscript letters are significantly different (p<0.05). Values were expressed as mean + SEM; GSI=gonadosomatic index; HSI= hepatosomatic index; Female LW=liver weight of female catfish; Female LL=liver length of female(cm).

Table 4 Effects of commercial, high carbohydrate (HC) and high carbohydrate + benfotiamine (HCB) diets on the reproductive performance and liver condition of the male Asian *catfish Clarias macrocephalus* subadults.

Diet	*Testes wt. (g)*	*GSI_male*	*HSI (male)*	*Male LW (g)*	*Male LL (cm)*
Com	0.30 ± 0.00^{c}	0.25 ± 0.00^{c}	0.41 ± 0.05^{b}	0.50 ± 0.06^{b}	1.20 ± 0.06^{b}
HC	0.50 ± 0.00^{b}	0.37 ± 0.00^{b}	0.64 ± 0.13^{ab}	0.87 ± 0.18^{ab}	1.57 ± 0.12^{a}
HCB	0.67 ± 0.03^{a}	0.46 ± 0.05^{a}	0.71 ± 0.08^{a}	1.03 ± 0.09^{a}	1.70 ± 0.06^{a}

Values in the same column with different superscript letters are significantly different (p<0.05). Values were expressed as mean + SEM; GSI=gonadosomatic index; HSI=hepatosomatic index; LW=liver weight (g); LL=liver length (cm).

Table 5 Effects of commercial, high carbohydrate (HC) and high carbohydrate + benfotiamine (HCB) diets on the reproductive performance of both male and female Asian catfish breeders.

Diet	*Fertilization rate (%)*	*Hatching rate (%)*	*Larval survival rate*
Com	54.24 ± 2.53^{a}	42.24 ± 5.30^{a}	44.71 ± 1.78^{b}
HC	52.51 ± 6.28^{a}	54.65 ± 7.05^{a}	48.69 ± 1.46^{b}
HCB	63.13 ± 10.03^{a}	62.28 ± 7.29^{a}	59.30 ± 1.84^{a}

Values in the same column with different superscript letters are significantly different (p<0.05). Values were expressed as mean + SEM.

Discussion

The role of dietary benfotiamine is to provide bioavailable thiamine in the form of either TDP or TPP which are active forms of this vitamin. Thiamine is a positively charged molecule that requires a carrier to cross biological membranes which make its absorption slower. In contrast, benfotiamine is primarily dephosphorylated in the intestinal villi by extracellular alkaline phosphatase to generate a more soluble compound, S-benzoylthiamine. This compound passes easily through the membranes of intestinal and endothelial cells and into the mesenteric circulation where it is taken up by the liver and hydrolyzed to thiamine by hepatocyte thioesterases (Volvert et al., 2008). The excess hepatic thiamine is released into the bloodstream where it is captured by erythrocytes and transformed into TDP by the action of TPP-kinase. Within a short period, benfotiamine was able to produce the highest thiamine (also TMP and TDP) concentration in rats as reported by Portari et al. (2013).

Thiamine occurs in the environment at extremely low concentrations; its availability depends on factors such as pH, ultraviolet radiation, and temperature. It is primarily produced by micro and macroalgae, as well as by some fungi and bacteria (Kraft & Anger 2017). Overall, thiamine has been linked to a number of important biological processes, especially during reproduction and early development (Wilson 1997). Also, there is the problem of the presence of a considerable amount of thiaminase, an enzyme that breaks down thiamine, which is present in the viscera of certain raw fishes and shellfish. Considering that *C. macrocephalus* is a carnivorous fish that feeds on worms, insects, crustaceans, and other benthic organisms (Coniza et al., 2008) which may contain a considerable amount of thiaminase, it is very likely that the Asian catfish in the wild might also be suffering from thiamine deficiency. Morito et al. (1986) observed a reduced growth rate when rainbow trout was exposed to a low-thiamine experimental diet. Estimating the amount of thiamine (vitamin B_1) in all the experimental diets in the present study to be 1.6 $mg{\cdot}kg^{-1}$ diet, it was doubtful whether it could provide for the thiamine requirement of the native catfish, which currently have not been determined. Considering that (a) the requirement range for freshwater fish was 0.5 to 10 $mg{\cdot}kg^{-1}$ (Mai et al., 2022), (b) that thiamine has low bioavailability and absorption in humans (Xie et al., 2014), and (c) is excreted quickly from the body due to its water-soluble characteristic (Beltramo et al., 2008), the amount provided by the vitamin premix might ultimately fell short of the requirement in the present study. Possibly, this could be the reason for the lower growth performance of fish fed Com and HC diets relative to those fed the HCB diet. There is a need to study the physiology of thiamine requirement in *Clarias macrocephalus* to give clarity to this hypothesis.

The similarity of the growth performance of the Com and HC groups indicated that *C. macrocephalus* could handle elevated dietary carbohydrates in the HC diet (from 7.70% dietary carbohydrate in the Com diet to 22.73% in the HC diet). This was expected considering that reportedly, it could shift from being carnivorous in the wild into being omnivorous in culture ponds (Coniza et al., 2008). High dietary carbohydrates usually result in persistent hyperglycemia, which is regarded as a physiological stress response leading to the growth retardation of fish (Hemre et al., 2002). In blunt snout bream (*Megalobrama amblycephala*), an herbivorous fish species, HSI values of fish fed the HC diet were all lower than that of the C group (fish fed low carbohydrate diet and no benfotiamine supplementation)(Xu et al., 2018). They ascribed this observation to its being herbivorous and logically, it follows that it had a higher glucose tolerance that led to the quick removal of excess glycogen and lipid from the liver. This is consistent with the findings of Lauzon (2020) who reported that Nile tilapia *Oreochromis niloticus* fed the HCB diet exhibited better glucose tolerance than did those fed the control (no benfotiamine, low carbohydrate) or HC diet. Supplementation of dietary benfotiamine to the HC diet (i.e. HCB diet) in the present study resulted in more efficient utilization of dietary carbohydrates that effectively and significantly increased the FABW, WG, SGR, FCR, and HSI in both males and female catfish indicating sufficient thiamine level that circulated in the system of catfish. The findings of the present study also suggested a beneficial effect of benfotiamine on the growth performance of a catfish-fed high carbohydrate diet at 0.02% (0.2 $g{\cdot}kg^{-1}$ diet). Huang et al. (2011) have demonstrated in *Cyprinus carpio var Jian* that adequate thiamine (the analog of benfotiamine) levels could promote the activities of intestinal digestive and brush border enzymes of fish, thus improving nutrient absorption and feed efficiency, and this is might have promoted growth performance. Benfotiamine administration could reduce the hyperglycemic damage in mammals through the inhibition of AGEs (advanced glycation end products from carbohydrate metabolism) formation and other metabolic pathways (Hammes et al., 2003). Xu et al. (2018) assert that it is possible that a similar mechanism also exists in fish but the hypothesis awaits further testing. Together, these effects could be beneficial to the glucose homeostasis of fish-fed carbohydrate-enriched diet that would result in improved carbohydrate utilization by fish.

Thiaminase, commonly found in fish meat, produces severe thiamine deficiency in wild stocks (Honeyfield et al., 2005). It leads to a decline in the overall egg thiamine content (Wiegand et al., 2011) which eventually causes early mortality syndrome and increased mortalities in fish larvae (Keinanen et al., 2012). They have demonstrated that thiamine injection has positive effects on reproductive performance in the sturgeon *Acipenser ruthenus,* and the negative impacts of anti-thiamine in the offspring can be reduced by the injection of this vitamin into the broodstock. In literature, there is a scarcity of information on how thiamine or its analog benfotiamine affects reproductive performance in fish. In the present study, dietary benfotiamine resulted in a heavier, bigger ovary and thus higher fecundity but not a bigger egg diameter. This is in contrast with the observation of Mitchell (2020) that a high-thiaminase diet (that caused thiamine deficiency) was associated with smaller egg diameter; it is also the observation in the wild-caught Atlantic salmon that exhibited symptoms of thiamine deficiency which had smaller eggs than salmon that did not exhibit symptoms (Amcoff et al., 2000). Thiamine injection in the sturgeon did not affect the plasma estradiol 17β concentration (Ghiasi et al., 2017). The working fecundity in the sturgeon was not affected by thiamine injection, but more eggs were obtained from fish treated with 50 $mg{\cdot}kg^{-1}$ BW of thiamine (Ghiasi et al., 2017). There is a scarcity of studies regarding the effect of thiamine on fecundity. In the present study, GSI and fecundity were significantly highest in the HCB group associated with body weight. Although the HCB group exhibited numerically the largest eggs, this was not significantly different from the other groups because of considerable variability. The increase in egg size could be due to the increase in vitellogenin deposition in eggs, as hypothesized by Ghiasi et al. (2017) in sturgeon.

Thiamine has been detected in the milt of Atlantic salmon, but its function is not well-characterized (Koski 2002). Mitchel (2020) did not find a significant effect of a high thiaminase diet (i.e. effectively a thiamine-deficient diet) on sperm count or sperm motility or sperm velocity. This observation agrees with the present study that the fertilization rate was unaffected by any of the diets. However, the larval survival rate of catfish was elevated by the dietary benfotiamine in the present study, in contrast with the results of Mitchell (2020) that no differences were observed in the mortality of the Atlantic salmon during the egg to alevin stage (or during the alevin to fry state). Thus, the importance of benfotiamine or thiamine in milt remains uncertain. Thiamine injection in male sturgeon (Ghiasi et al., 2017) caused a significant increase in plasma testosterone concentration in those fish injected with 50 $mg{\cdot}kg^{-1}$ BW of thiamine. This observation was the first on the effect of thiamine on sex steroids, and yet, there is no clear explanation regarding this observation offered in the literature.

In fish, GSI has often been used to indicate relative gonadal development or activity (Rizzo & Bazzoli, 2020). In the present study, dietary benfotiamine resulted in significantly highest GSI values in male and female Asian catfish isometrically associated with body weight. Gonadal recrudescence in female fish involves an accumulation of lipid and protein stores within the developing oocytes (Mahboob et al., 1990) which may come from the liver. In contrast with GSI, HSI values in the female were not affected by the dietary treatments, although female LW and LL were significantly higher in the HCB group than in the female catfish in the Com or HC group. This indicated that the female liver's size (i.e., LW and LL) was isometrically associated with body weight. Since the HSI value reflected liver participation in vitellogenesis, the dietary treatment did not affect this process in the female catfish. Male HSI values were significantly increased in those fed either the HC or HCB diet, and the increase was numerically higher than just an isometric association with body weight. This indicates that high dietary carbohydrates, whether or not supplemented with benfotiamine, resulted in higher energetic conditions or energy reserves in the male catfish (Lamber & Dutil, 1997).

In conclusion, elevating dietary carbohydrates in the diet from 7.70% to 22.73% (from dietary carbohydrate levels in the Com and HC diets, respectively) did not negatively affect the growth and reproductive performance of the Asian catfish. Supplementing benfotiamine at 0.02% to the high carbohydrate diet significantly enhanced the growth and reproductive performance of the sub-adult catfish.

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References

Amcoff, P., Akerman, G., Borjeson, H., Tjarnlund, U., Norrgren, L., Balk, L., 2000. Hepatic activities of thiamine-dependent enzymes, glucose-6-phosphate dehydrogenase and cytochrome P4501A in Baltic salmon (*Salmo salar*) yolk-sac fry after thiamine treatment. *Aquat. Toxicol.* 48, 391–402. https://doi.org/10.1016/S0166-445X(00)00089-8

Arinez, J.B., Deallo, A.G., Tumbokon, B.L.M., Serrano Jr., A.E., 2020. Evaluation of two extant population sources of *Clarias macrocephalus* using liver transcriptomic profiles towards reintroduction. *The Israeli Journal of Aquaculture-Bamidgeh, IJA_71*.2020.1118385, 12 pages. https://doi.org/10.46989/001c.19030

Balakumar, P., Rohilla, A., Krishan, P., Solairaj, P., Thangathirupathi, A., 2010. The multifaceted therapeutic potential of benfotiamine. *G. Pharmacol. Res.,* 61(6):482-488. https://doi.org/10.1016/j.phrs.2010.02.008

Beltramo, E., Berrone, E., Tarallo, S., Porta, M., 2008. Effects of thiamine and benfotiamine on intracellular glucose metabolism and relevance in the prevention of diabetic complications. Acta Diabetol. 45: 131-141. https://doi.org.10.1007/s00592-008-0042-y

Bryhn, A., Sundelöf, A., Lingman, A., A.B. Florin, A.B., Petersson, E., Vitale, F.,Sundblad, G., Strömberg, H., Wennhage, H., Wickström, H., AhlbeckBergendahl, I., Olsson, J.,Lövgren, J., Persson, J., Sundin, J., Lundström, K., L. Edsman, L., Ogonowski, M., Ulmestrand, M., Lindmark, M., Bergenius, M., Sjöberg, M., N., Holmgren, N., Renman, O., Naddafi, R., Fredriksson, R., Larsson, S., Axenrot, T., Dekker, W., Hekim Pekcan, Z., 2020. Fisk-och skaldjursbestånd i hav och sötvatten 2019: Resursöversikt [*Fish and shellfish stocks in marine and freshwater areas 2019: Synthesis of the fisheries resources*] (In Swedish).

Coniza, E.B., Catacutan, M.R., Tan-Fermin, J.D., 2008. Growth and yield of Asian catfish *Clarias macrocephalus* (Gunther) fed different grow-out diets. *The Israeli Journal of Aquaculture – Bamidgeh* 55(1), 2003, 53-60.

Coniza, E.B., Catacutan, M.R., Tan-Fermin, J.D., 2008. Grow-out culture of the Asian catfish Clarias macrocephalus (Gunther). *Aquaculture Extension Manual* No. 41. SEAFDEC AQD *Tigbauan, Iloilo, Philippines*.

Conlu, P.V., 1986. Guide to Philippine flora and fauna. Vol. 9. Fishes. Quezon City: Natural Resources Management Center, *University of the Philippines*, 495p.

Hammes, H.P., Du, X., Edelstein, D., Taguchi, T., Matsumura, T., Je, Q., Lin, J., Bierhaus, A., Nawroth, P., Hannak, D., Neumaier, M., Bergfeld, T., Giardino, I., Brownlee, M., 2003. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat. Med., 9:294–299. https://doi.org/10.1038/nm834

Fisher, J. P.,Fitzsimons, J.D.,Combs Jr., G.F.,Spitsbergen, J.M., 1996. Naturally occurring thiamine deficiency causing reproductive failure in Finger Lakes Atlantic salmon and Great Lakes lake trout. *Transactions of the American Fisheries Society* 125:167–178. https://doi.org/10.1577/1548-8659(1996)125<0167:NOTDCR>2.3.CO;2

Ghiasi, S., Falahatkar, B., Arslan, M., Dabrowski, K., 2017. Physiological changes and reproductive performance of Sterlet sturgeon Acipenser ruthenus injected with thiamine. *Anim. Reprod. Sci.* 178:23–30. https://doi.org/10.1016/j.anireprosci.2017.01.005

Gustafsson, J., Strom, K., Arvstrand, L., Forlin, L., Asplund, L., Balk, L., 2021. Adult female European perch (*Perca fluviatilis*) from the Baltic Sea show no evidence of thiamine deficiency. *J. Sea Res.*, 174:102081. https://doi.org/10.1016/j.seares.2021.102081

Hammes, H. P., Du, X., Edelstein, D., Taguchi, T., Matsumura, T., Ju, Q., et al., 2003. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat. Med.* 9, 294–299. doi: 10.1038/nm834

Harder, A.M., Ardren, W.R., Evans, A.N., Futia, M.H., Kraft, C.E., Marsden, J.E.,Richter, C.A., Rinchard, J., Tillitt, D.E., Christie, M.R., 2018. Thiamine deficiency in fishes: causes, consequences, and potential solutions. *Rev. Fish Biol. Fish.*, 28: 865-886. https://doi.org/10.1007/s11160-018-9538-x

Hemre, G. I., Mommsen, T. P., and Krogdahl, A., 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquac. Nutr.* 8, 175–194. https://doi.org.10.1046/j.1365-2095.2002.00200.x

Honeyfield, D.C., Hinterkopf, J.P., Fitzsimons, J.D., Tillitt, D.E., Zajicek, J.L., Brown, S.B., 2005. Development of thiamine deficiencies and early mortality syndrome in lake trout by feeding experimental and feral fish diets containing thiaminase. *J. Aquat. Anim. Health* 17, 4–12. https://doi.org/10.1577/H03-078.1

Huang, H.H., Feng, L., Liu, Y., Jiang, J., Jiang, W.D., Hu, K., Li, S.H., Zhou, X.Q., 2011. Effects of dietary thiamin supplement on growth, body composition and intestinal enzyme activities of juvenile Jian carp (*Cyprinus carpio* var. Jian*). Aquac. Nutr.* 17:233-240 https://doi.org/10.1111/j.1365-2095.2010.00756.x

Johnston, T.A., Wiegand, M.D., Mittermuller, S., Casselman, J.M., Pyle, G.G., Leggett, W.C., 2008. Metal provisioning of ova in walleye and lake whitefish. *Aquaculture* 281, 131–137. https://doi.org/10.1016/j.aquaculture.2008.05.039

Keinänen, M., Uddström, A., Mikkonen, J., Casini, M., Pönni, J., Myllylä, T., Aro, E., Vuorinen, P.J., 2012. The thiamine deficiency syndrome M74, a reproductive disorder of Atlantic salmon (*Salmo salar*) feeding in the Baltic Sea, is related to the fat and thiamine content of prey fish. ICES J. Mar. Sci. 69, 516–528. https://doi.org/10.1093/icesjms/fss041

Koski, P. 2002. Parental background predisposes Baltic salmon fry to M74 syndrome. *Acta Veterinaria Scandinavica*, 43(2): 127-130. https://doi.org/10.1186/1751-0147-43-127

Kraft, C. E. and Angert, E. R. 2017. Competition for vitamin B1 (thiamin) structures numerous ecological interactions. *The Quarterly Review of Biology*, 92(2): 151-168.

Lambert, Y., Dutil, J.-D., 1997. Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of cod (*Gadus morhua*)? *Can. J. Fish. Aquat. Sci.,* 54*:*104-112. https://doi.org/10.1139/f96-149

Lauzon, Q.D., Canillo, S.D.T., Tumbokon, B.L.M., Serrano Jr., A.E., 2019. Effects of High Carbohydrate and Benfotiamine on the Growth and Feed Efficiency of Juvenile Nile Tilapia, *Oreochromis niloticus*. The Israeli Journal of Aquaculture-Bamidgeh, IJA_71.2019.1635, 8 pages.

Lauzon, Q.D., 2020. Evaluation of dietary benfotiamine to enhance dietary carbohydrate utilization of Nile tilapia. Ph.D. Dissertation. University of the Philippines Visayas. Iloilo, Philippines. 116 pages.

Mahboob, S., Sheri, A.N., Javed, M., 1990. Observation on the biology of *Cyprinus carpio*. The 10th Congress of Zoology 26 27 May, Khanspur (Murree). *Pak. J. Zool. Abstract*, Series No. 10.

Mai, K., Waagbo, R., Zhou, X.Q., Ai, Q.H., Feng, L., 2022. Vitamins. In *Fish Nutrition* (Hardy, R.W., Kaushik, S.J., Eds). Elsevier Inc., Oxford, UK. P. 57-179.

Mills, E. L., Casselman, J. M., Dermott, R., Fitzsimons, J. D., Gal, G., Holeck, K. T., Millard, E. S. 2003. Lake Ontario: Food web dynamics in a changing ecosystem (1970-2000). Canadian *Journal of Fisheries and Aquatic Sciences,* 60(4): 471-490. https://doi.org/10.1139/f03-033

Mitchell, K.T., 2020. Effects of dietary thiaminase on reproductive traits in three populations of Atlantic salmon targeted for reintroduction into Lake Ontario. M.S. Thesis. *The University of Western Ontario. Repository*. 6826. https://ir.lib.uwo.ca/etd/6826

Morito, C. L. H., Conrad, D.H., J. W. Hilton, J.W., 1986. The thiamin deficiency signs and requirement of rainbow trout (*Salmo gairdneri*, Richardson). *Fish Physiology and Biochemistry* 1:93–104. https://doi.org/10.1007/BF02290209

Na-Nakorn, U., Kamonrat, W., Ngamsiri, T., 2004. Genetic diversity of walking catfish, *Clarias macrocephalus*, in Thailand and evidence of genetic introgression from introduced farmed **C. gariepinus**. Aquaculture 240: 145–163. https://doi.org/10.1016/j.aquaculture.2004.08.001.

Nwadukwe, F.O., Ayinla, O.A., Abby-Kalio, N. J., 1993. Effect of various doses of Acetone-dried powdered Carp pituitary extract and season on hatchery propagation of *Heterobranchus longifilis* (Val.1840) (Pisces: Clariidae), J. Aqua. Trop. 8:33-40.

Nelson, D.L., Cox, M.M., 2017. Principles of Biochemistry. Addison-Wesley 7th Ed. https://doi.org/10.1007/978-3-662-08289-8

Portari, G.V., Vannucchi, H., Jordao, A.A., 2013. Liver, plasma and erythrocyte levels of thiamine and its phosphate esters in rats with acute ethanol intoxication: a comparison of thiamine and benfotiamine administration. *Eur. J. Pharm. Sci.,* 48:799–802. https://doi.org/10.1016/j.ejps.2013.01.010

Rizzo, E., Bazzoli, N., 2020. Reproduction and embryogenesis. In Biology and Physiology of Freshwater Neotropical Fish. Academic Press, Inc. 287-313. https://doi.org/10.1016/B978-0-12-815872-2.00013-0

Santos, B.S., Vesagas, F.P.C., Tan, M.T.C., Jumawan, J.C., Quilang, J.P., 2015. Status assessment of *Clarias* species in the Philippines: insights from DNA barcodes. *Science Diliman* 27(2):21-40.

Tan, M.T.C., Jumawan, J.C., Quilang, J.P., 2016. Low genetic diversity in *Clarias macrocephalus* Günther, 1864 (Siluriformes: Clariidae) populations in the Philippines and its implications for conservation and management. *J. Threat. Taxa* 8(6): 8849-8859. https://doi.org/10.11609/jott.2261.8.6.8849-8859.

Viveen, W.J.A.R, Richter, C.J.J., Van-Ordt, P.G., Janseen, J.A.L., Huisman, E.A., 1985. *Practical Manual for the culture of the African Catfish (Clarias gariepinus).* Section for Research and Technology, Box 20061, 5600 EB. The Hague, The Netherlands, Pages: 121.

Volvert ML, Seyen, S., Piette, M., Evrard, B., Gangolf, M., Plumier, J.P., Bettendorff, L., 2008. Benfotiamine, a synthetic S-acyl thiamine derivative, has different mechanisms of action and a different pharmacological profile than lipid-soluble thiamine disulfide derivatives.
BMC Pharmacology 2008: 8-10. https://doi.org/10.1186%2F1471-2210-8-10

Wiegand, M.D., Johnston, T.A., Brown, L.R., Brown, S.B., Casselman, J.M., Leggett, W.C., 2011. Maternal influences on thiamine status of walleye (*Sander vitreus*) ova. *J. Fish Biol.* 78, 810–824. https://doi.org/10.1111/j.1095-8649.2010.02895.x

Xie, F.F., Cheng, Z.N., Li, S.W., Liu, X.I., Guo, X., Yu, P., Gu, Z.K., 2014. Pharmacokinetic study of benfotiamine and the bioavailability assessment compared to thiamine hydrochloride. J. Clin. Pharmacol. 54: 688-695. https://doi.org/10.1002/jcph.261

Xu, C., Liu, W.-B., Zhang, D.-D., Shi, H.-J., Zhang, L., Li, X.-F., 2018. Benfotiamine, a lipid-soluble analog of vitamin B_1, improves the mitochondrial biogenesis and function in blunt snout bream (*Megalobrama amblycephala*) fed high-carbohydrate diets by promoting the AMPK/PGC-1β/NRF-1 Axis. *Front. Physiol.,* 03 September 2018 https://doi.org/10.3389/fphys.2018.01079