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Effect of Niacin and Folic Acid in Feed Rations on Growth and Live Weights of Green Catfish (*Mystus nemurus* Valenciennes 1840)

[1]D.V. Hien and [2]S. Doolgindachbaporn
[1]Division of Aquaculture, Faculty of Agriculture-Forestry-Aquaculture,
Quangbinh University, Quangbinh, Vietnam
[2]Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract: This study aims to justify effects of niacin and folic acid in feed rations on growth and survival rate of the fish under *in vitro* conditions. A Completely Randomized Design (CRD) with four replications for Experiment I and three replications for Experiment II was used. Niacin (0, 5, 10, 20 and 40 mg kg^{-1}) and folic acid (0, 2, 4, 6 and 8 mg kg^{-1}) were used for Experiments I, and II, respectively. The cement tanks were used for Experiment I and fibre tanks for Experiment II. A static water system was used. Water temperature was maintained at a range from 26 to 30°C with pH values from 7.4 to 7.9. The results showed that niacin at a rate of 10 mg kg^{-1} feed ration gave significant differences on weight gained %, survival %, protein efficiency ratio, specific growth rate % and feed conversion ratio with mean values of 99.92, 100, 1.18, 5.77 and 1.35, respectively. Folic acid at a rate of 2 mg kg^{-1} gave significant differences on weight gained %, survival %, protein efficiency ratio, specific growth rate % and feed conversion ratio with mean values of 108.88, 100, 1.22, 6.13 and 1.43, respectively. Niacin at a rate of 10 and 2 mg kg^{-1} of folic acid were the best rates for *in vitro* culture of Green catfish.

Key words: Folic acid, Green catfish, growth rate, *Mystus nemurus*, niacin

INTRODUCTION

In carrying out fish experiment with many important fish species for economic purposes, one must involve with many research aspects in fish culture in order to attain the utmost outcome of the work. It has been recognized that some vitamins derive from growth substances such as niacin and folic acid have played its important role in increasing growth and production of many fish species. For niacin substance, there had been a large number of experiments carried out by different authors, e.g., Ng *et al.* (1997) carried out an experiment in the USA with the use of Channel catfish for 12 weeks they used different rates of niacin in feed ration. They reported in Experiment 1 that niacin concentrations increased fish liver NAD concentrations linearly (r = 0.98). An amount of 7.4 mg kg^{-1} diet gave a rapid growth. In contrast, Morris *et al.* (1998) also with a niacin experiment in the United Kingdom where they used African catfish being fed with higher rates of niacin in feed diet and reported that after 126 days of feeding, the fish fed with diets containing niacin less than 33.1 mg kg^{-1} resulted in sub-optimal feed efficiency and poor protein utilization. They recommended that niacin rate for African catfish culture should be no less than 33.1 mg kg^{-1} diet. Mohamed and Ibrahim (2001) carried out experiment in India with Indian catfish reported that niacin at a rate of 20 mg kg^{-1} diet gave the highest growth rate than the rest and the differences were large and statistically significant. For the work of Ahmed (2010) carried out in India with Indian major carps viz., *Labeo rohita* and *Cirrhinus mrigala* reported that the recommended rates of niacin for the two fish species were 33 and 30 mg kg^{-1} diet for *L. rohita* and *C. mrigala,* respectively. These published information emphasized the significant role of niacin substance yet some other workers have paid their attention in culturing fish with the use of folic acid where they aimed to achieve a similar result as that of the niacin substance, e.g., Cowey and Woodward (1993) carried out the work in Canada with rainbow trout and Duncan *et al.* (1993) carried out an experiment with Channel catfish. These workers have reported the rapid growth of their fish and signified the important role of folic acid in fish culture. In addition, there had been a large number of publications concerning the use of folic acid added to their feed rations aiming to achieve a rapid growth of the fish, e.g., Shiau and Huang (2001a) carried out the work in Taiwan with juvenile Tilapia (*Oreochromis niloticus* x *O. aureus*), with

Corresponding Author: Dr. Sompong Doolgindachbaporn, Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

eight levels (0, 0.3, 0.6, 1.0, 3.0, 6.0, 10.0 and 20 mg kg^{-1} feed ration). Shiau and Huang (2001b) with eight levels (0, 1, 2, 5, 10 15, 30 and 60 mg kg^{-1} feed ration) where they used Grass shrimp (*Penaeus monodon*) for the experimental work, whilst Barros *et al.* (2009) with Nile Tilapia also used eight levels of folic acid, i.e., 0, 0.5, 1.0, 2.0, 3.0, 4.0 and 6.0 mg kg^{-1} feed ration.

Green catfish (*Mystus nemurus* Valenciennes 1840), one of the important economic fish in Southeast Asia have been commonly found in many wild inhabitations in Thailand and Indonesia (Rainboth, 1996). This type of fish has been widely accepted in the markets both domestic and overseas due to its high palatability of flesh and a good flesh texture. It seems more likely that data on Green catfish with the use of niacin and folic acid as sources of vitamins are limited. Thus it may be of tangible value to carry out experiments on Green catfish (*Mystus nemurus* Valenciennes 1840) with the use of different rates of both sources of soluble vitamins. The objectives in carrying out this experiment include: (1) the search for the information on the survival rate and growth rate of Green catfish in relation to different rates of both niacin and folic acid in feed ration and (2) the most appropriate rates on growth of the fish due to both niacin and folic acid when added in the feed rations on live-weight of Green catfish.

MATERIALS AND METHODS

There were two experiments carried out, i.e., Experiments I was carried out with the use of five different rates of niacin, i.e., 0 (T_1) control, 5 (T_2), 10 (T_3), 20 (T_4) and 40 mg kg^{-1} dry diet (T_5) and the Experiment II was dealing with five different rates of folic acid, i.e. 0 (T_1) control, 2 (T_2), 4 (T_3), 6 (T_4) and 8 mg kg^{-1} dry diet (T_5). Thus each experiment had five treatments. The experiments were carried out at the Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Thailand during a period from March to June 2010 for both experiments. Both experiments were laid in a Completely Randomized Design (CRD) with four replications for the Experiment I and three for the Experiment II. The formula or recipe of feed ration used for both experiments was similar to the formula reported by Boonarsa and Doolgindachbaporn (2009). This previous recipe of ration had been confirmed its adequate amountof nutritive value for growth of Green catfish (Boonarsa and Doolgindachbaporn, 2009). The ration formula composed of many important ingredients such as fish meal, soybean meal, maize meal, soybean oil and others. Each ingredient has its specific amount (Table 1). This feed ration formula had a crude protein content of 31.17% (on dry weight basis). When it comes to treatments used, each rate of both niacin and folic acid was thoroughly mixed by hand into their respective treatments with the use of distilled water (approximately 300 g kg^{-1} diet) and then the stuffs were made into pellets and then fan-dried at room temperature to attain a moisture content of approximately 9.33%. This value of moisture content was attained when tested in an oven of 100°C with the use of the initial feed weight before and after being placed in the ventilated hot air oven for 12 h.

Table 1: Different kinds of ingredients and its respective amounts being used in the formula of feed ration being used for Green catfish experiments

Item	Amount
Ingredients (g kg^{-1} diet)	
Fish meal	300.00
Soybean meal	179.00
Rice meal	209.00
Rice bran	11.00
Powder	84.165
Coru meal	100.00
Full fat soybean	64.00
Soybean oil	45.00
Vitamin mixture[1]	0.198
Mineral mixture[2]	7.637
Proximate composition based on dry weight (%)	
Crude protein	31.17
Crude lipid	9.38
Crude ash	8.09
Moisture	9.33
Crude fiber	0.85
Cross energy (kcal/100 g feed)	470.35

[1]Vitamin mixture (g kg^{-1} mixture): retinyl acetate (500 000 IU g^{-1}), 0.06 g; cholecalciferol (500 000 IU g^{-1}), 0.012 g; D,L-a-tocopherol acetate, 0.009 g; menadione, 0.00525 g; thiamin nitrate, 0.00375 g; riboflavine, 0.006 g; pyridoxine hydrochloride, 0.006 g; niacin, 0.045; folic acid (96%), 0.00075 g; cyanocobalamin (10%), 0.00005 g; ascoryl acetate (92%), 0.045 g; Ca pantothenate, 0.04 g. [2] Mineral mixture (g kg^{-} mixture): $FeSO_4.7H_2O$, 0.03 g; $CuSO_4.5H_20$ (25.00% copper), 0.006 g; $ZnSO_4.7H_2O$ (22.50% zinc), 0.6 g; $MnSO_4.H_2O$ (31.80% manganese), 1 g; KI (3.8% iodine), 0.001 g; $CaCO_3$, 6 g

The catfish fingerlings were attained from the Sakolnakorn Freshwater Fisheries Centre, Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand. The fish were acclimatized to laboratory conditions for two months in a 1,000 L fibre-tank upon arrival. They were fed with a commercial catfish diet (in land production) where the formula consisted of crude proteins (40%), lipid (3%), fibre (3%), with moisture content of 12%. For Experiment I, 500 individuals Green catfish of a similar size (18.72 g) were transferred into 20 cement tanks (each tank has dimensions of 103.5×78 cm in breadths and 28 cm in height) and each tank has a population of 25 fish with a volume of freshwater of 125 L. With Experiment II, 15 fibre tanks were used and each was allocated with 200 L of freshwater. 600 individuals Green catfish of a similar size (20.02 g) were allocated into their respective treatments, 120 fish for each treatment, i.e. 40 fish for each replication. Experiment I was carried out at one week earlier than Experiment II. That is

why the weight of fish was slightly greater for Experiment II than Experiment I. A static water system was used in each experiment to provide adequate aeration and for the first four weeks an approximately 25% of water in each tank was taken away daily at 7 a.m. and this amount was replaced by freshwater immediately. Likewise, an amount of 50% of water in each tank was taken out where the same manner was repeated during the final eight weeks. The feed diets were hand-fed daily to the fish at a rate of 2.5% of body weight. This feeding rate was similar to the maximum amount of ration being fed to Green catfish as reported by Ng *et al.* (2000). The fish were weighed out tank by tank each week and the amount of feed being given was adjusted accordingly. A weekly cleaning of the tanks was carried out, i.e. the tank walls were scrubbed away and washed before refilling the new freshwater and then returned the fish into their respective treatments. The feeding trial of both experiments was carried out for 12 weeks duration. Throughout the experimental period, water temperature was maintained at 26-30°C, salinity at 0-2 ppt, pH values from 7.4-7.9. Dissolved O_2 was no less than 6.0 mg L^{-1}, and the ammonia content was not determined. The calculations were carried out followed the tools used by Aliyu-Paiko *et al.* (2010):

$$\text{Weight Gained (WG \%)} = \left[\frac{(W_f - W_i)}{W_i}\right] \times 100$$

$$\text{Specific Growth Rate (SGR \%)} = \left[\frac{(\ln W_f - \ln W_i)}{T}\right] \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total feed intake (g)}}{\text{Total wet weight gained (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Final number of surviving fish}}{\text{Initial number of fish}} \times 100$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Wet weight gained (g)}}{\text{Total protein intake (g)}}$$

All of the collected results were calculated with the use of a computer programme (SAS, 1998).

RESULTS

Initial body weight, weight gained, survival rate, protein efficiency ratio, specific growth rate and feed conversion ratio: For Experiment I, the effect due to different rates of niacin on different measured parameters of the Green catfish showed that mean values of initial weights of individual fish ranged from 18.70 to 18.78 g for T_4 and T_1, respectively (Table 2). The mean values of weight gained of the fish (taken from four replications of each treatment) ranged from 50.04 to 99.92 g for T_1 and T_3, respectively. The mean values of survival rate expressed in percentages ranged from 86 to 100 for T_1 and T_3, respectively. Mean values of protein efficiency ratio ranged from 0.63 to 1.18 g for T_1 and T_3, respectively. Mean values of specific growth rate (% $week^{-1}$) ranged from 3.38 to 5.77 for T_1 and T_3, respectively and finally mean values of feed conversion ratio ranged from 1.35 to 1.69 g for T_3 and T_1, respectively.

With the Experiment II with Green catfish on the effect due to Folic Acid (FA) the results revealed that mean values of initial body live weights of individual fish of the five treatments ranged from 19.89 to 19.93 g for T_2 and T_1, respectively (Table 3). There was no statistical difference found among the fish used at the beginning of the experimental period. The results on final weights gained after 12 weeks revealed that mean values of live weights gained (taken as a mean from replications in each treatment) ranged from 57.35 to 108.88 g for T_1 and T_2, respectively. All fish treated with folic acid gave significantly higher values of live weights than the control treatment (T_1). There was no statistical difference found among the Green catfish treated with folic acid (T_2 to T_5). For survival rate which expressed in terms of percentages, the results showed that mean values of survival % ranged from 91.67 to 100 for T_1 and T_2, respectively. The results on Protein Efficiency Ratio (PER) showed that mean values of PER ranged from 0.79 to 1.22 g for T_1 and T_2, respectively. All fish treated with folic acid gave significantly greater values than the control treatment (T_1). There was no statistical difference found among the fish treated with folic acid (T_2 to T_5). With the results on

Table 2: Mean values of Initial Weight (W), Weight Gained (WG), Survival Rate (SR), Protein Efficiency Ratio (PER), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of Green catfish as influenced by different rates of niacin in feed ration, *in vitro* cultured for 12 weeks duration at Khon Kaen University

Treatments (mg kg^{-1} of niacin)	W (g)	WG (%)	SR (%)	PER	SGR (%)	FCR
T_1 (0)	18.78^{a}	50.04^{c}	86.00^{b}	0.63^{c}	3.38^{c}	1.69^{a}
T_2 (5)	18.70^{a}	69.84^{cb}	97.00^{a}	0.88^{b}	4.40^{bc}	1.50^{b}
T_3 (10)	18.70^{a}	99.92^{a}	100.0^{a}	1.18^{a}	5.77^{a}	1.35^{c}
T_4 (20)	18.70^{a}	73.96^{b}	97.00^{a}	0.90^{b}	4.56^{b}	1.50^{b}
T_5 (40)	18.72^{a}	75.47^{b}	98.00^{a}	0.91^{b}	4.63^{b}	1.51^{b}

Letter(s) represent least significant differences of Duncan's Multiple Range Test at probability (p) = 0.05

Table 3: Mean values of Initial Weight (W), Weight Gained (WG), Survival Rate (SR), Protein Efficiency Ratio (PER), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of Green catfish as influenced by different rates of folic acid in feed ration, *in vitro* cultured for 12 weeks duration at Khon Kaen University

Treatments (mg kg^{-1} folic acid)	W_i (g)	WG[2] (%)	SR[3] (%)	PER[4]	SGR[5] (%)	FCR[6]
T_1 (0)	19.93[a]	57.35[b]	91.67[b]	0.79[b]	3.78[b]	1.66[b]
T_2 (2)	19.89[a]	108.88[a]	100.00[a]	1.22[a]	6.13[a]	1.43[a]
T_3 (4)	19.91[a]	94.92[a]	98.33[a]	1.11[a]	5.55[a]	1.46[a]
T_4 (6)	19.91[a]	97.78[a]	100.00[a]	1.14[a]	5.67[a]	1.46[a]
T_5 (8)	19.91[a]	92.84[a]	100.00[a]	1.11[a]	5.45[a]	1.50[a]

Letter(s) represent least significant differences of Duncan's Multiple Range Test at probability (p) = 0.05

Specific Growth Rate (SGR) being expressed in terms of percentages $week^{-1}$, mean values ranged from 3.78 to 6.13% $week^{-1}$ for T_1 and T_2, respectively. A similar trend due to treatments as found with PER was attained. For Feed Conversion Ratio (FCR), the results showed that mean values of FCR ranged from 1.50 to 1.66 g for T_5 and T_1, respectively. The poorest FCR value was attained with the control treatment (T_1) and the highest was found with T_5, yet no statistical difference was found among the folic treated fish (T_2 to T_5).

DISCUSSION

An *in vitro* cultured of Green catfish in cement tanks carried out at Khon Kaen University, Thailand with the use of different rates of niacin revealed that the fish treated with niacin at a rate of 10 mg kg^{-1} feed ration gave significantly higher value of the final weight gained than control and other treatments. This trend was also found with other measured parameters, i.e., survival %, Protein Efficiency Ratio (PER), Specific Growth Rate (SGR) and feed conversion ratio. The results indicated that an *in vitro* cultured of the Green catfish required an amount of niacin at a rate of 10 mg kg^{-1} in feed ration and other higher rates of niacin added to the feed ration significantly decreased body live weight gained, PER, and SGR although survival % were similar among the niacin treated fish but their values were relatively lower. Feed Conversion Ratio (FCR) was also highest with niacin at a rate of 10 mg kg^{-1} feed ration, whilst others were relatively poorer. The differences found in all measured parameters must be attributable to the significant effect of niacin where the appropriate rate promotes a rapid growth of the Green catfish. The attained results were similar to the results carried out by Shiau and Suen (1994) yet the niacin rates were much higher than the present work. However, a higher rate of 14 mg kg^{-1} feed ration possessed significant effect on growth of Channel catfish better than any other rates. This finding has been reported by Andrews and Murai (1978). In contrast, Ng *et al.* (1997) reported that Channel catfish required only 7.4 mg kg^{-1} in feed ration. The low level of niacin being used in the latter work could have been possibly attributed to the differences in protein and other elements contained in the feed rations, e.g., Shiau and Suen (1992) used two feed formulae, i.e., the first one contained glucose of 38 g per 100 g ration and the second ration contained dextrin at a rate of 38 g per 100 g ration. They reported that the first formula of glucose required niacin at a rate of 26 mg kg^{-1} in feed ration, whilst the second formula of dextrin required niacin at a rate of 121 mg kg^{-1} in feed ration. Both rates of niacin in both rations gave maximal growth of Tilapia. Thus different formulae of rations require different rates of niacin for maximum growth of fish. A similar rate of niacin (10 mg kg^{-1} diet) to the present work had been proved to show significant effect when used with Rainbow trout. This result was reported by Poston and Wolfe (1985). Thus it could be inferred that different species of fish and different formulae of feed rations required different rates of niacin for the utmost growth of fish such as niacin at the rate from 150 – 200 mg kg^{-1} for Pacific salmon (Halver, 1989), 63 – 83 mg kg^{-1} for Gilthead sea bream (Morris and Davises, 1995), 33 mg kg^{-1} for African catfish (Morris *et al.,* 1998), 20 mg kg^{-1} diet for Indian catfish (Mohamed and Ibrahim, 2001) and the rates of 33 and 30 mg kg^{-1} diet for two Indian major carps, *L. rohita* and *C.mrigala*, respectively (Ahmed, 2010). For the effect due to folic acid added to the feed ration where the experiment was carried out with the fibre tanks, the results showed that a rate of 2 mg kg^{-1} feed ration of T_2 gave significant effect on Weight Gained Ratio (WGR), Survival Rate (SR), Protein Efficiency Ratio (PER), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of the Green catfish. Further added rates to the feed ration gave a similar effect as that of T_2 in all measured parameters. The results evidently showed that an *in vitro* cultured of Green catfish required this maximum amount of folic acid (2 mg kg^{-1} feed ration) for utmost growth parameters. The results confirm the work reported by Shiau and Huang (2001a, b) when they used the same rate of folic acid to Grass shrimp. However, with other experimental works reported by several workers with the use of different fish species, it was found that the requirement for folic acid for some fish species were differently found, e.g., an amount of folic acid at a rate of 0.3-0.6 mg kg^{-1} diet was best suited

for Rainbow trout (Cowey and Woodward, 1993), 0.82 mg kg^{-1} diet for Tilapia (Shiau and Huang, 2001a, b), and the rates between 0.5 to 1 mg kg^{-1} diet for Nile Tilapia (Barros *et al*., 2009). The differences in the amount of folic acid being used by different authors could possibly be attributable to the differences in animal species and environmental conditions. Therefore, it may be inferred that an *in vitro* cultured of Green catfish required only at a rate of 2 mg kg^{-1} feed ration.

CONCLUSION

It was found that *in vitro* cultured of Green catfish of 12 weeks duration required the maximum amount of 10 mg kg^{-1} feed ration of niacin for all growth measured parameters and a rate of 2 mg kg^{-1} of folic acid in feed ration gave the highest growth parameters of the fish.

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