J. Fish. Sci. Tech. 1(2), 192~200, 1998

Effect of Variable Feed Allowance with Constant Protein Input on Water Quality in Channel Catfish Production Ponds

Sung Hwoan Cho

Department of Fisheries and Allied Aquacultures, Auburn University, AL 36832, USA

(Received April 1998, Accepted June 1998)

This study was carried out to evaluate the effect of feeding higher protein feeds with lesser amount, but feeding the constant total protein input for all treatments, on water quality and nitrite toxicity in channel catfish ponds.

There was no significant difference in survival rate among treatments ($P>0.05$). Specific growth rate (SGR) for Treatment 1 (28% protein and 100% of satiation) was significantly higher ($P<0.05$) than for Treatment 3 (36% protein and 87.5% of satiation), but not significantly higher than for Treatment 2 (32% protein and 77.8% of satiation) at constant digestible energy (DE), 3.08 kcal/g (treatments 1, 2 and 3). At constant DE/P (treatments 4, 2 and 5), no significant difference in SGR was observed among treatments. Feed conversion ratio (FCR) slightly improved or improved as dietary protein level increased from 28% to 32 % and feed allowance decreased by 12.5%, but did not improve as dietary protein level increased from 32% to 36% and feed allowance decreased by 22.2%, at constant DE and constant DE/P.

There was no significant difference in water quality variables, such as total ammonia nitrogen (TAN), nitrite, chlorophyll a, soluble phosphorous concentrations among treatments, but significant difference in water quality variables over time as amount of feed fed increased ($P<0.0001$). There was a trend toward increase in TAN and nitrite over time. A strong linear regression was observed between mean total ammonia nitrogen and nitrite for all treatments Y (Nitrite) = 0.04 X (TAN) + 0.01, $R^2=0.89$.

Methemoglobin percent in the blood of catifish was not significantly different among treatments. And its mean value was 7.5%, which was relatively low, so that it was not serious problem in catfish production pond under these experiment conditions. There was the stronger linear regression between the percentage of Methemoglobin and the molar ratio of nitrite to chloride rather than nitrite alone: Y (Methemoglobin %) = 58.45 X (NO^{2-}/Cl^-) + 0.41, $R^2=0.60$.

These results indicate that deterioration of water quality has no strong impact on poor weight gain for 36% dietary protein in this study.

Key words: channel catfish, protein, feed allowance, water quality, Methemoglobin %

Introduction

Deterioration of the water quality becomes serious problem at high stocking density in aquaculture. Especially, high concentration of ammonia products and phosphorous in water in ponds have a great influence not only on raising fish in ponds, but also on the natural environments when discharged. Those in water largely come from uneaten feeds or excretion of fish. Approximately 10 to 20% of nitrogen and phosphorous in feeds are retained by the fish and the remainder go into the pond (Lovell 1990; Boyd and Tucker 1995), which are adsorbed by mud or consumed by phytoplankton (Boyd 1990; Gross et al., 1998). Understanding of nutrients requirements in the feed for fish can help to reduce wasted feed and to minimize nitrogen excretion from fish. When fish were fed to satiation, more feed were likely to be wasted and water quality were easily deteriorated and resulted in decrease of production of fish. Li and Lovell (1992 b) reported that when catfish were fed to satiation with high dietary protein, catfish production decreased due to the increase of total ammonia nitrogen and nitrite concentrations in water resulted in nitrite toxicity, called Methemoglobinemia,

*To whom correspondence should be addressed.

especially, when the molar ratio of nitrite to chloride in water was high. Also Tucker et al., (1979) and Cole and Boyd (1986) showed that high ammonia concentration in water resulted from high feeding rate had adversely effect on growth rate of catfish.

The objective of this study was to evaluate the effect of feeding higher protein feeds with lesser amount, but feeding the constant total protein input for all treatments, on water quality and nitrite toxicity in channel catfish ponds.

Materials and Methods

An average fish weight of 16.7 g of catfish fingerlings were purchased from a commercial farm and were stocked in twenty 0.04-ha earthen ponds at a density of 13,750 fish/ha. A 2-m diameter of feeding ring made from 5-cm diameter black plastic pipe with a 20-cm deep plastic net attached around the perimeter of the ring was placed in each pond to retain the floating feed. A 0.25-kw lift-type aerator with time actuated switches (Model AF-53, Air-O-Lator Co.) was placed in each pond.

Feeds were similar to commercial feeds and prepared to contain three percentages (28%, 32%, or 36%) of protein (P) at constant and variable digestible energy (DE) concentrations. One group of feeds contained a constant DE, 3.08 kcal/g at each protein percentage. Another group contained 2.70, 3.08, and 3.41 kcal of DE per gram of feed at each protein percentage so that the digestible energy/protein (DE/P) would be the same. Table 1 gives the ingredient and nutrient composition of the experimental feeds. The feed containing 28% protein and 3.08 kcal/g DE was the control which was fed at satiation rate. Approximately 30 minutes was required for the control fish to finish feeding. Feed allowance for the control fish was increased by 10% every 3 to 5 days, based upon observed feeding activity. The daily allowance of all of the other feeds was based upon the amount of protein consumed by the control fish; all treatments received the same daily protein allowance. Thus, the fish fed 32% protein received 12.5% less feed than the control and those fed 36% protein received 22.2% less feed than the control. The low-energy, 28 % protein treatment received the same feed allowance as the high-energy 28% protein feed, which was the control. Each treatment was assigned to four replicate ponds. Feed was supplied between 17:30 and 19:00 daily.

Table 1. Composition of the experimental diets

Component	Diets				
	1	2	3	4	5
Ingredient (%)					
Corn	46.8	36.3	26.6	34.2	17.6
Soybean meal	38.3	49.5	60.0	39.9	61.7
Wheat middlings	5.0	5.0	5.0	5.0	5.0
Fish meal	7.5	6.8	6.0	7.5	6.0
Catfish oil	1.0	1.0	1.0	0	8.3
Alfalfa meal	0	0	0	12.0	0
Dicalcium phosphate	1.4	1.4	1.4	1.4	1.4
Trace mineral mix[1]	0.01	0.01	0.01	0.01	0.01
Vitamin mix[2]	0.01	0.01	0.01	0.01	0.01
Vitamin C, stable[3]	0.05	0.05	0.05	0.05	0.05
Nutrient					
Crude protein (%)	28	32	36	28	36
Digestible energy (kcal/g diet)[4]	3.08	3.08	3.08	2.70	3.41
DE/P (kcal/g)	11.0	9.6	8.6	9.6	9.5
Total phosphorous (%)	1.03	1.01	0.91	0.92	0.87

[1] Trace mineral mix provided the following minerals per kg of feed: Zn, 150 mg; Fe, 44 mg; Mn, 25 mg; I, 5 mg; Cu, 3 mg; Co, 0.05 mg.

[2] Vitamin mix provided all of the following vitamins in the amounts presented per kg of feed: retinyl acetate, 4000 IU; vitamin D_3, 2000 IU; alpha tocopherol acetate, 50 mg; menadione, 10 mg; choline chloride, 500 mg; niacin, 80 mg; riboflavin, 12 mg; pyridoxine, 10 mg; thiamin, 10 mg; pantothenic acid, 32 mg; folic acid, 2 mg; vitamin B_{12}, 8 g; ethoxyquin (antioxidant), 125 mg.

[3] Ascorbyl-1-phosphate, contains 15% vitamin C.

[4] Digestible energy was calculated from tabular values of the feed ingredients (National Research Council, 1993).

Dissolved oxygen (DO) and water temperature were monitored at 18:30 daily with a YSI-57 oxygen meter. Aerators were used to maintain dissolved oxygen at a concentration of no less than 3.0 mg/ℓ. During the latter part of the feeding periods, whenever DO concentration was expected to be low, the aerators came on at 23:00 by time actuated switches. At least twice each week, DO concentration was measured in the early morning.

Criteria measured were weight gain, specific growth rate (SGR), feed conversion, water quality and Methemoglobin % induced from nitrite toxicity in blood of catfish. The following water quality were monitored every month; total ammonia nitrogen (TAN), nitrite and chlorophyll a. All analyses were followed by standard methods for analysis of waste water (Pechar 1987; Boyd and Tucker 1992; van Rijn 1993). Methemoglobin was measured by Tietz's method (1970) in eight fish from each pond immediately after harvesting for possible nitrite toxicity.

The mean differences were tested using Duncans New Multiple range (Duncan 1955). Monthly changes of water quality variables were tested by using ANOVA with repeated measurement designs (Cody and Smith 1991). All statistical analysis was performed on SAS version 6.11 (SAS Institute, Cary, NC).

Results

The range of temperature for treatment 1, 2, 3, 4 and 5 were 23~34℃ (mean; 28.7℃), 23~34℃ (28.7℃), 23~34℃ (29.0℃), 23~35℃ (28.8℃) and 23~34℃ (29.1℃), respectively.

Table 2 gives survival rate, weight gain, SGR and feed conversion ratio (FCR). There was no significant difference in survival rate among treatments (P>0.05). Mean suvival rates for all treatments were over 90%. SGR for treatment 1 (28% protein and 100% of satiation) was significantly higher than for treatment 3 (36% protein and 77.8% of satiation), but not significantly higher than for treatment 2 (32% protein and 87.5% of satiation) at constant DE (treatments 1, 2 and 3). At constant DE/P (treatments 4, 2 and 5), no significant difference in SGR was observed among treatments. At constant DE, feed conversion ratio (FCR) slightly improved as dietary protein level increased from 28% to 32% and feed allowance decreased from 100% of satiation to 87.5% of satiation, but did not improve as dietary protein level increased from 28% to 36% and feed allowance decreased from 100% of satiation to 77.8% of satiation. At constant DE/P, FCR improved as dietary protein level increased from 28% to 32% increased and feed allowance decreased by 12.5%, but did not improve as dietary protein level increased from 28% to 36% and feed allowance decreased by 22.2%.

Monthly changes in TAN concentration are shown in Fig. 1. The range for TAN was 0.07~3.45 mg/ℓ. The concentration of TAN was not statistically different among treatments (P>0.05). But there was a trend toward increase in TAN over time as amount of feed fed increased. The highest concentration of TAN for all treatments occurred just before the harvesting. At the end of experiment, concentration of TAN in pond receiving treatment 3 was highest, 3.45 mg/ℓ and the lowest was 1.89 mg/ℓ in pond receiving treatment 4.

Table 2. Means for survival rate (%), weight gain (g/fish), and SGR of channel catfish fed expeimental diets containing variable protein and energy levels in earthen ponds[1]

	Treatments				
	1	2	3	4	5
Survival rate (%)[2]	94.4[a]	94.0[a]	93.5[a]	91.0[a]	89.5[a]
Initial weight (g/fish)	10.6	10.6	10.6	10.6	10.6
Final weight (g/fish)	282.9	278.6	250.9	273.6	259.7
Weight gain (g/fish)	266.2[a]	261.9[a]	234.2[b]	256.9[ab]	243.0[ab]
SGR[3]	2.56[a]	2.54[ab]	2.46[b]	2.53[ab]	2.48[ab]
Feed conversion ratio	1.40[ab]	1.31[b]	1.30[b]	1.49[a]	1.33[ab]

[1] 1 pond was excluded because of poor feeding activity and disease.

[2] Pooled SE for survival rate, SGR and feed conversion ratio were 4.7, 0.06 and 0.10, respectively.

[3] SGR=[(logW_2-logW_1)x100/lengh of period], where W_1 and W_2 stand for initial and final weight of fish, respectively.

Different superscript letters mean significant difference (P<0.05).

Monthly changes in nitrite concentration are given in Fig. 2. The range for nitrite was 0.01~0.15 mg/ℓ. The concentration of nitrite was not statistically different among treatments (P>0.05). But there was a trend toward increase in nitrite over time as amount of feed fed increased. The highest concentration of nitrite occurred just before the harvesting. At the end of experiment, a rapid increase of nitrite was observed in pond receiving treatments 1 and 2. The highest nitrite concentration was 0.15 mg/ℓ in pond receiving treatment 1 and the lowest was 0.07 mg/ℓ in pond receiving treatment 4.

Monthly changes in chlorophyll a are given in Fig. 3. The range for chlorophyll a was 0.01~0.17 mg/ℓ. The concentration of chlorophyll a was not statistically different among treatments (P>0.05). But there was significant difference in chlorophyll a over time (P<0.0001). At the end of experiment, chlorophyll a did not change much, except treatment 2 showing a dramatical decrease.

Monthly changes in soluble phosphorous concentration are given in Fig. 4. The range for soluble phosphorous was 0.01~0.07 mg/ℓ. The soluble phosphorous concentration was not statistically different among treatments (P>0.05). But there was significant difference over time (P<0.0001).

The relationship between mean TAN and nitrite for all treatments at each month during experiment

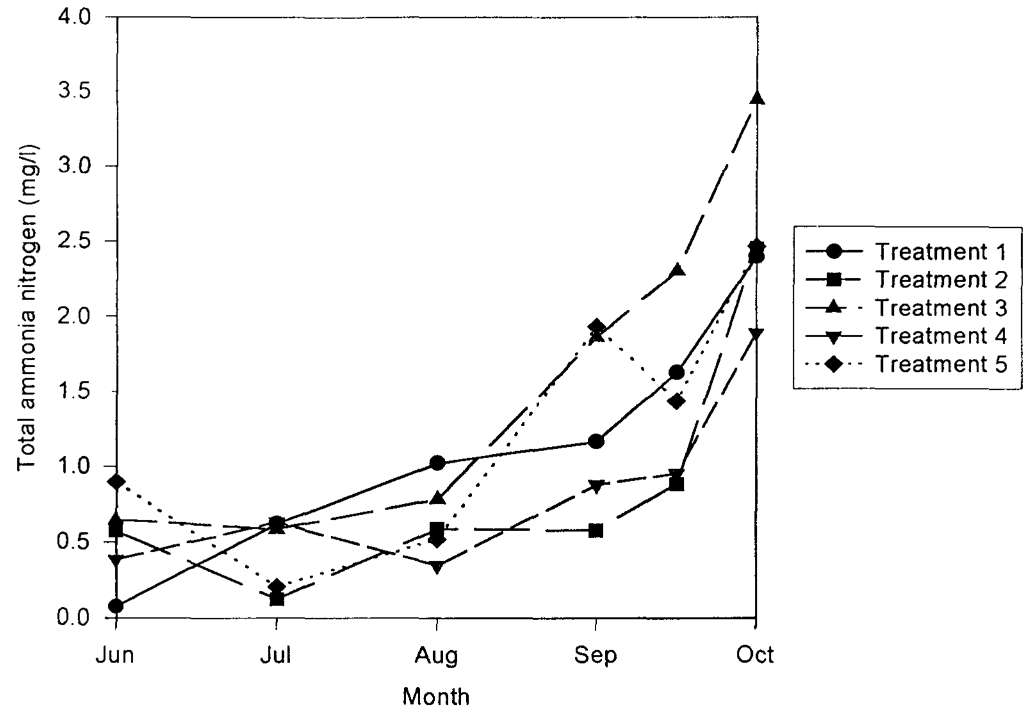

Fig. 1. Monthly changes in total ammonia nitrogen (TAN) in ponds receiving diets containing variable protein and digestible energy levels.

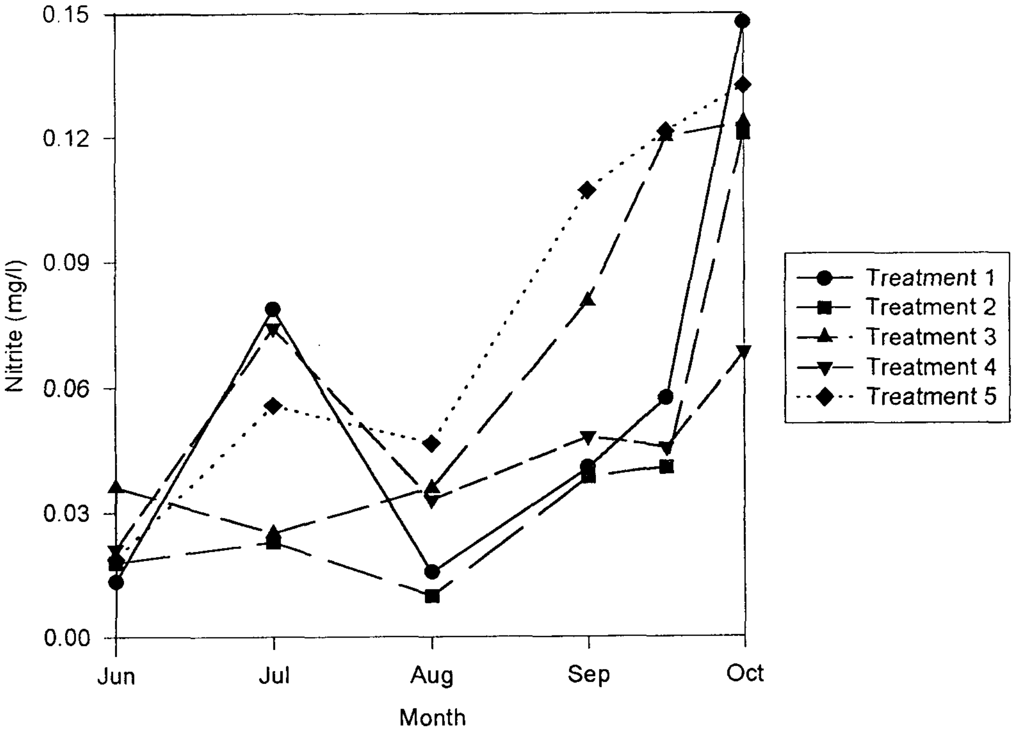

Fig. 2. Monthly changes in nitrite in ponds receiving diets containing variables protein and digestible energy levels.

is plotted in Fig. 5. As TAN increased over time due to increase of amount of feed fed, nitrite also increased in pond water. The following linear regression was observed; Y (Nitrite) = 0.04 X (TAN) + 0.01, $R^2 = 0.89$ ($P < 0.005$).

Total amount of feed fed, protein and phosphorous fed, ammonia products, chloride concentration, and the molar ratio of nitrite to

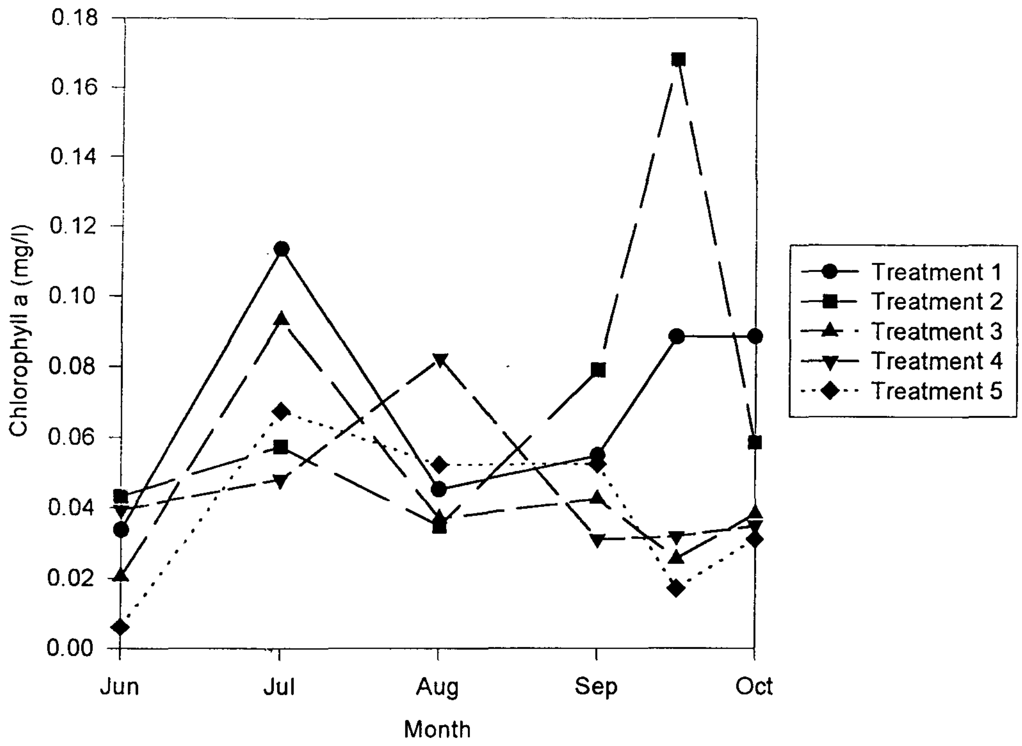

Fig. 3. Monthly changes in chlorophyll a in ponds receiving diets containing variable protein and digestible energy levels.

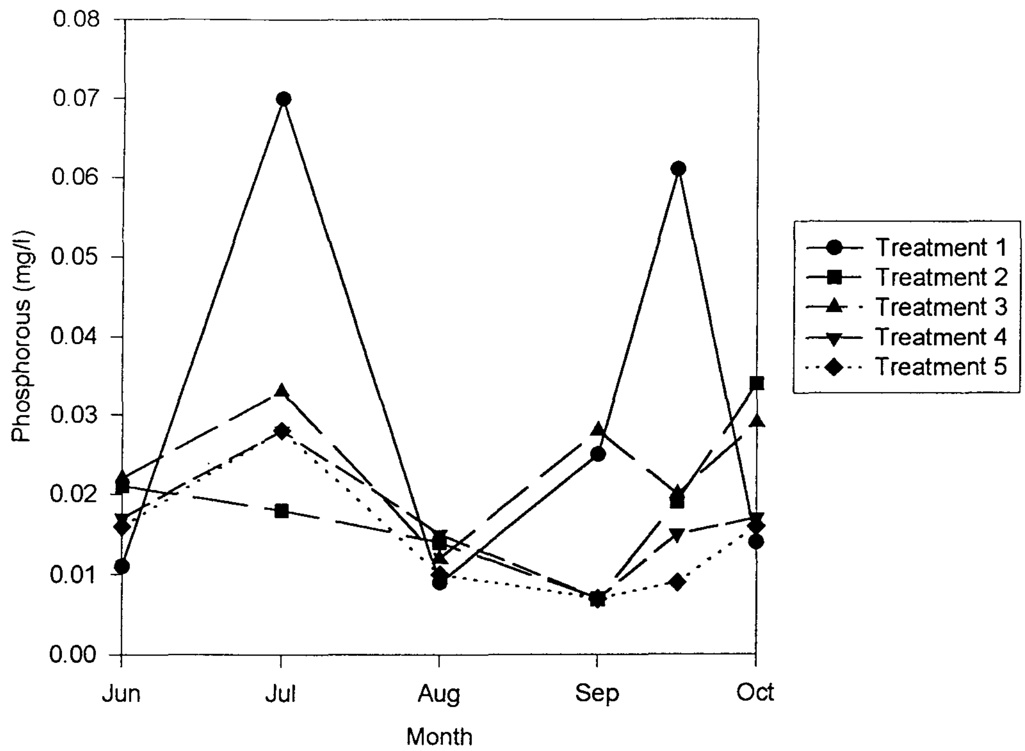

Fig. 4. Monthly changes in soluble phosphorous in ponds receiving diets containing variable protein and digestible energy levels.

chloride in ponds at the end of experiment and Methemoglobin % in the blood of catfish are shown in Table 3. Maximum daily feed allowance for 28, 32 and 36% dietary protein treatments reached 95.8, 83.8 and 74.5 kg/ha, respectively, at the end of experiment. Amount of protein and nitrogen fed was almost same for all treatments as designed. Amount of phosphorous fed linearly decreased as dietary protein level increased and feed allowance decreased. Since phosphorous content for Diet 1

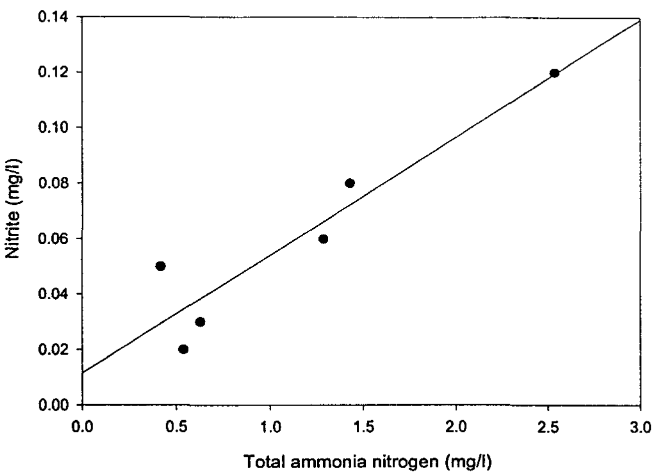

Fig. 5. Relationship of mean total ammonia nitrogen and nitrite for all treatments during the experiment; Y (Nitrite) = 0.04 X (Total ammonia nitrogen) + 0.01, $R^2 = 0.89$.

Table 3. Means for protein, nitrogen and phosphorous fed in ponds where channel catfish fed expeimental diets in earthen ponds[1] and total ammonia nitrogen (TAN), nitrite (NO^{2-}), NO^{2-}/Cl^- in the ponds water at the end of experiment and Methemoglobin percent in the blood of catfish

	Treatments				
	1	2	3	4	5
Feed fed (kg/ha)	4,815.0	4,392.5	3,882.5	4,777.5	3,927.5
Protein fed (kg/ha)	1,347.5	1,405.0	1,397.5	1,337.5	1,415.0
Nitrogen fed (kg/ha)	215.6	224.8	223.6	214.0	226.4
Pond TAN (mg/ℓ)	2.40[a]	2.46[a]	3.45[a]	1.89[a]	2.47[a]
Pond NO^{2-} (mg/ℓ)	0.15[a]	0.12[a]	0.12[a]	0.07[a]	0.13[a]
Pond Cl^-	2.83	2.35	2.57	2.35	2.90
NO^{2-}/Cl^-	0.15[a]	0.15[a]	0.11[a]	0.06[a]	0.12[a]
Methemoglobin %	10.7[a]	10.0[a]	4.7[a]	6.0[a]	5.9[a]

[1] 1 pond was excluded because of poor feeding activity and disease.
Pooled SE values for TAN, NO^{2-}, NO^{2-}/Cl^- and Methemoglobin % are 1.09, 0.04, 0.04 and 3.07, respectively.
Means in rows with same superscript letter are not different at $P<0.05$.

was higher than for Diet 4, total amount phosphorous fed during the experiment was greater for treatment 1 than for treatment 4. The range of chloride concentration was 2.35~2.90 mg/ℓ. The molar ratio of nitrite to chloride for treatment 4 was the lowest, 0.06, because of low concentration of nitrite. And the molar ratio of nitrite to chloride for treatments 1 and 2 were highest and both were 0.15. The molar ratio of nitrite to chloride for treatments 3 and 5 were 0.11 and 0.12, respectively. Methemoglobin % was not significantly different among treatment because of wide variation within same treatment ($P<0.05$). The range of Methemoglobin % for all treatments was 4.7~10.7%.

Relationship between Methemoglobin % and the molar ratio of nitrite to chloride or nitrite are plotted in Fig 6. and 7. There was a stronger linear regression between Methemoglobin % and the molar ratio of nitrite to chloride (Fig. 6) rather than nitrite (Fig. 7) alone; Y (Methemoglobin %) = 58.45 X (NO^{2-}/Cl^-) + 0.41, $R^2 = 0.60$ ($P<0.001$), and Y (Methemoglobin %) = 38.91 X (NO^{2-}) + 2.51, $R^2 = 0.24$.

Discussion

As dietary protein level increased from 28% to 32% and feed allowance decreased from 100% of satiation to 87.5% of satiation, weight gain was maintained, but dietary protein level increased from

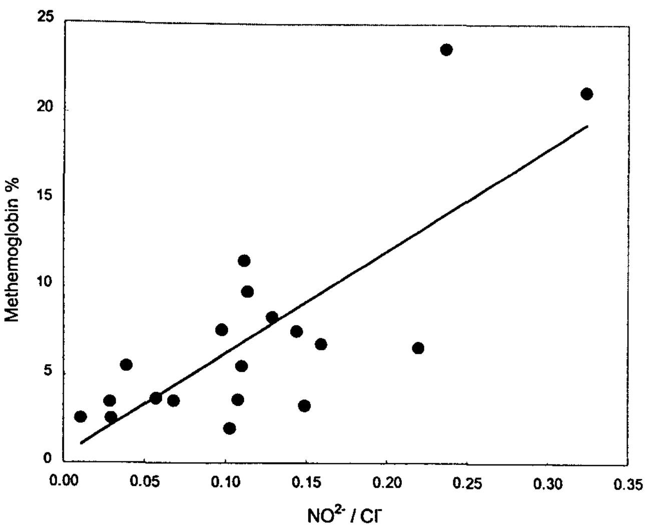

Fig. 6. Relationship between Methemoglobin percent and the molar ratio of nitrite to chloride (NO^{2-}/Cl^-) in pond; Y (Methemoglobin) = 58.45 X (NO^{2-}/Cl^-) + 0.41, $R^2 = 0.60$ ($P<0.001$).

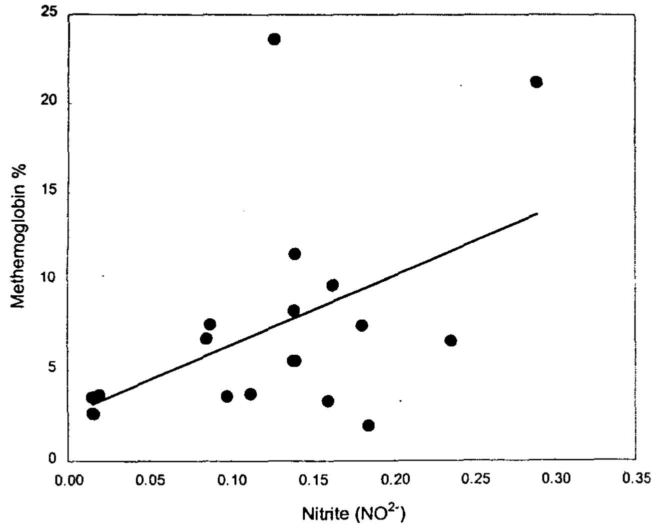

Fig. 7. Relationship between Methemoglobin percent and nitrite (NO^{2-}) in pond; Y (Methemoglobin) = 38.91 X (NO^{2-}) + 2.51, $R^2 = 0.24$.

28% to 32% and feed allowance decreased from 100% of satiation to 77.8% of satiation, weight gain reduced (Table 2). As a result, SGR did not reduce as dietary protein level increased from 28% to 32% and feed allowance decreased by 12.5%, but did as dietary protein level increaed from 28% to 36% and feed allowance decreased by 22.2%. FCR improved as dietary protein level increased from 28% to 32% and feed allowance decreased by 12.5%, but did not improve as dietary protein level increased from 28% to 36% and feed allowance decreased by 22.2%.

Generally speaking, when fish were fed to satiation, more feed were likely to be wasted and water quality were easily deteriorated and resulted in decrease of production of fish. Effects of protein levels in feeds and feed allowance on fish growth and possible water quality deterioration were reported (Li and Lovell 1992a; Li and Lovell 1992 b). Unlike above studies, in this experiment, when fish were fed to less than satiation, water quality variables (TAN, nitrite, chlorophyll a) in treatments 2, 3 and 5 were not improved than treatments 1 and 4 received satiation feeding. This is probably because of constant protein input for all treatments. But for all treatments, there was a trend toward increase in TAN and nitrite over time as amount of feed fed increased.

Amount of phosphorous fed linerly decreased as feed allowance decreased (Table 3), but there was no significant difference among treatments. The reason there was no significant increase in soluble phosphorous in the ponds where received 100% of satiation feeding is because the pod mud was able to absorb the phosphorous from the water and phytoplankton was abgle to use it (Maskey and Boyd 1986; Boyd 1990; Gross et al., 1998).

As feeding rate increased, water quality variables, such as chlorophyll a, chemical oxygen demand, carbon dioxide and TAN increased (Tucker et al., 1979; Cole and Boyd 1986), and therefore, fish production decreased at over feeding rate of 112 kg /ha/day. High concentration in ammonia had adversely effect on growth rate of catfish. Ammonia is the end-product of protein metabolism in fish and is excreted through gills. High ammonia concentration in the water caused degenerative tissue damage to gills and kidney in rainbow trout and cutthroat trout (Smart 1976; Thurston et al., 19 78), and increases in oxygen consumption, respiratory rate, and heart rate (Smart 1978). Also Colt (1978) observed reduction of catfish growth due to high ammonia concentration in water. The concentrations of un-ionized ammonia, 0.048 g/ℓ caused reduction of growth for catfish fingerling and no growth occurred at 0.967 g/ℓ.

The primary toxic effect of nitrite is the formation of Methemoglobin, which is unable to transport oxygen, causing brown blood disease (Lovell 1979). Palachek and Tomasso (1984) showed that channel catfish was the least resistant to nitrite among three species of fish (channel catfish, tilapia, largemouth bass) under similar water quality conditions and Methemoglobin for channel catfish was significantly higher than that for other fish at variably tested nitrite concentrations. Also Hanson (1983) reported that channel catfish exposed to a certain concentration of nitrite easily infected with bacterial disease and caused high mortality.

In this study, the maximum amount of feed allowance for 36% dietary protein (treatments 3 and 5) reached 74.5 kg/ha at the end of experiment, which were lower than in Li and Lovell (1992b)'s study. Thus, nitrite concentration in pond was relatively low and it resulted in low value of the molar ratio of nitrite to chloride (NO^{2-}/Cl^-). The ratio of NO^{2-}/Cl^- for all treatments was lower than 0.20. And low value of Methemoglobin % for all treatments was observed and there was no significant difference among treatments because of wide variation within the same treatment. Mean of Methemoglobin % for all treatments was 7.5 ± 2.68% (s.d.). Therefore, nitrite toxicity was not seriously concerned in this study.

The formation of Methemoglobin % in the blood of catfish was proportional to the molar ratio of nitrite to chloride rather than nitrite alone. This is consistent with other researches (Schwedler and Tucker 1983; Tucker and Schwedler 1983; Li and Lovell 1992b). Tucker et al., (1989) reported that nitrite toxicity of channel catfish exposed to different levels of nitrite for a long period of time up to 21 days in tanks. Channel catfish exposed to 0.92 mg/ℓ nitrite concentration (nitrite:chloride= 0.12) did not become anemic because of less than 20% of methemoglobin and developed only mild Methemoglobinemia. Bower et al., (1983) proposed that establishing a Cl^{-}:NO^{2-} ratio of 3:1 in the pond and maintaining dissolved oxygen level over 5 mg/ℓ be effective to prevent Methemoglobinemia for catfish based on short-term test in aquarium. Methemoglobinemia is less of a problem in water with high hardness and alkalinity. Also, nitrite toxicity is not concerned in marine fish as much as freshwater fish because of high concentrations of chloride in water (Scaramo et al., 1984).

These results indicate that the reduction of growth for channel catfish fed on 36% protein diets not result from deterioration of water quality or nitrite toxicity.

Acknowledgements

The author wishes to express appreciation to Dr. A. Gross helped to do water quality analysis.

References

Bowser, P.R., W.W. Falls, J. VanZandt, N. Collier, and J. D. Phillips. 1983. Methemoglobinemia in channel catfish: methods of prevention. Prog. Fish-Cult. 45 (3), 154~158.

Boyd, C.E. 1990. Water Quality in Ponds for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, AL, USA, 482 pp.

Boyd, C.E. and C.S. Tucker. 1992. Water quality and pond soil analyses for aquaculture. Alabama Agricultural Experiment Station. Auburn University, Alabama, USA.

Boyd, C.E. and C.S. Tucker. 1995. Sustainability of channel catfish farming. World Aquaculture 26 (3), 45~53.

Cody, R.P. and J.K. Smith. 1991. Applied statistics and the sas programming language. 3rd edition. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Cole, B.A. and C.E. Boyd. 1986. Feeding rate, water quality and channel catfish production in ponds. Prog. Fish-Cult. 48, 25~29.

Colt, J.E. 1978. The effect of ammonia on the growth of channel catfish, *Ictalurus punctatus*. Ph.D. dissertation. University of California, Davis, California.

Duncan, D.B. 1955. Multiple range and multiple F Tests, Biometrics, Vol.11.

Gross, A., C.E. Boyd, R.T. Lovell and J.C. Eya. 1998. Phosphorous budget for channel catfish ponds receiving diets with different phosphorous concentrations. J. World Aqua. Soc. 29 (1), 31~39.

Hanson, L.A. 1983. The effects of sublethal nitrite exposure on the predisposition of channel catfish to bacterial disease. Masters thesis, Auburn University, Auburn, Alabama.

Li, M. and R.T. Lovell 1992a. Growth, feed efficiency and body composition of second- and third-year channel catfish fed various concentrations of dietary protein to satiety in production ponds. Aquaculture 103, 153~162.

Li, M. and R.T. Lovell 1992b. Effect of dietary protein concentration on nitrogenous waste in intensively fed catfish ponds. J. of World Aqua. Soc. 23 (2), 122~127.

Lovell, R.T. 1979. Brown blood disease in pond-raised catfish. Commercial Fish Farmer 5 (3), 33.

Lovell, R.T. 1990. Feeds and feed management in catfish ponds. Symposium on managing fish farm effluents through feed management. American Fisheries Society Annual Meeting. Pittsburgh. PA: Aug. 27~31.

Maskey, S. and C.E. Boyd. 1986. Seasonal changes in phosphorous concentrations in sunfish and channel catfish ponds. J. Aqaua. Trop. 1, 35~42.

National Research Council. 1993. Nutrient requirements of fish. National Academy Press, Washington DC, 114 pp.

Palachek, R.M. and J.R. Tomasso. 1984. Toxicity of nitrite to channel catfish (*Ictalurus punctatus*), tilapia (*Tilapia aurea*), and largemouth bass (*Micropterus slamoides*): evidence for a nitrite exclusion mechanism. Can. J. Fish. Aqu. Soc. 41, 1739~1744.

Pechar, L. 1987. Use of an acetone:methanol mixture for the extraction and spectrophotometric determination of chlorophyll a in phytoplankton. Arch. Hydrobiol. Suppl. 78 (1), 99~117.

Scarano, G., M.G. Saroglia, R.H. Gray, and E. Tibaldi. 1984. Hematological responses of sea bass *Dicentrarchus labrax* to sublethal nitrite exposures. Trans. Amer. Fisheries Soc. 113, 360~364.

Schwedler, T.E. and C.S. Tucker. 1983. Empirical relationship between percent methemoglobin in channel catfish and dissolved nitrite and chloride in ponds. Trans. Amer. Fisheries Soc. 112, 111~119.

Smart, G. 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). J. Fish Biol. 8, 471~475.

Smart, G. 1978. Investigation of the toxic mechanisms of ammonia to fish-gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acutely lethal concentrations. J. Fish Biol. 12, 93~104.

Thurston, R.V., R.C. Russo and K.B. Davis. 1978. Acute toxicity of ammonia and nitrite to cutthorat trout fry. Trans. Amer. Fisheries Soc. 107, 361~367.

Tietz, N.W., editor. 1970. Fundamentals of clinical chemistry. W.B. Saunders, Philadelphia, Pennsylvania, USA.

Tucker, L., C.E. Boyd and E.W. McCoy. 1979. Effects of feeding rate on water quality, production of channel catfish and economic returns. Trans. Amer. Fisheries Soc. 108, 389~396.

Tucker, C.S. and T.E. Schwedler 1983. Variability of percent methemoglobin in pond populations of nitrite-exposed channel catfish. Prog. Fish-Cult. 45 (2), 108~110.

Tucker, C.S., R. Francis-Floyd and M.H. Beleau. 1989. Nitrite induced anemia in channel catfish, *Ictalurus punctatus* Rafinesque. Bull. Environ. Contam. Toxicol. 43, 295~301.

Van Rijn, J. 1993. Methods to Evaluate Water Quality in Aquaculture. Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel.