**Original Article****Residual effect of oxytetracycline medicated diet on shelf life of mrigal carp (*Cirrhinus cirrhosus*) stored in ice under laboratory condition**S. A. Haque^{1*}, M. K. Rahman², M. N. A. Khan³, M. S. Reza⁴¹Department of Fisheries, Bangamata Sheikh Fojilatunnesa Mujib Science and Technology University, Melandah, Jamalpur-2012, Bangladesh;²Bangladesh Fisheries Research Institute, Mymensingh-2201, Bangladesh;³Department of Fishing & Post-Harvest Technology, Chattogram Veterinary and Animal Sciences University, Chattogram-4225, Bangladesh;⁴Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.**ABSTRACT**

Among the six common classes of antimicrobials approved for aquaculture by World Health Organization (WHO), the use of oxytetracycline (OTC) is common in the aquaculture of Bangladesh. Since OTC hampers the growth and reproduction of fish microbial communities including the commensal and pathogenic flora, efforts were made to investigate the residual effect of OTC via medicated feed on the shelf life of apparently healthy mrigal carp (*Cirrhinus cirrhosus*) during ice storage. A group of live fish (average weight: 18 ± 0.05 g) were fed OTC supplemented diet (2g OTC /kg diet) twice a day for 21 days and compared to another group fed with basal diet (control). After a brief withdrawal period (14 days), fish were killed by ikejime fish-slaughtering method and kept in separate ice boxes containing 1:1 ice to fish ratio for determining their shelf life using organoleptic, biochemical and bacteriological aspects of fish. Organoleptically, control fish were found to be acceptable up to 11 days whereas fish of treated group were found acceptable up to 15 days, showing significant rise in shelf life for OTC-treated fish. Proximate analysis showed a slight increase in moisture and a gradual decrease in protein and ash contents that were statistically insignificant ($p > 0.05$) between control and OTC-treated group. The decline in lipid content was, however, significantly different between the two groups ($p < 0.05$). Muscle pH increased sharply other than control fish as well as total volatile based nitrogen (TVB-N), peroxide value (PV) increased rapidly. The aerobic plate count (APC) was significantly higher for control fish than OTC-treated fish during storage time. According to the parameters assessed, the OTC-treated fishes were found to be delayed spoilage along with a prolonged shelf life in ice storage condition.

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Introduction

Antibiotics are effective therapeutical agents used to treat certain bacterial infections either by stopping them from reproducing or destroying them. Sometimes they are incorporated intentionally as feed additives in animal husbandry and fisheries and exert growth promoting effect in fish (Kennedy, 1980; Pollmann, 1980; Ahmed and Matty, 1989) or enter the pond ecosystem unintentionally (Islam *et al.*, 2014). Introduction of these antibacterial agents coincided with intensification of culture systems where they improved feed conversion and animal growth and reduced

morbidity and mortality due to clinical and subclinical diseases (Butaye *et al.*, 2003). Sanchez-Martinez *et al.* (2008) studied the effect of supplementing channel cat fish (*Ictalurus punctatus*) feeds with oxytetracycline (OTC) and observed that treated fish exhibited a significant increase in weight suggesting a growth promotion action of the antibiotic agent used. The bacterial composition inside fish skin, gill, gut as well as aquaculture systems also changed (Haque *et al.*, 2013; 2014a, 2014b). These changes in bacterial flora may be of significance in fish spoilage (Kaneko, 1971).

Until now more than 100 different kinds of antibiotics have been discovered (Nigam *et al.*, 2014). Among these antibiotics, only a small number of them are approved by World Health Organization (WHO), Food and Drug Administration (FDA) and the European Union (EU) for use in aquaculture. They have approved 5 (five) different drugs for use in aquaculture as long as the seafood contains less than a mandated maximum residue limit (GAO, 2011). Among the antibiotics used, OTC is the most commonly administered antibacterial in aquaculture production (Erdogdu, 2012). In Bangladesh, it is also used frequently in fish and shrimp farms to treat disease affected fish and/or as a prophylactic in fresh water aquaculture (Ali *et al.*, 2016). With the expanding aquaculture practice in the country, a number of indigenous fish species including mrigal carp (*Cirrhinus cirrhosus*) culture has increased in semi-intensive and extensive systems in ponds. After they are harvested from water, ice is generally used for transportation from farm to the market. In the market, the wholesaler uses crushed ice to keep the quality of fish. So, a long time pass over the fish covering with the ice (Alam, 2007) during transportation. Previously, we reported antibacterial activity of OTC on microbial ecology in a number of commercially important fish species of Bangladesh (Haque *et al.*, 2014a, 2014b; Mannan *et al.*, 2020). However, indiscriminate use of OTC in aquafarms as feed additives for prophylaxis and growth promotion without prescription has led us to believe that the quality and safety aspect of wet fish is influenced to some extent. Until now there were no systematic studies of OTC-treated fish on fish spoilage bacteria that ultimately influence the shelf life of fish under different storage condition. The aim of the present study was, therefore, to determine the shelf life of mrigal carp for OTC-treated group during ice storage condition.

Materials and Methods

Experimental design

The research was based on randomized controlled study where one hundred (100) mrigal carp (*Cirrhinus cirrhosus*) (average body weight 18 ± 0.05 g) in six (6) glass aquaria (size 37cm×30cm×60cm) were randomly distributed and allowed to adapt to the rearing conditions. For this work, all aquaria were set at the Laboratory of Fish Harvesting, Department of Fisheries Technology, Bangladesh Agricultural University during autumn (September-October) and were operated under the same conditions. Fish in the three (3) aquaria were used for OTC-treated group and three (3) for control group. Water in these aquaria was changed every day. Two types of feed, basal feed and basal feed supplemented with OTC at 2 g/kg diet were prepared at the laboratory (Table 1) and fed twice per day (once at 9:00 a.m. and second at 5:00 p.m.) for 21 consecutive days. After 14 days of withdrawal period fish were then killed humanely by Japanese ikejime fish-slaughtering method where a spike is pushed through the pineal window to penetrate the brain (Robb *et al.*, 2000; Lines and Spence, 2014). Then fish were transferred to an insulated box where freshly prepared crushed ice at ratio of ice and fish at 1:1 (w/w) was used for icing the samples. Ice was replenished on every alternate day until the termination of experiment. The icebox had a number of holes at the bottom to drain out the melted water. The quality change in ice stored samples was evaluated after every 4 (four) days interval by determining the chemical and bacteriological tests. The organoleptic quality changes were assessed everyday during ice storage condition.

Table 1. Pelleted feed ingredients

Basal feed		Basal feed supplemented with OTC	
Ingredients	Amount (%)	Ingredients	Amount (%)
Wheat bran	11.90	Wheat bran	11.86
Rice bran	19.75	Rice bran	19.75
Maize flour	13.85	Maize flour	13.84
Soybean meal	14.85	Soybean meal	14.83
Fishmeal	33.70	Fishmeal	33.60
Molasses	3.98	Molasses	3.96
Vitamin premix	1.00	Vitamin premix	1.00
Salt	0.97	Salt	0.96
		Antibiotics	0.20

Sampling procedure and sample preparation

At described intervals (starting from first day), 4 to 5 fish were randomly sampled and their sensory attributes were evaluated while keeping the fish in iced condition. The fish were subjected to sensory analysis; 2 to 3 fish for chemical and rest for bacteriological analyses. The muscle was skinned, deboned and homogenized by mincing, and was taken for aerobic plate count (APC).

Organoleptic quality assessment

Sensory methods were used to assess the degree of freshness based on organoleptic characteristics such as odor, color, general appearance, eyes, slime and consistency of flesh. Starting from first day, 2 to 3 fish were randomly sampled and their raw sensory attributes were evaluated. The organoleptic characteristics were judged everyday by a trained panel of expert members during the storage period. They were evaluated by grading fish using the score from 1 to 5 based on organoleptic characteristics. The grading of fish using score on the characteristics has been followed by ECC freshness grade for fishery products with slight modification (Howgate *et al.*, 1992) to judge the quality of the fish.

Proximate composition analyses

Moisture content was determined by air drying of a given sample in a thermostat oven (Gallenkamp, HOT-BOX, Manchester, UK) at 105°C for 24 hours until constant weight. Ash content was determined by igniting the sample in a muffle furnace at a temperature of 550°C for 6 hours. Crude protein was determined by the Macro Kjeldahl method by determining total nitrogen and applying the protein conversion factor of 6.25 to the results to convert total nitrogen into total protein, assuming that fish protein contained 16% nitrogen, and lipid content was determined by extracting required quantity of samples with petroleum ether for 16–18 hours in a ground joint Soxhlet apparatus. The oil obtained by evaporation of the solvent on a steam bath was weighed in a sensitive balance and percent lipid was calculated.

Chemical analysis

pH was determined by homogenizing 1g of pooled fish muscle in 10 ml of distilled water with a pH meter (Hanna Instruments Ltd., Germany). TVB-N was determined according to the standard method described by (EC, 1995) with some modifications. Estimation of TVB-N was done at every 4 days interval up to 16 days of iced storage. Peroxide value (PV) was determined according to Lima Dos Santos *et al.* (1981).

Bacteriological analysis

About 10-15g of whole fish sample was blended with appropriate volume of 0.2% peptone water in a sterilized blender for a few min until homogenous slurry was obtained. Bacteriological analyses of ice stored fishes were done at every 4 days interval until termination of experiment. Total APC expressed as colony forming units per gram of muscle (cfu/g) of the representative samples was determined by standard plate count methods using plate count agar (Hi-media, Mumbai, India) according to Collins and Lyne (1976).

Statistical analysis

Data obtained in the experiment were recorded and preserved in computer and paired t-test was done by using SPSS 11.0 (Statistical Package for the Social Science, Chicago, USA). Significant differences were determined among treatments at the 5 % level ($p < 0.05$).

Results and discussion

Changes in organoleptic qualities of fish during ice storage

The organoleptic qualities of control and OTC-treated groups of mrigal carp during iced storage are presented in Table 2. The organoleptic characteristics indicated that OTC-treated mrigal carp was excellent condition up to the 5 days whereas the control fish was up to the 4 days in ice. Here, all parameters in this randomized controlled experiment between OTC-treated group and control fish were kept similar, ranging from water quality parameters, feeding as well as sampling producers. The apparent difference is quality indices between the two groups can, therefore, be attributed to OTC-treatment. After this brief period, the samples gradually lost their characteristics of freshness with the lapse of storage period. However, the experimented group of fish was judged of good quality up to 9 days and acceptable up to the 15 days of iced storage. On the other hand, the control fish stored in ice were found organoleptically good up to 7 days, acceptable up to 11 days, and finally was rejected after 12 days, indicating significant increase in keeping time of OTC-treated group in ice, an increment of 4 days compared to non-added antibiotic.

The pattern of changes in organoleptic qualities of control and OTC treated mrigal carp can roughly be divided into four phases as reported by Reza *et al.* (2009). For control mrigal carp the periods of 0 to 4, 5 to 7, 8 to 10 and 11 to 12 days in ice storage condition. In phase I (0 to 4 days), the mrigal carp just passed the rigor mortis and there was a very little change in texture. At this stage, samples were excellent condition with fresh bright shining and iridescent appearance, firm, consistent and elastic texture, characteristic white flesh color and natural odor and color. In phase II (5 to 7days), there was slight dullness and loss of brightness and slight softening of texture. In phase III (8 to 10 days), there was considerable loss of brightness and softening of texture. In phase IV (11 to 12 days), there was definite dullness, loss of bloom, cloudy lens and sunken eye cap, brown and bleached gill, soft flesh and sour odor. As the days passed the off flavor become too intense. Similarly, in case of OTC-treated group, the pattern of organoleptic quality changes was more or less similar, except for phase IV (11 to 16 days) where the fish showed a significant loss of brightness and softening the texture.

Although the estimated shelf life differs from those reported by Dhanapal *et al.* (2013) where he reported shelf life of

rohu carp (*Labeo rohita*) was 17 days. The decreased shelf life in the present study may be related to size variation of fish, feeding condition and environmental factors where the average weight of fish used in that experiment was 884.40 ± 114.77 g. Another study by Mehta and Shamasundar (2015) also reported that Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) were organoleptically rejected after 17 days of ice storage. In the present study, fish supplied with antibiotic mediated feed in aquarium condition had lower level of bacterial flora in the different parts of the body as observed for bacteriological studies. Thus, the quality deterioration process due to microbes during ice storage condition of fish was probably slow. The apparent rise in TVB-N values also coincides well where the values were significantly higher for control fish compared to OTC-treated fishes. So, the use of OTC supplemented diet in mrigal can extend the total shelf life by few more days.

Table 2. Changes in organoleptic quality of control and OTC-treated mrigal carp during ice storage in laboratory condition

Ice storage period (days)	Control mrigal carp		OTC-treated mrigal carp	
	Defect Points	Overall quality	Defect Points	Overall quality
1	1.21 (A)	Excellent	1.21 (A)	Excellent
2	1.46 (A)	Excellent	1.32 (A)	Excellent
3	1.64 (A)	Excellent	1.47 (A)	Excellent
4	1.85 (A)	Excellent	1.66 (A)	Excellent
5	2.10 (B)	Good	1.87 (A)	Excellent
6	2.43 (B)	Good	2.10 (B)	Good
7	2.74 (B)	Good	2.34 (B)	Good
8	3.50 (B)	Acceptable	2.59 (B)	Good
9	3.95 (B)	Acceptable	2.85 (B)	Good
10	4.41 (B)	Acceptable	3.16 (B)	Acceptable
11	4.93 (B)	Acceptable	3.50 (B)	Acceptable
12	5 (C)	Rejected	3.85 (B)	Acceptable
13	-	-	4.21 (B)	Acceptable
14	-	-	4.58 (B)	Acceptable
15	-	-	4.95 (B)	Acceptable
16	-	-	5 (C)	Rejected

Values in the parentheses are on grade for respective defect points of fish.

Changes in proximate composition during ice storage condition

Proximate composition of control and OTC-treated group stored in ice was determined at every 4 days interval under laboratory condition and resulted gradual increase in moisture content of control and OTC treated mrigal carp was observed throughout the 16 days of ice storage (Fig.1A) where the moisture content reached to 78% and 82% for control and OTC treated fish samples, respectively. This sort of increased moisture content during ice stored fish is possibly linked to uptake of water during storage period as reported by previous studies (Reza *et al.*, 2009; Islam *et al.*, 2015). More or less similar results was observed for ash contents where the initial values were 2.3% and 3.2% and after 16 days of ice storage condition increased to 3.8% and 4.7% respectively for control and OTC treated fish (Fig.1B). Generally, ash content is decreased with ice storage, but in the present study it was found to have increased. Rising in ash content may be related to experimental error. The protein content, on the other hand, was 14.9%, and 16.7%, in the first day of storage that decreased to 12.9% and 13.5% for the control and OTC treated fish at the final day of storage

condition (Fig. 1C). The decrease in crude protein during 10 days of iced storage in both control and OTC treated fish was due to the formation of free drip accompanied by some sarcoplasmic protein. Tarr (1965) reported that some loss of organic nitrogenous constituents, largely sarcoplasmic protein and inorganic salts with free drip are probable contributing factor of such loss of protein contents in chilled fish. In the present study, an inverse relationship was observed between moisture and lipid contents which were similar to the findings of Stansby (1962). The initial levels of lipid were 1.9%, and 2.0% at the start of ice storage which decreased to 1.3% and 1.9% for the control and OTC treated fish (Fig.1D) respectively. At the end of storage period, the lipid content differed significantly between control and OTC treated groups due to the experimental error.

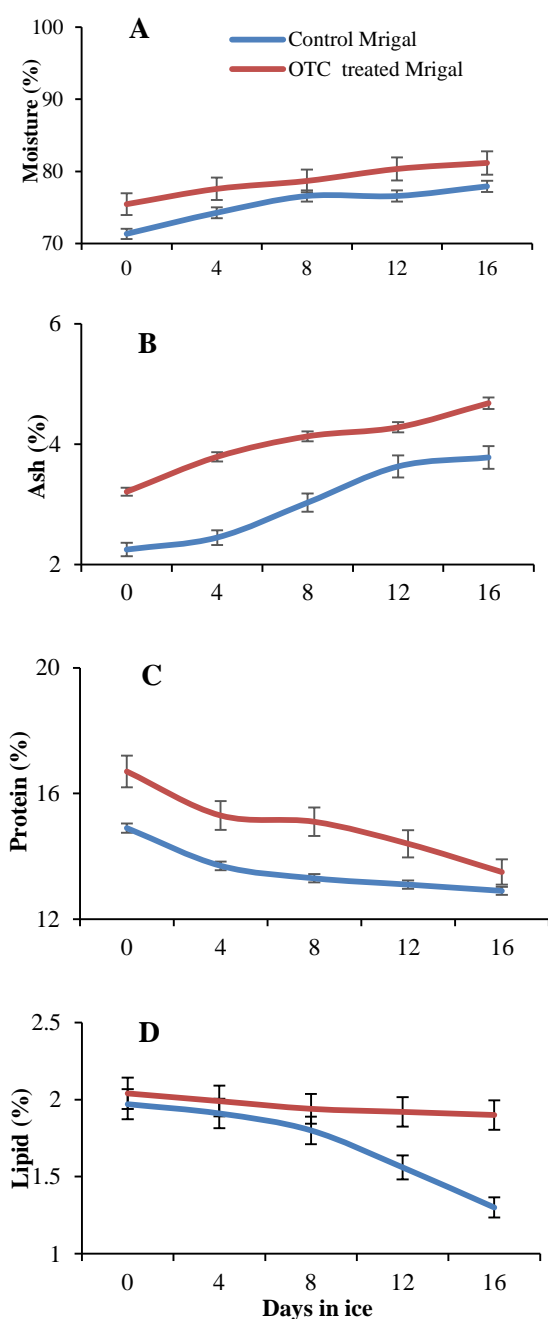


Fig. 1: Changes in proximate composition of control and OTC treated mrigal carp during ice storage. A, Moisture; B, Ash; C, Protein; and D, Lipid. Vertical bars denote SD. Statistical difference is shown by asterisk ($p < 0.05$).

Changes in chemical composition during ice storage condition

The changes in fish muscle pH of ice stored control and OTC-treated group during 16 days of ice storage condition were observed and showed in Fig.2A. The initial pH was 6.5 and 6.2 at the first day of storage condition and increased gradually to 8.9 and 7.3 respectively at the final day of storage period for the control and experimented mrigal fish. The low pH values encountered a few hours after harvesting may also indicate that the fish were not harvested in a rested state and that they had been stressed (Kyra *et al.*, 1997). The curves were obtained for different days although the pH in all the samples gradually increased with the lapse of storage period, and at the end of 16 days of storage the pH increased up to the range of 8.9 and 7.3 with the lowest value in the antibiotic treated fish. The initial low pH was because of form action of lactic acid during anaerobic glycolysis in fish muscle. The gradual increase in pH with 16 days of ice storage period indicates the accumulation of alkaline compounds, such as ammonia compounds and TMA, mainly derived from microbial action (Hebard *et al.*, 1982). This is related to the increased production of various amines and basic substances from proteolytic products derived from microbial activity. A good relationship between changes in pH and organoleptic qualities of the fish samples was observed where the organoleptic characteristics greatly deteriorated with the increase in pH values above 7.5. A high degree of association was observed between pH of the flesh and either APC (log cfu/g) or TVB-N level in the spoiling fish.

The inverse relationship found in NPN contents where the initial values were 0.0032%, and 0.0042% at the preliminary day of storage condition and a very little decreased to 0.0011% and 0.003% respectively for the control and experimented fish at the 16th day of storage condition (Fig.2B). The initial TVB-N contents were 2.7 and 2.00 at the first sampling day and reached 33.00 and 27.5 mg/100gm respectively, at the final day of the sampling in the mrigal and OTC-treated mrigal fish (Fig.2C). TVB-N is used for the determination of the spoilage level during the storage period (Cobb and Venderzont, 1975). In the studies of the storage of different time interval of two ice stored fish, it was suggested that TVB-N value may change depending on the spoilage flora and analysis method (Antonacopoulos and Vyncke, 1989). The concentration of TVB-N in freshly caught fish is typically between 5 and 20 mg TVB-N 100/g flesh (Connell, 1995). However, latest reports showed an initial TVB-N value of <20 mg/100g flesh (Kyra *et al.*, 1997; Rodriguez *et al.*, 2004). The available reports suggest that, the upper limit of 30 mg 100/g TVB-N with the lapse of storage may be attributed to bacterial spoilage, and pH and TVBN levels were found closely related well with the changes in quality (Kietzmann *et al.*, 1969; Cobb and Venderzont, 1975). Peroxide formation in ice stored fish, proved to be very slow during storage period as can be observed in (Fig.2D). The initial PVs were below 4 meq kg/oil, which increased gradually with the lapse of storage period. At the end of 16 days of storage, the values for control and OTC treated mrigal exceeded 23 and 16 meq kg⁻¹ oil which is much more than the recommended value of 10-20 suggested by Connell (1995). Interestingly, mrigal carp, which is a lean fish, also had a high PV after 16 days of ice storage condition. At the value above 20, the fish will probably smell and taste rancid. The PV gives a measure of the first stages of oxidative

rancidity, which did not necessarily correlate well with the sensory assessment of rancidity.

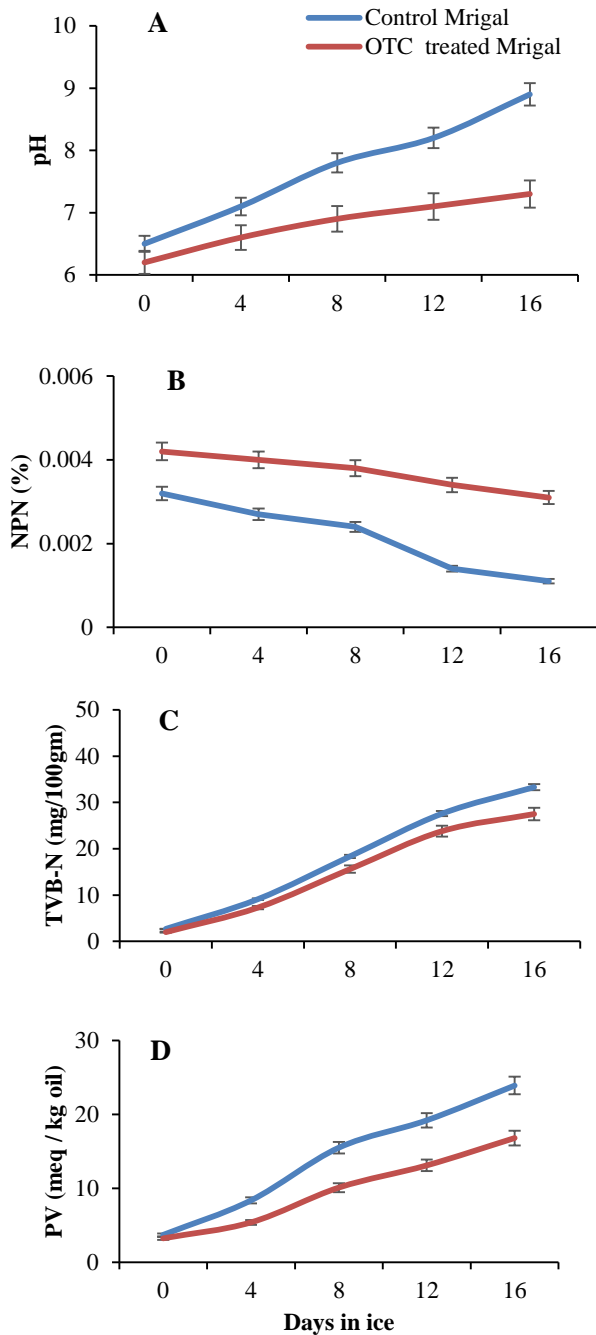


Fig. 2: Changes in chemical composition of control and OTC-treated mrigal carp during ice storage. A, pH; B, NPN; C, TVB-N; and D, PV. Vertical bars denote SD.

Changes in bacterial load during ice storage

The initial APC at the first day of sampling before ice storage was very low. In the fish species, these values increased gradually until the end of the experiment. But it was interesting to see that the rate of increase in APC was slow process in case of OTC-treated mrigal. The initial bacterial loads were 3.46 and 2.8 log cfu/g, which reached to a value of 9.8 and 6.5 log cfu/g after 16 days of ice storage in control and OTC treated mrigal fish (Fig.3), which indicates that OTC in experimental fish had some effect on viability of total bacterial load. Islam et al. (2015) reported that the APC is significantly reduced after the treatment of OTC in *Barbonymus gonionotus* which was similar to our findings. The APC was significantly reduced in gill, intestine

and skin at the end of 21 days of OTC treated feed (Depaola et al., 1995) which was more or less similar to our study. Kashem (2012) reported that the aerobic plate count of control pond and OTC treated ponds were significantly varied which was agreed with our findings.

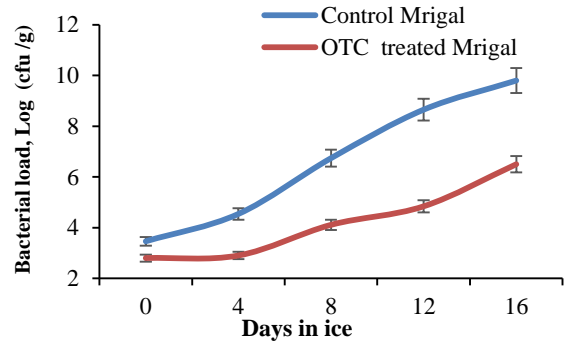


Fig. 3: Changes in APC values of control and OTC-treated mrigal carp during ice storage in laboratory condition. Vertical bars denote SD. Statistical difference is shown by asterisk (p<0.05).

This increase in bacterial load was also positively correlated (0.999 for control fish and 0.951 for OTC-treated fish) to the increase in TVB-N values found in both groups of ice stored fishes (Fig. 4). The corresponding equations are $y = 4.7142x - 13.152$ for control fish and $y = 6.6824x - 13.027$ for OTC-treated fish. Similar results were also reported by Reza et al. (2009).

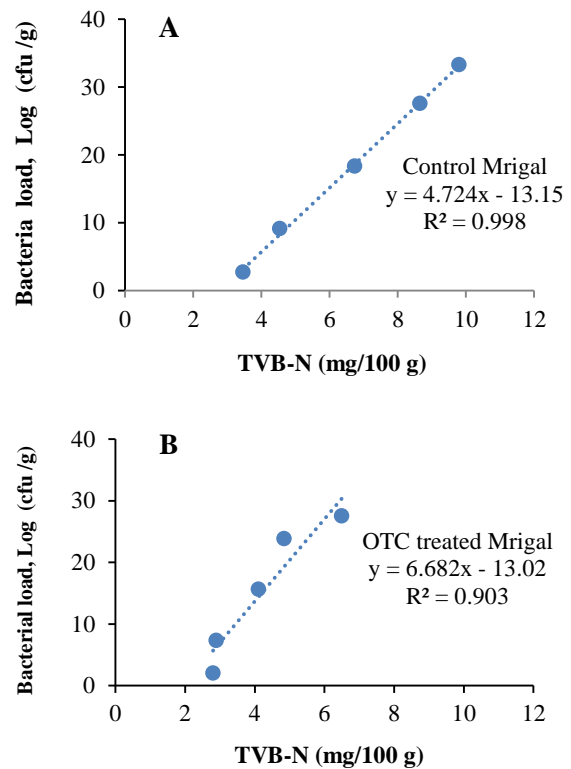


Fig. 4: Regression equation of TVB-N on bacterial load (APC) during ice storage. A, Control Mrigal; and B, OTC treated Mrigal. Plot of linear regression with time.

Conclusion

Shelf life of OTC treated mrigal carp was determined by organoleptic, biochemical, chemical and bacteriological



method which revealed that fish can be kept in iced condition for 16 days and had significant variation is existed between control and OTC supplemented fish. The OTC treated fish exerted significant effect for reducing bacterial population on fish stored in ice and extend the shelf life. However, more studies are needed whether such antibiotics remain in fish body or not as residues are great concern for food safety as well as human health.

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