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Shelf life of antibiotic treated rohu fish, *Labeo rohita* (Hamilton) under ice storage condition

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Abstract

The study was conducted to evaluate the effect of antibiotic on shelf life in rohu fish, *Labeo rohita* (Hamilton) under ice storage condition. Oxytetracycline (OTC), the most widely used antibiotic, was fed to rohu (average body weight 16.0 g) at the rate of 2 g/kg through fish diet for 5 days and their shelf life was determined in iced condition. Organoleptically, fish were found to be acceptable up to 16 days before becoming inedible compared to 15 days for control fish which received pelleted diet with no antibiotic under the same condition. Initial moisture, ash, protein, lipid, NPN and TVB-N values were 70.42±1.91%, 2.80±0.10%, 17.90±0.50%, 3.12±0.04%, 0.0086±0.01% and 17.43±0.60 mg/100g respectively in the control which reached at values of 78.45±1.50%, 3.84±0.10%, 13.47±1.00%, 2.80±0.08%, 0.0053±0.001%, 26.17±0.76 mg/100g, respectively after 16 days of ice storage. There was no significant difference for these values compared to control group. In case of total bacterial load, values of aerobic plate count (APC) was $2.0 \pm 0.1 \times 10^3$ during the start of ice storage condition which increased significantly to $5.6 \pm 0.38 \times 10^7$, exceeding the acceptable limit for ice stored fish. The APC values also did not show any significant variation compared to control fish, suggesting that the use of antibiotic in fish diet had little or no effect on shelf life of rohu fish during ice storage condition.

Keywords: Antibiotic, Ice storage condition, Proximate composition, Bacterial load, *Labeo rohita*, Shelf life

Introduction

With the expansion and intensification of aquaculture in south Asian countries and other parts of the world, antibiotics have become an integral part to treat bacterial infections of fish, shrimp and other aquatic organisms to ensure health and productivity. These drugs are applied as a means to treat diseased fishes or for prevention of diseases (Christensen *et al.,* 2006) as the production may get hampered by unpredictable mortalities that may be due to negative interactions between fish and pathogenic bacteria. Although they are used to improve aquaculture production, residues of these antibiotics and chemicals could influence quality of the final product. Such influences, in general, may include shelf life of the fish and fishery product under different storage conditions, nutritional aspects and food safety.

Among the antibiotics used to treat different infectious diseases in human, poultry, cattle, aquaculture and other animal husbandry, only a small number of them were approved by EU, FDA and other organizations for use in aquaculture.The FDA has approved five different drugs for use in aquaculture as long as the seafood contains less than a mandated maximum residue limit such as florfenicol, sulfamerazine, chorionic gonadotropin, oxytetracycline dihydrate, oxytetracycline hydrochloride, as well as a drug combination of sulfadimethoxine and ormetoprim (United States Government Accountability Office, GAO, 2011). Therefore, oxytetracycline (OTC) has been used as the preferred antibiotic in aquaculture. It is also the most widely used antibacterial agents in aquaculture worldwide (Smith *et al.,* 1994). The vast majority of OTC supplied in mediated feed can be released to the culture system via fish excreta and even the portion of uneaten feed (Hektoen et al. 1995). These chemicals have effect on the bacterial population and also on the storage condition of fishes. Sometimes these antibiotics are indiscriminately used in the aquaculture system which may result in long term effect on fish and also to its consumers.

Rohu fish, *Labeo rohita* (Hamilton) is a member of the family Cyprinidae within the order Cypriniformes. It is native to the river systems of Bangladesh, India, Pakistan, Myanmar (Talwar and Jhingran, 1991). With the expanding aquaculture practice in the country, rohu culture is practicing in semi-intensive and extensive systems in ponds, low-lying areas like haor and baor. The features that made this species a potential candidate for aquaculture include faster growth rate, higher market price, greater feed utilization and ability to feed in all three columns in a water body. In 2008-09, rohu production from culture fishery was 226,585 mt, covering 24.84% of total aquaculture production in Bangladesh (BBS, 2009). It is expected that its production will continue to increase.

Although there were no systematic studies conducted in the past, the available reports suggest that considerable post-harvest changes take place during storage condition in ice or frozen for antibiotic treated fishes. Considering the importance of rohu fish in Bangladesh aquaculture, the study was carried out to determine the changes in its post-harvest quality fed with antibiotic treated feed and control and bacterial population during ice storage condition.

Materials and Methods

Experimental design

Six glass aquaria (size 37cm×30cm×60cm) were set at the Laboratory of Fish Harvesting, Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh where 3 aquaria were used for antibiotic treatment and 3 as control. These aquaria were filled to a depth of 15 cm with tap water. Rohu fry (average body weight 16g) was collected from Field Laboratory Complex, Faculty of Fisheries, BAU and released into the aquarium. Aerators were set in each aquarium and water was changed everyday. Antibiotic medicated feed was prepared at the rate of 2g OTC / kg feed and fed to the fish at 3-5% of their body weight for consecutive 5 days. Fish were killed by insertion of sharp pointer into the head and then transferred into an insulated box. Fresh block ice at ratio of ice and fish at 1:1(w ⁄ w) was used for icing the samples and 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 days interval by determining the organoleptic, chemical and bacteriological tests.

Sampling procedure and sample preparation

At described intervals (starting from 0 day), 4 to 5 fish were randomly sampled and their sensory attributes were evaluated while keeping the fish in iced condition. The whole fishes were subjected to sensory analysis; 2 to 3 fish for chemical and rest for microbiological analysis. The muscle was skinned, deboned and homogenized by mincer, and was taken for the determinations of bacteriological aspects.

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 0 day, 4 to 5 fish were randomly sampled and their raw sensory attributes were evaluated. The organoleptic characteristics were judged by a trained panel of expert members during the storage period. 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 and chemical 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 h until constant weight. Ash content was determined by igniting the sample in a muffle furnace at a temperature of 550°C for 6 hrs. Crude protein was determined by the Macro Kjeldahl method through determination of 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 hrs in a ground joint Soxhlet apparatus at 70^0C. The oil obtained by evaporation of the solvent on a steam bath was weighed in a sensitive balance and percent lipid was calculated. The 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 ice storage fish.

Bacteriological analyses

About 15 g 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 were done using SPSS 11.0 (Statistical Package for the Social Science, Chigago, USA).

Results and Discussion

Changes in organoleptic qualities during ice storage condition

The changes in quality of chilled rohu fish during storage were assessed by organoleptic assessment. On the basis of the scores, all the fish samples were found in acceptable condition for up to 16 days in ice storage before they became inedible (Table 1).

Table 1. Changes in organoleptic characteristics of antibiotic treated rohu fish stored in ice

Days in ice	Organoleptic qualities	Defect points	Grade	Overall Quality
0	Full bloom, bright and shining skin; convex, transparent eye cap; bright red gills; firm and elastic flesh; natural fishy odour	1.25	A	Excellent
4	Full bloom and shining appearance; transparent eye cap & black pupil; red gills; elastic flesh; natural fishy odour	1.58	A	Excellent
8	Bright skin; slightly plane eye; slightly pinkish gills; some loss of elasticity of flesh; natural odour	2.21	B	Good
12	Loss of bloom; cloudy lens; pink gill; softening of flesh; moderately sour odour	3.45	B	Acceptable
16	Dull, no bloom of skin; concave eye cap; gray gills; lump flesh; sour odour	5	C	Rejected

The organoleptic characteristics of quality changes that occurred during storage period can be roughly divided into four phases corresponding to periods of 0–3, 4–7, 8–11 and 12–16 days in ice. In the phase 1, the fishes were very fresh with species taste and natural flavor and odor. At this stage, the fish samples had the characteristics of freshly caught fish. In phase 2, there was some slight change but similar to the first phage. In phase 3, there was little deterioration apart from some slight loss of natural flavour and characteristic odor. In phase 4, there was slight dullness with some off-flavour, but in acceptable condition. Although the estimated shelf life differed slightly from those reported by Dhanapal *et al.* (2013) where he reported shelf life of rohu was 17 days. It may be related to seasonal variation, size and other environmental factors. As the fish were supplied with antibiotic mediated feed in aquarium condition so the bacterial flora in the different parts of fish body was very low. Thus the quality deterioration process in the ice storage condition of fish was very slow.

Changes in chemical characteristics during ice storage condition

Proximate composition of rohu fish was determined during ice stored condition at an interval of 4 days interval is given in Table 2.

Table 2. Proximate composition of antibiotic treated rohu fish stored in ice

Days of storage	Moisture (%)	Ash (%)	Crude Protein (%)	Lipids (%)	NPN (%)	TVB-N (mg/100g)
Control fish						
0	72.2±1.80	3.0±0.22	18.1±0.60	3.5±0.10	0.0060±0.50	4.2±0.50
4	73.2±0.50	3.0±0.15	17.8±0.25	3.5±0.10	0.0062±0.10	8.5±0.30
8	75.1±0.35	3.2±0.60	16.2±0.20	3.2±0.20	0.0062±0.05	8.2±0.50
12	75.8±0.30	3.5±0.20	15.0±0.50	2.8±0.20	0.0060±0.05	12.4±0.40
16	79.2±0.30	3.6±0.25	12.9±0.80	2.2±0.30	0.0060±0.50	28.7±0.65
Antibiotic treated fish						
0	70.4±1.91	2.8±0.10	17.9±0.50	3.1±0.04	0.0086±0.01	7.4±0.60
4	73.0±2.00	3.2±0.26	17.6±0.10	3.1±0.04	0.0069±0.02	8.0±0.60
8	74.3±1.00	3.4±0.6	16.2±0.23	3.0±0.06	0.0062±0.02	14.3±0.49
12	76.5±0.51	3.5±0.10	15.5±0.50	2.8±0.06	0.0055±0.01	19.4±0.45
16	78.4±1.50	3.8±0.10	13.4±1.00	2.8±0.08	0.0053±0.01	26.1±0.76

Results (mean±S.D)

It was found that moisture content of fish stored in ice condition was increased with increase in storage period. The initial moisture content was 70.4±1.91%, which increased to 78.45±1.50% at the end of the experiment. These values were not significantly different for those that obtained for control rohu fish (Table 1). The higher moisture content during ice storage was probably because of uptake of water during storage period (Reza *et al*., 2009). Similar condition was found for protein and lipid contents where the values were highest with 17.9±0.50% and 3.1±0.04% on 0 day respectively which decreased to values of 13.4±1.00% and 2.8±0.08%, on 16th day respectively in carcass of fish feed an antibiotic treated feed. Changes in moisture content were followed by a reverse change in lipid content indicating an inverse relationship between water and fat content (Stansby, 1962).

The loss of crude protein in fish during ice storage was due to leaching of water soluble protein fraction from fish muscle.The initial ash and TVB-N contents of fish fed antibiotic treated feed were 2.80±0.10, 7.43±0.60 on 0 day and reached to 3.84±0.10, 26.1±0.76 respectively on 16th day. The level of increasing in the next was slow.The initial level of crude protein, lipid, and NPN content of fish were 17.90±0.50, 3.12±0.04, 0.0086±0.01, and reached 13.47±1.00, 2.80±0.08, and 0.0053±0.01 respectively in the last day of storage condition. Interestingly for ash content, statistically significant change was observed for rohu fish during 16 days of ice storage. It is well known that the variations in chemical composition of fish was closely related to feed intake and reproduction cycle.The most dramatic changes that occured in chemical components were water and fat content in the fish species. There were also significant differences in composition of muscles lipid and water content. An inverse relationship existed between lipid and moisture content where approximates to 80%, which was also more or less in agreement with the general rule formulated by (Stansby, 1962). The TVB-N was used for the determination of the spoilage level during the storage period (Cobb & Venderzont, 1975). 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 was typically between 5 and 20 mg TVB-N 100/g flesh (Connell, 1995). However, latest reports showed an initial TVB-N value of <20 mg100/g flesh (Kyrana *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 TVB-N levels were found closely related well with the changes in quality (Kietzmann *et al.* 1969; Cobb and Venderzont, 1975).

Changes in bacterial load during ice storage

The changes of bacterial flora during ice storage condition at an interval of 4 days interval were determined and data are shown in Table 3.

Table 3. Changes of bacterial load in antibiotic treated rohu fish stored in ice

Days of ice storage	Bacterial load (cfu/g)	
	Control fish	Antibiotic treated fish
0	$2.75{\pm}0.20{\times}10^{3}$	$2.02{\pm}0.01{\times}10^{4}$
4	$4.00{\pm}0.12{\times}10^{3}$	$9.10{\pm}0.05{\times}10^{4}$
8	$2.50{\pm}0.01{\times}10^{5}$	$7.31{\pm}1.06{\times}10^{5}$
12	$3.50{\pm}1.30{\times}10^{6}$	$3.03{\pm}1.53{\times}10^{6}$
16	$2.60{\pm}1.50{\times}10^{8}$	$5.60{\pm}0.38{\times}10^{7}$

Results (mean±S.D) are expressed as CFU/g

Initial APC at 0 day sample before ice storage was very low. These values increased gradually until the end of the experiment. But it was interesting to see that the rate of increase in APC was very slow. The initial bacterial load was $2.02{\pm}0.01{\times}10^{4}$, which reached to a value of $5.60{\pm}0.38{\times}10^{7}$ cfu/g after 16 days of ice stored fish. It was found that the growth of bacteria was one log higher in control fish i.e., $2.60{\pm}1.50{\times}10^{8}$ cfu/g, which indicates that OTC in experimental fish had some effect on viability of total bacterial load. Similar results were also found by Yeasmin *et al.* (2010) where they reported that bacterial growth was significantly lower in formalin treated rohu fish under ice storage condition compared to control group. This increase in bacterial load was also positively correlated to the increase in TVB-N values found in both groups of ice stored fishes.

Conclusion

Shelf life of antibiotic treated rohu fish was determined by organoleptic, biochemical and bacteriological method and it was found that fish can be kept in iced condition for 16 days which was slightly higher as compared control fish which were acceptable up to 15 days. The loss of quality in these fishes was due to bacterial and enzymatic activities during long time storage in ice although antibiotics added in experimental fish diet exerted little effect for reducing bacterial population on fish stored in ice. However, more studies are needed whether such antibiotic remain in fish body as residues or not, as they are great concerns of food safety.

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