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FULL LENGTH ARTICLE

The combined effect of environmental thermal drop and isotonicity on metabolic stores of the teleost, *Oreochromis niloticus*

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Winter Stress Syndrome;
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Abstract Four experimental regimes were designed to test the capacity of the Nile tilapia *Oreochromis niloticus* to make use of the energy saved form osmoregulation in an isotonic medium in overcoming the Winter Stress Syndrome (WSS). Fish either pre- acclimated to freshwater or isotonic salinity at 25 °C were transferred directly to freshwater or an isotonic medium (ca. 12‰) at 14 °C. Fish were killed 3, 6, 24, 48, 72 and 168 h after transfer. The mobilisation and use of lipids from perivisceral and muscle fat observed in the study seem to be a direct response to cold stress as well as the associated fasting, these effects were salinity dependent being lesser at the isotonic salinity. The energy needs of fish diminish in cold conditions when the salinity of the environment approaches the isotonicity. The absence of any significant changes in muscle and water content indicated that the changes of muscle lipid are true and lipid was the sole source of energy upon thermal drop. The changes of blood haemoglobin levels throughout the four experimental regimes may indicate that the isotonic medium acclimated tilapia showed less haematological disturbance due to cold stress. Results from this experimental study recommend that the pre-acclimation of the Egyptian strain of Nile tilapia, *O. niloticus*, to an environmental salinity close to the isotonicity may improve fish cold tolerance.

Introduction

Winter Stress Syndrome (WSS) is a term coined by Lemly (1993) to describe a condition of metabolic distress in warm-water fish. The syndrome develops when external stressors that cause increased metabolic demands are present concurrently with the arrival of cold water temperature in late autumn. Cold weather and the associated short photoperiod of winter

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environmentally "program" warm-water fish like tilapia for reduced activity and food intake, and they do not respond to stressors with increased feeding. If the elevated metabolic demands persist, stored body lipid necessary for overwintering is depleted, body condition deteriorates, and the fish may die. To the best of our knowledge, few studies have been conducted to investigate the lipid requirement of tilapia under different salinities. Recently, El Sayed et al. (2005) have tackled this issue during their investigation on the effect of lipid source on the reproductive performance of Nile tilapia broodstock reared at different salinities (0.7‰ and 14‰). They found that fish reared in brackishwater required n-3 HUFA for optimum spawning performance, while those reared in freshwater were not affected by dietary lipid source. The optimum protein requirement at different salinities of the Nile tilapia *Orechromuis niloticus* was studied by El Salhia (1999), she found that fish reared in 20‰ salinity consumed less dietary protein and grow faster than those reared in other salinities or freshwater.

Many previous works revealed that fish in an isotonic medium have better physiological conditions and growth than those in other salinities (Schofield et al., 2010; Hassan, 2011; Hassan et al., 2013). Kang'ombe and Brown, 2008, studied the effect of salinity on growth, feed utilisation, and survival of Juvenile *Tilapia rendalli* in tanks, and found that salinity of 10‰ is optimal for *T. rendalli* in tank culture.

Haemoglobin is particularly important in fish adaptation as it constitutes an interface between the organism and the environment (Landini et al., 2002). Inoue et al. (2008) evaluated the haematology (haematocrit and haemoglobin) in the Amazonian warm-water fish matrinxã (*Brycon amazonicus*) subjected to an acute cold shock (cold shock from 28 °C to 18 °C) and sampled after 1, 3, 6, 12, 24 h, and recorded that blood haematocrit and total haemoglobin were not affected throughout the study in either control or cold shock groups.

The present study was designed to test the capacity of the fish, *O. niloticus*, to make use of the spared energy for osmoregulation in an isotonic medium to improve their cold tolerance. The experiments were conducted to determine the changes of energy sources of muscle lipid and protein in response to cold stress at both freshwater (FW) and isotonic point water (IP). Blood haemoglobin, as indicator of fish health status, was also measured.

Materials and methods

Preparation of the fish for experiments, preparation of the isotonic medium (IP), preparation of the cold system, experimental protocol and sample collection of Blood and tissue are given in detail elsewhere (Hassan et al., 2013).

Analytical techniques

Haemoglobin content in blood was determined by using the Diamond diagnostic haemoglobin kit (Wintrobe, 1965). Muscle protein concentration was measured by the method of biuret (Gornall et al., 1949). Muscle lipid content was determined with an adaptation of the sulphophosphovanilin method described by Knight et al. (1972). Muscle water content was analysed by taking a piece of a specific weight of white epaxial muscle and drying it to constant weight at 100 °C for 24 h. It was then reweighed after drying, and then the water content was measured as a percentage to the muscle weight.

Statistical analysis

Data were analysed using Student's *t*-test. Statistical significance is judged on overlap of 95% confidence intervals ($p < 0.05$). Comparisons were made against controls at 25 °C. The fish transferred from fresh water to fresh water or to isotonic point water were compared with the corresponding values of fresh water acclimated fish as control and those transferred from isotonic point to fresh water or to isotonic point water were compared with the corresponding values of isotonic point acclimated fish as control. Values in the tables are expressed as mean ± standard error of mean (SEM). If there was a significant deviation of the experimental from the control means, the points in the table were marked with one star at level of 0.05 ($p < 0.05$) and two stars if at the level of 0.01 ($p < 0.01$). The program used for statistical analysis is SPSS.

Results

Changes of muscle lipid content (Table 1)

The muscle lipid content of fish decreased significantly three hours after they were transferred from FW (25 °C) to FW (14 °C) ($p < 0.01$, 20%) and remained low throughout the following time until it recovered only by the end of exposure. Similar but lesser initial (3hr, $p < 0.01$, 11%) reduction of lipid in fish muscle was recorded when they were transferred from FW (25 °C) to IP (14 °C). Muscle lipid content remained more or less unchanged during the whole experimental time without any significant variation as compared to the control when the fish were either transferred from IP (25 °C) to FW (14 °C) or from IP (25 °C) to IP (14 °C).

Changes of haemoglobin content (Table 2)

Haemoglobin content decreased significantly compared to the control by 3 hour exposure 3 ($p < 0.01$, 20% or 40%) thereafter it tends to recover throughout the rest of the experimental time. These changes were recorded in fish after transferred either from FW (25 °C) to FW (14 °C) or from FW (25 °C) to IP (14 °C) respectively. No significant variations were measured during the whole experimental periods during adaptation after transfer from IP (25 °C) to FW (14 °C) or from IP (25 °C) to IP (14 °C).

Changes of muscle water content (Table 3)

During all of the four experimental conditions, muscle water content remained more or less unchanged as compared to the corresponding controls.

Changes of muscle protein content (Table 4)

The changes of muscle protein content recorded during all experimental conditions were not significantly different from their corresponding controls.

Table 1 Changes of muscle lipid content during the four experimental conditions (g/100 g tissue).

Time (h)	FW 25 °C to FW 14 °C	FW 25 °C to IP 14 °C	IP 25 °C to FW 14 °C	IP 25 °C to IP 14 °C
0	1.59 ± 0.110	1.59 ± 0.133	1.59 ± 0.150	1.59 ± 0.105
3	1.22 ± 0.120**	1.26 ± 0.122*	1.56 ± 0.120	1.57 ± 0.107
6	1.22 ± 0.122**	1.13 ± 0.0150*	1.58 ± 0.123	1.56 ± 0.100
24	1.23 ± 0.130**	1.22 ± 0.120*	1.53 ± 0.150	1.57 ± 0.109
48	1.16 ± 0.123**	1.26 ± 0.154*	1.58 ± 0.125	1.56 ± 0.110
72	1.20 ± 0.112**	1.24 ± 0.110*	1.58 ± 0.123	1.57 ± 0.120
168	1.66 ± 0.125	1.56 ± 0.120	1.57 ± 0.100	1.57 ± 0.125

* Mean values are significant at the level of $p < 0.05$.
** Mean values are significant at the level of $p < 0.01$.

Table 2 Changes of haemoglobin content during the four experimental conditions (g/dl).

Time (h)	FW 25 °C to FW 14 °C	FW 25 °C to IP 14 °C	IP 25 °C to FW 14 °C	IP 25 °C to IP 14 °C
0	6.47 ± 0.11	6.47 ± 0.11	6.00 ± 0.20	6.00 ± 0.20
3	4.18 ± 0.08**	4.89 ± 0.06**	6.92 ± 0.08	6.93 ± 0.11
6	6.47 ± 0.11	6.42 ± 0.07	6.00 ± 0.38	5.91 ± 0.14
24	6.45 ± 0.16	6.47 ± 0.00	6.98 ± 0.12	5.95 ± 0.12
48	6.40 ± 0.09	6.47 ± 0.13	5.95 ± 0.08	5.90 ± 0.04
72	6.40 ± 0.09	6.49 ± 0.11	6.00 ± 0.13	6.00 ± 0.09
168	6.40 ± 0.11	6.55 ± 0.10	5.99 ± 0.14	5.90 ± 0.11

** Mean values are significant at the level of $p < 0.01$.

Table 3 Changes of muscle water content during the four experimental conditions (%).

Time (h)	FW 25 °C to FW 14 °C	FW 25 °C to IP 14 °C	IP 25 °C to FW 14 °C	IP 25 °C to IP 14 °C
0	76.19 ± 0.00	76.19 ± 0.003	77.92 ± 0.003	78.92 ± 0.003
3	76.45 ± 0.007	76.96 ± 0.002	78.30 ± 0.003	78.49 ± 0.004
6	77.67 ± 0.006	77.11 ± 0.002	78.87 ± 0.005	77.85 ± 0.013
24	77.25 ± 0.003	75.46 ± 0.002	79.57 ± 0.004	78.43 ± 0.002
48	77.84 ± 0.004	77.58 ± 0.008	78.99 ± 0.008	77.75 ± 0.003
72	77.43 ± 0.026	75.42 ± 0.011	77.10 ± 0.006	79.49 ± 0.002
168	75.01 ± 0.009	78.79 ± 0.009	78.71 ± 0.003	78.39 ± 0.001

Table 4 Changes of muscle protein content during the four experimental conditions (g/100 g tissue).

Time (h)	FW 25 °C to FW 14 °C	FW 25 °C to IP 14 °C	IP 25 °C to FW 14 °C	IP 25 °C to IP 14 °C
0	25.63 ± 0.004	25.63 ± 0.004	25.63 ± 0.004	25.63 ± 0.004
3	24.85 ± 0.004	25.61 ± 0.005	24.30 ± 0.003	24.78 ± 0.003
6	24.75 ± 0.003	24.97 ± 0.004	24.06 ± 0.004	24.74 ± 0.003
24	24.37 ± 0.003	24.82 ± 0.003	25.87 ± 0.003	25.28 ± 0.003
48	25.22 ± 0.003	24.20 ± 0.003	24.39 ± 0.004	25.17 ± 0.003
72	26.39 ± 0.002	25.34 ± 0.003	24.91 ± 0.004	25.04 ± 0.004
168	24.61 ± 0.005	24.35 ± 0.004	24 ± 0.001	24.52 ± 0.002

Discussion

Lipid content of tilapia muscle has been studied by many researchers (Nguyen et al., 2010; Thiansilaku et al., 2010; Teoh et al., 2011; Ng and Wang, 2011; Yarnpakdee et al., 2012). In the present experiments the muscle lipid content dropped significantly during transfer from fresh water at 25 °C to fresh water at 14 °C, the reduction was observed during the whole experimental time with tendency to be recovered to the control level only after one week of exposure to cold temperature. Similar changes were also recorded during transfer from freshwater at 25 °C to isotonic concentration at 14 °C, however the magnitude of lipid drop during the later trail (FW, 25 °C to IP, 14 °C) was smaller than that during the formal one (FW, 25 °C to FW, 14 °C) which may indicate that the increase of the salinity of the media to which the fish were transferred lowered the effect of cold stress that reflected in a lower energy requirement and hence lower lipid depletion. Therefore, the

probability that a given temperature drop leads to WSS could be minimised by increasing the water osmotic pressure to the isotonic point and that is in a good agreement with our assumption. Charo-Karisa et al. (2005) investigated the cold tolerance of juvenile Nile tilapia, *O. niloticus*, and they concluded that the most appropriate way of enhancing cold tolerance of tilapia juveniles is by husbandry practices that increase pre-winter body weights.

With an emphasis on the limiting level for lipid depletion upon increasing the salinity of the media to the isotonic point, we designed two experiments during which tilapia after being adapted to isotonic salinity at 25 °C have been transferred directly either to an isotonic medium at 14 °C or to FW at 14 °C. No significant depletion of the muscle lipid content was observed in the isotonic medium pre-acclimated tilapia. These results have thus supported and are in line with the assumption made before that increasing salinity to the isotonic point may support tilapia survival at cold stress. A similar conclusion was given by Schofield et al. (2010), who revealed that however, survival was much lower when fish were exposed to winter temperatures (14 °C), only at 10‰ Nile tilapia survived well at winter temperatures.

The results of this experiment indicated that tilapia can adjust to direct decrease in temperature when they are kept at an isotonic salinity. When the temperature was maintained at 14 °C and the salinity of the environment was 12‰, fish experienced no sign of WSS irreversible harm, such as losing equilibrium, cessation of respiration, cessation of food consumption. In other words, cold tolerance of *O. niloticus* can be enhanced by increasing the environmental salinity to isotonic point.

Upon a further decrease of water temperature below 14 °C, tilapia become more easily infected by disease (Sifa et al., 2002). We suggested that freshwater temperature for Nile tilapia overwintering in earthen pond should be above 16 °C and at isotonic salinity not below 14 °C, which can assure that fish have normal activity and a high survival rate. This assumption that made by us is supported by Peterson et al. (2005) who determined the cold tolerances of juvenile blue tilapias *Oreochromis aureus* at salinities ranging from 0‰ to 35‰ in the laboratory by decreasing temperatures of 1 °C/d until fish died, and they found that fish maintained in isosmotic media (11.6‰) survived at lower temperatures than those in water of higher or lower salinity.

The mobilisation and use of lipids from perivisceral fat and muscle observed in our study seem to be a direct response to cold stress as well as the associated fasting, these effects were salinity dependent being lower at the isotonic salinity. The energy needs of fish diminish in cold conditions when the salinity of the environment approaches the isotonicity.

From our present and previous studies (Hassan et al., 2013) we can conclude that the changes of muscle lipid content and of plasma glucose concentration are in favour of improving the capability of the fish *O. niloticus* to overcome the cold stress during winter in earthen ponds simply by saving more energy via decreasing the energy requirements for osmoregulation. The simultaneous decrease of lipid proved that most of the energy needed, in the form of glucose generated ATP, for cold tolerance came from the stored triacylglycerols which catabolised to glycerol and was used in gluconeogenesis and glucose production, this result is in good agreement with those observed by Ibarz et al. (2005) in gilthead sea bream (*Sparus aurata*). The absence of any significant changes in total muscle protein supported the assumption that the sole source of glucose and hence energy was the muscle lipid.

Moreover, the lack of any significant changes in muscle water content during all experimental trials indicates without any doubt that the changes of muscle lipid content were actual changes and not due to dehydration or hydration of muscle tissues.

Haemoglobin is particularly important in fish adaptation as it constitutes an interface between the organism and the environment (Landini et al., 2002). In the present study cold stress in the fresh water pre-acclimated tilapia induced a decrease in the blood haemoglobin content. These results match with Diouf et al. (2000) who revealed that blood haemoglobin concentration is decreased in cold-stressed fish, which could be the result of haemodilution. The results are in odd with Inoue et al. (2008) who recorded that blood haematocrit and total haemoglobin were not affected by cold shock. Despite the decrease of blood haemoglobin in the freshwater pre-acclimated tilapia exposed to cold stress, an insignificant variation is noticed in the isotonic point water pre-acclimated tilapia after cold stress.

The isotonic point water pre-acclimated tilapia showed higher blood haemoglobin than those in the freshwater pre-acclimated tilapia. These results differ from those found by Karsi and Yavuzcan Yildiz (2005), who stated that the salinity levels of freshwater, 9‰ and 18‰ did not influence haematocrit values in the Nile tilapia.

The present investigation indicates that the isotonic medium pre-acclimated tilapia showed a less haematological disturbance due to cold stress than that observed in the freshwater pre-acclimated ones.

Conclusion

In the light of our study, it is recommended, firstly, that pre-acclimation of the Egyptian strain of Nile tilapia, *O. niloticus*, to an environmental salinity close to the isotonicity can improve the cold tolerance and secondly, the increment of the muscle lipid content prior to the winter onset can also enhance cold tolerance, which may be provided by increasing the lipid content of the ration and hence pre- winter body weight.

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