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Myo-inositol enhances the low-salinity tolerance of turbot (*Scophthalmus maximus*) by modulating cortisol synthesis

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ABSTRACT

Myo-inositol is a major intracellular osmolyte that can be accumulated to protect cells from a variety of stresses, including fluctuations in the osmolality of the environment, and cortisol is thought to be an osmotic hormone in teleost fish. In this study, dietary *myo*-inositol resulted in increased Na^+-K^+-ATPase activity and gene expression of partial ion channel genes and prolonged survival time of turbot (*Scophthalmus maximus*) under low salinity. The cortisol regulated by dietary *myo*-inositol also was correlated with these outcomes. The optimal concentrations of cortisol stimulated gill Na^+-K^+-ATPase activity and increased the expression of ion channel genes to enhance low salinity tolerance, as indicated by longer survival time under low salinity. When cortisol level was suppressed, *myo*-inositol failed to increase the survival time of turbot under low salinity, and strong correlations between cortisol concentration and Na^+-K^+-ATPase activity, expression of partial ion channel genes, and survival time of turbot were detected. These results showed that *myo*-inositol enhanced the low salinity tolerance of turbot by modulating cortisol synthesis.

1. Introduction

Myo-inositol is a B-complex vitamin that classified as an essential vitamin for most animals [1]. It is synthesized by various animal tissues and is an essential dietary ingredient for most aquatic animals [2]. It is also a major intracellular osmolyte that can be accumulated to protect cells from a variety of stresses including increases in the osmolality of the extracellular environment [3]. Sakaguchi et al. [4] described the specific mechanism of *myo*-inositol action in the eel gill. in which the chondrocytes of the gill cartilage may function to produce and release inositol into the bronchial circulation, allowing the epithelial cells to take up the osmolyte via active membrane transport systems such as the sodium-dependent *myo*-inositol transporter.

Cortisol is a glucocorticoid hormone and the major corticosteroid in teleost fish. It also has a mineralocorticoid function [5]. Accumulating evidences suggested that the hypo-osmoregulatory ability of euryhaline fishes can be improved by cortisol treatment [6]. Kiilerich et al. [7] reported that cortisol stimulated the transcript levels of ion transporters in the gill of Atlantic salmon in freshwater. McCormick found that during seawater acclimation, cortisol stimulated chloride cell numbers and Na^+-K^+-ATPase activity in the gill and increases overall salinity tolerance [8]. Watanabe et al. [9]summarized cortisol's osmoregulatory role as a promoter of electrolyte conservation in freshwater teleosts and as a promoter of ion excretion during seawater acclimation. Cruz et al. [10] presented solid molecular evidence that the effects of cortisol on ionocyte development and function were mediated by the glucocorticoid receptor that is present on ionocytes.

Cruz et al. recently reported that exogenous cortisol promoted epidermal ionocyte progenitor differentiation in zebrafish (*Danio rerio*) [11].

Turbot (*Scophthalmus maximus*) have the ability to adapt to opposing osmotic challenges and are an excellent model for studying the physiological adaptations of flounder associated with osmoregulatory plasticity [12]. The physiological and molecular adaptability of turbot to salinity have been reported [13]. However, the utility of *myo*-inositol in osmoregulation has been reported in only a few fish, such as zebrafish (*Danio rerio*) [11], and little is

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known about the effects of *myo*-inositol on osmotic regulation in turbot. Ma et al. [14] reported osmoregulation via the *myo*-inositol biosynthesis pathway in turbot *S. maximus*, but the specific mechanism of action remains unknown.

To study the role of *myo*-inositol on osmoregulation in turbot, Na^+-K^+-ATPase activity, expression of ion channel genes, and survival time under low salinity were analyzed after dietary delivery of exogenous *myo*-inositol, cortisol, and metyrapone (a cortisol inhibitor). Both the pattern of *myo*-inositol in osmotic regulation of turbot and the functional mechanism of action were assessed.

2. Materials and methods

2.1. Fish

Turbot (total length 18.29 ± 0.49 cm) were obtained from Yantai Tianyuan Aquaculture Co., Ltd. (Shandong, China). Healthy fish were stocked in circular fiberglass tanks supplied with seawater for 2 weeks to adapt them to the experimental facilities and conditions (photoperiod, 14 h light: 10 h dark; temperature, 14.0 ± 1.0 °C; pH 8.1). The use of fish in this study was approved by the Experimental Animal Ethics Committee, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China.

2.2. Experimental diets containing myo-inositol and the challenge trial

To investigate the potential effects of *myo*-inositol on cortisol synthesis and osmoregulation of turbot, experimental diets containing *myo*-inositol were created and used in the salinity challenge trial. The formulation of the basal diet and preparation of the *myo*-inositol concentrations were the same as those described by Ma et al. [14]. *Myo*-inositol (Solarbio, Beijing, China) was added to the basal diet to provide graded concentrations of 300, 600, 900, and 1200 mg MI kg^{-1} [14,15]. A diet containing no *myo*-inositol was used as the control.

A total of 300 fish from the acclimatization aquarium were randomly distributed into 15 groups (triplicate groups per treatment). The experimental conditions were the same as those in the acclimatization aquarium. The fish were fed a commercial diet containing *myo*-inositol twice a day (8:00 and 18:00 h) for 2 weeks at a feeding rate of 1% body weight during the feeding trial. At the end of the feeding trial, the gill and arterial blood were sampled from 6 turbot from each group, and 10 fish from each group were acutely transferred to lethally low salinity 0‰. The survival time of fish was recorded for each treatment.

2.3. Experimental diets containing cortisol and the challenge trial

To investigate the potential effects of cortisol on osmoregulation of turbot, experimental diets were created and a salinity challenge trial was carried out. Analytical grade cortisol (Hydrocortisone; H4001-1G; ≥99% HPLC; CAS No:50-23-7) was purchased from Solarbio (Beijing, China). The stock solution of cortisol was prepared by dissolving it in ethanol (0.1%) and sterile water. Cortisol was added to the basal diet to provide graded concentrations of 125, 250, 500, and 750 mg kg^{-1} [16]. A diet containing no *myo*-inositol was used as the control.

A total of 450 fish from the acclimatization aquarium were randomly distributed into 15 groups (triplicate groups per treatment). The experimental conditions were the same as those in the acclimatization aquarium. The fish were fed as described in section 2.2. At the end of the feeding trial, the gill and arterial blood were sampled from 6 turbot from each group, and 20 fish from each group were acutely transferred to lethally low salinity 0‰. The survival time of fish was recorded for each treatment. After half of the fish in a tank had died, the remaining 10 fish were transferred to normal seawater (salinity 30) for 14 days to test whether cortisol improved their osmotic regulation. The number of live fish and changes of skin characteristics were observed and recorded.

2.4. Experimental diets containing myo-inositol and metyrapone and the challenge trial

To investigate the potential effects of *myo*-inositol on osmoregulation of turbot in the context of cortisol suppression, experimental diets containing *myo*-inositol and metyrapone were created and used in the salinity challenge trial. Metyrapone (Cat No: HY-B1232, CAS No: 54-36-4) was purchased from MedChem Express (Monmouth Junction, N J, USA). It was dissolved in sterile water at 38 mg mL^{-1} and further diluted to the indicated concentrations in culture medium before use. Metyrapone was added to the basal diet to provide graded concentrations of 300, 600, 900, and 1200 mg kg^{-1}, and *myo*-inositol was added to each basal diet to provide a concentration of 1200 mg kg^{-1} [16]. A diet containing no metyrapone was used as the control.

A total of 300 fish from the acclimatization aquarium were randomly distributed into 15 groups (triplicate groups per treatment). The experimental conditions were the same as those in the acclimatization aquarium. The fish were fed as described in section 2.2. At the end of the feeding trial, the gill and arterial blood were sampled from 6 turbot from each group, and 10 fish from each group were acutely transferred to lethally low salinity 0‰. The survival time of fish was recorded for each treatment.

2.5. Plasma cortisol test

The concentration of cortisol in the plasma of experimental fish was measured using the Fish Cortisol ELISA Kit following the manufacturer's protocol (Jianglaibio, Shanghai, China). Before the experiment, the reliability and stability of the kit were validated by serial dilution curve and high temperature (37 °C) destructive experiments, respectively.

2.6. Na^+-K^+-ATPase activity assay

The Na^+-K^+-ATPase activity in the gills of the experimental fish was measured using a Na^+-K^+-ATPase assay kit following the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) [17].

2.7. Fluorescence quantitative real-time PCR (qRT-PCR)

The qRT-PCR assays were conducted to detect the expression profiles of ion channel genes in gill tissues of turbot. The experimental procedures and all primers (Table S1) for qPCR were described in previous studies [14,18]. The *18S* gene was used as the internal control. All data are expressed as the change with respect to the corresponding *18S* Ct level.

2.8. Statistical analysis

The results are reported as mean ± SEM. SPSS19.0 software (SPSS, Chicago, IL, USA) was used to test for significant differences among the means of different groups. $P < 0.05$ was considered to be statistically significant. Normality and homoscedasticity assumptions were tested prior to the analysis. The *t*-tests (for two groups), two-way analysis of variance (ANOVA), and the Pearson's correlation test were used to analyze the data.

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3. Results

3.1. Responses of turbot after consuming myo-inositol

To determine the effects of dietary *myo*-inositol on osmoregulation in turbot, the Na^+-K^+-ATPase activity assay was conducted. The Na^+-K^+-ATPase activity was down regulated and then up regulated as the concentration of dietary *myo*-inositol increased. Na^+-K^+-ATPase activity was highest without dietary *myo*-inositol, and lowest for the 600 mg kg^{-1} diet (Fig. 1A). After consuming *myo*-inositol, *AQP1*, *AQP3*, *AQP11*, *NHE3*, *14-3-3*, and *CFTR* were expressed regularly (Fig. 1B). Exception for the control group, expression levels of *AQP1* and *AQP3* were first up regulated and then down regulated as the *myo*-inositol concentration increased. The expression of AQP11 gradually decreased and those of *NHE3*, *14-3-3*, and CFTR increased as the *myo*-inositol concentration increased. The survival time of turbot with *myo*-inositol feeding was longer than that of fish without dietary *myo*-inositol. Survival time was longest in the 600 mg kg^{-1} *myo*-inositol group (Fig. 1C).

These results demonstrated that *myo*-inositol improved low salinity adaptation by mediating osmotic regulation as represented by ion channels and Na^+-K^+-ATPase activity. To determine whether *myo*-inositol regulates osmoregulation of turbot by mediating cortisol synthesis, the cortisol concentration after *myo*-inositol feeding was studied. The ELISA assay showed that the average concentration of cortisol slightly decreased and then increased as the concentration of dietary *myo*-inositol increased. The 600 mg kg^{-1} *myo*-inositol group had the lowest concentration of cortisol (Fig. 1D).

We conducted Pearson's correlation analysis to examine the correlation between the cortisol concentrations and Na^+-K^+-ATPase activity, expression of ion channel genes, and survival time. We found a strong positive correlation (R = 0.9) between cortisol concentration and Na^+-K^+-ATPase activity and a strong negative correlation between cortisol concentration and expression of ion channel genes *AQP1* and *AQP3* (Table S2). Correlations between *myo*-inositol concentration in diets and expression of ion channel genes *AQP11*, *NHE3*, *14-3-3*, and *CFTR* were statistically significant ($p < 0.05$), except for the control group. A significant negative correlation (R = −0.994) between cortisol concentration and survival time was detected ($p < 0.05$).

3.2. Responses of turbot after consuming cortisol

To study the effects of cortisol consumption on cortisol content *in vivo*, the cortisol concentrations in the plasma after cortisol consumption were measured. The cortisol content in the plasma gradually increased as the concentration of dietary cortisol increased (Fig. 2A). The Na^+-K^+-ATPase activity assay and relative expression of ion channel genes by quantitative real-time PCR were performed to study the effects of cortisol on osmoregulation. As the cortisol content in the plasma gradually increased, the average level of Na^+-K^+-ATPase activity in the gill was up regulated, except for the 750 mg kg^{-1} group (Fig. 2B). The Na^+-K^+-ATPase activity was significantly and positively correlated (R = 0.925) with the cortisol concentrations, except for the 750 mg kg^{-1} group ($p < 0.05$). The expression of ion channel genes was regularly expressed as the concentration of dietary cortisol increased. In the gill of turbot,

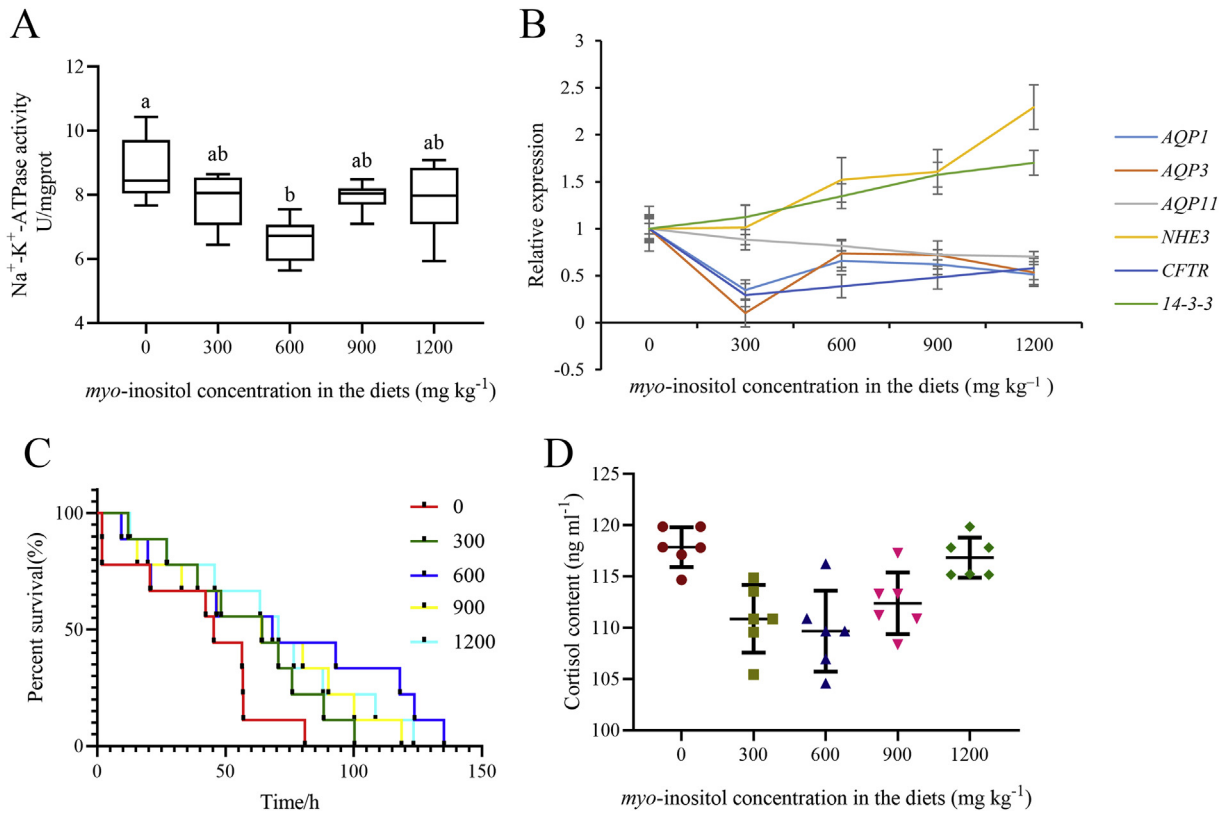

Fig. 1. Responses of turbot after consuming *myo*-inositol. (A) Na^+-K^+-ATPase activity after *myo*-inositol feeding, (B)relative expression of ion channel genes after *myo*-inositol feeding, (C) survival time of turbot under low salinity after *myo*-inositol feeding, (D) cortisol concentration in plasms after *myo*-inositol feeding. *AQP1*, Aquaporin 1; *AQP3*, Aquaporin 3; *AQP11*, Aquaporin 11; *CFTR*, cystic fibrosis transmembrane regulator; *NHE3*, Na^+/H^+ exchanger 3. Different letters represent significant differences ($p < 0.05$) between the columns.

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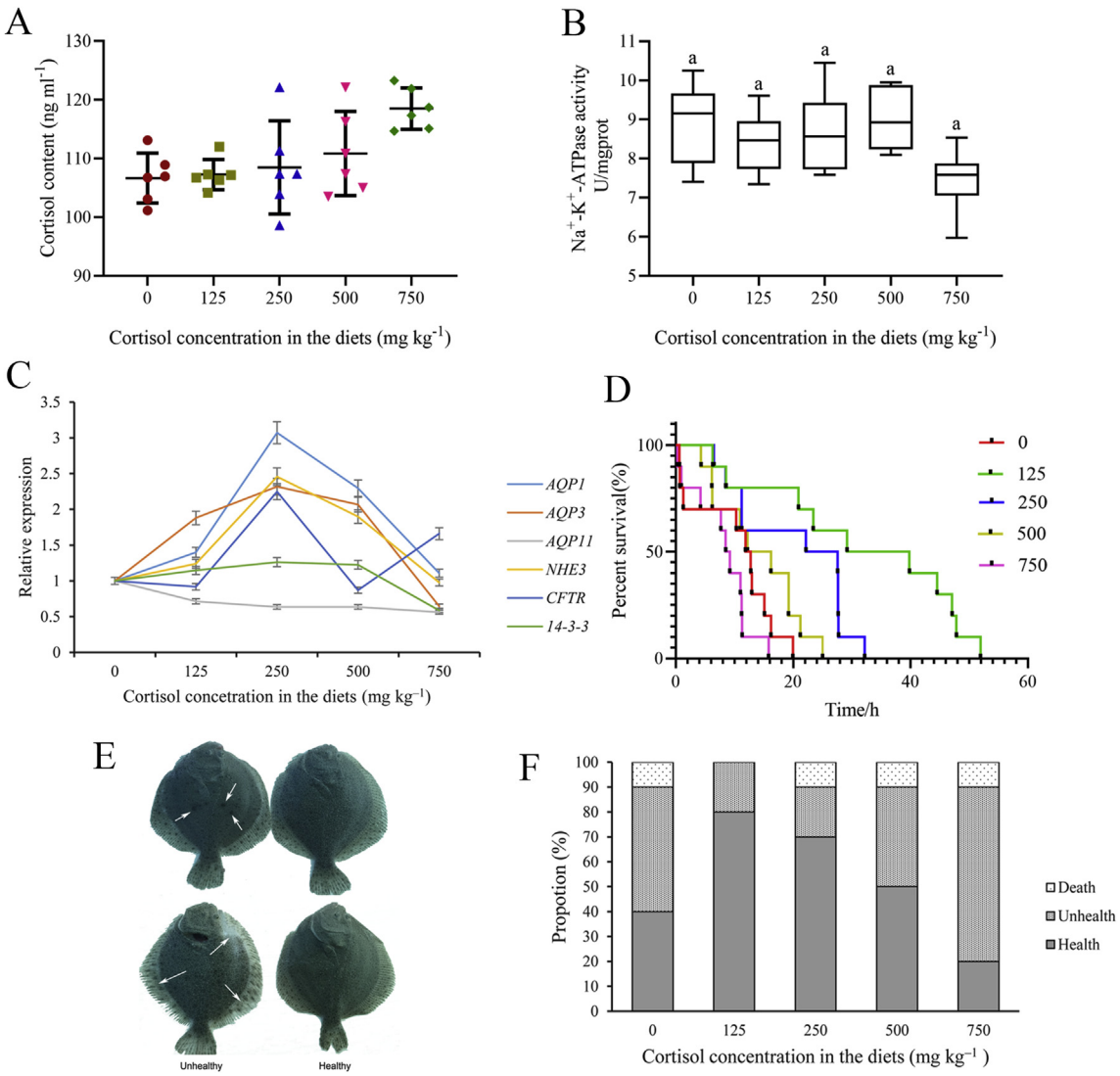

Fig. 2. Effects of consuming cortisol on turbot. (A) Cortisol concentration in the plasma, (B) Na^+-K^+-ATPase activity, (C) relative expression of ion channel genes, (D) survival time of turbot under low salinity, (E) body characteristic of turbot in seawater after low salinity stress (white arrow indicate Unhealthy characteristics), (F) healthy fish rate in seawater after low salinity stress. Different letters represent significant differences ($p < 0.05$) between the columns.

expression of *CFTR* exhibited zigzag-like changes. As the concentration of cortisol in the feed increased, expression of *AQP1*, *AQP3*, *NHE3*, and *14-3-3*, which are related to ion transport, were up regulated first and then down regulated, whereas expression of *AQP11* was down-regulated (Fig. 2C). The survival time of turbot under salinity 0‰ increased after cortisol consumption but was lower with each increase in cortisol concentration, and the survival time was shorter in the 750 mg kg^{-1} group compared to the control group (Fig. 2D). Pearson's correlation analysis detected a strong negative correlation between cortisol concentration and survival time (R = −0.857). To further probe the effects of cortisol on low salinity tolerance of turbot, recovery of turbot in seawater after low salinity exposure and cortisol consumption was assessed. The number of healthy fish was defined as the total fish minus the dead and the unhealthy fish (Fig. 2E). The number of healthy fish increased after cortisol consumption but decreased as the cortisol concentration increased. The number of healthy fish was lower in the 750 mg kg^{-1} group compared to the control group (Fig. 2F). The correlation between cortisol concentration and healthy ratio was strong (R = −0.863).

3.3. Responses of turbot after consuming myo-inositol and metyrapone

To investigate the effects of metyrapone-mediated inhibition of cortisol, the cortisol level in the plasma after fish had consumed *myo*-inositol and metyrapone was measured. The cortisol level was depressed, yet its effect decreased with the increase of metyrapone concentration (Fig. 3A). The Na^+-K^+-ATPase activity assay and relative expression of ion channel genes by quantitative real-time PCR were performed to investigate the potential effects of *myo*-inositol on osmoregulation of turbot in the context of cortisol suppression. Although the turbot were fed with a high concentration of *myo*-inositol, the average level of Na^+-K^+-ATPase activity in the gill was gradually down regulated as metyrapone content increased (Fig. 3B). The ion channel genes were expressed regularly as the metyrapone concentration increased. With increasing metyrapone content in the diet, the expression of *AQP1*, *AQP11*, and *NHE3* was up regulated first and then down regulated, while the expression of *AQP3*, *CFTR*, and *14-3-3* was continuously down-regulated (Fig. 3C). The survival time of turbot under salinity

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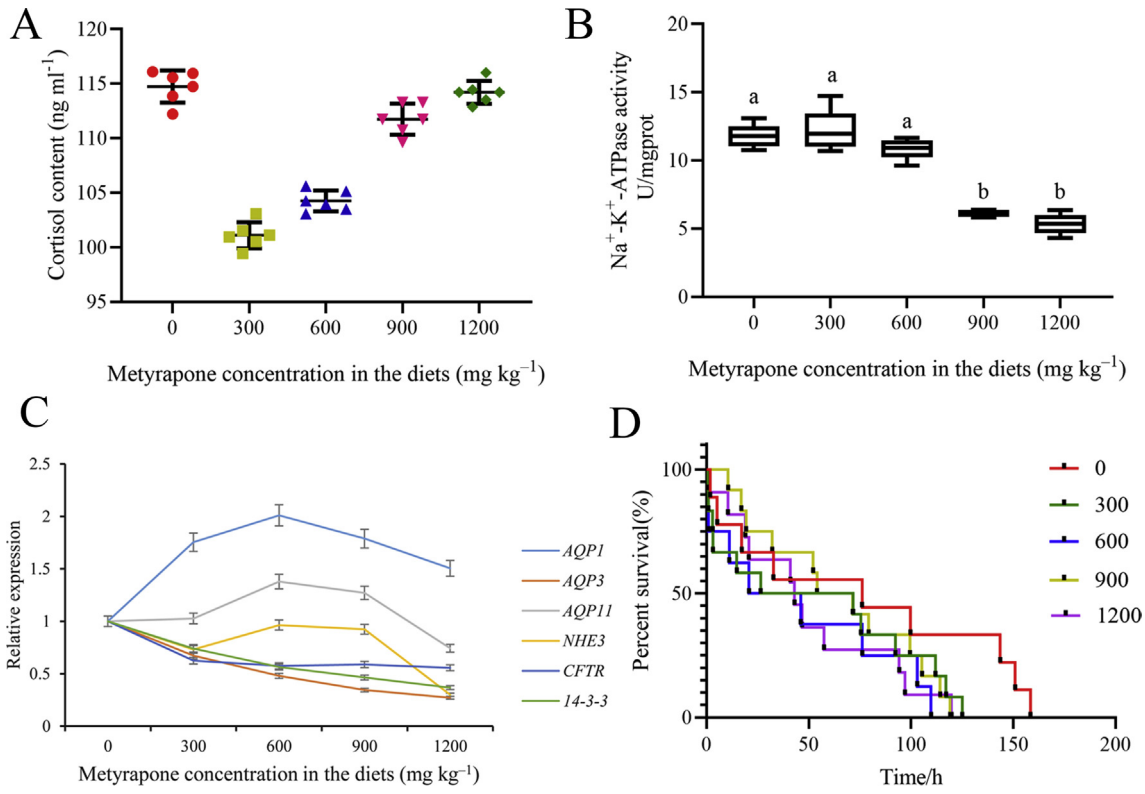

Fig. 3. Responses of turbot after feeding *myo*-inositol and metyrapone. (A) Cortisol concentration in the plasma, (B) Na^+-K^+-ATPase activity, (C) Relative expression of ion channels, (D) Survival time of turbot under low salinity. Different letters represent significant differences ($p < 0.05$) between the columns.

0 after consuming *myo*-inositol and metyrapone was also recorded to study the fish's tolerance to low salinity. Although the survival time of some individuals in the 900 mg kg^{-1} and 1200 mg kg^{-1} groups was longer than that in the control group, the survival time of the other fish was lower than that of the control group (Fig. 3D).

Pearson's correlation analysis was performed to examine the correlation between cortisol concentration and Na^+-K^+-ATPase activity, expression of ion channel genes, and survival time under low salinity. Na^+-K^+-ATPase activity was significantly and negatively correlated ($R = -1$) with the cortisol concentrations, except for the control group ($p < 0.01$). Additionally, significantly negative correlations were identified between cortisol concentration and expression of ion channel genes *AQP3*, *14-3-3*, and *CFTR* ($p < 0.05$) (Table S3). There also was a remarkably negative correlation between cortisol concentration and expression of *AQP1* (Table S3). Cortisol concentration and survival time also were strongly correlated.

4. Discussion

Myo-inositol is a major intracellular osmolyte that can be accumulated to protect cells from a variety of stresses, including fluctuations in the osmolality of the environment [19,20]. In present study, we found that dietary *myo*-inositol resulted in regular changes in gene expression and Na^+-K^+-ATPase activity in the turbot gill. Consistent with a study in rats [21], in which *myo*-inositol administration improved survival, dietary *myo*-inositol prolonged the survival time of turbot under low salinity. Although animals utilizing *myo*-inositol for osmoregulation was known previously [22], its specific mechanism of action remains unclear. We detected a tendency for regular change of cortisol content in the plasma of turbot as the dietary *myo*-inositol concentration increased, and this change was remarkably correlated with Na^+-K^+-ATPase activity, expression of partial ions channel genes, and survival time under low salinity. Consistent with results of a previous study, cortisol was significantly regulated by dietary *myo*-inositol [23], and the process involved in the seawater adaptation by stimulating gill Na^+-K^+-ATPase activity and transport of Na^+, Cl^-, and H_2O [24]. These results indicated that the actions of *myo*-inositol on osmoregulation may be mediated by its anabolite cortisol to regulate Na^+-K^+-ATPase activity and expression of partial ion channel genes.

Cortisol is the major corticosteroid in teleost fish, and it is considered to be dual regulator of chloride cell differentiation and function [7]. It also is thought to be a seawater-adapting hormone, as it promotes salt excretion in hypo-osmoregulating fish [25]. The effects of cortisol on osmoregulation have been investigated in numerous fish species [7,26,27], but the role of cortisol in the osmotic regulation of turbot is still unknown. In present study, increased plasma cortisol induced by dietary cortisol resulted in changes in osmoregulation under low salinity stress in turbot. As described by Madsen [25], the optimal concentration of cortisol could stimulate gill Na^+-K^+-ATPase activity and increase the expression of ion channel genes to enhance low-salinity tolerance, as indicated by longer survival time under low salinity. Strong correlations between cortisol concentrations and expression of ion channel genes in turbot were detected, which emphasized the important role of cortisol in regulating osmoregulatory gene expression in the teleost fish gill [7]. The correlations between cortisol concentrations and survival time and health in between different treatment groups and the control group in this study also demonstrated that exogenous cortisol may affect the development of osmoregulatory mechanisms and, consequently, the physiological features associated with ion and osmoregulation [24].

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Consequently, all of the evidence suggesting that cortisol directly plays an important role in ionic and osmotic regulation [28,29], argues for an adaptive role for cortisol during salinity stress in fish.

Metyrapone was used to investigate the potential effects of *myo*-inositol on osmoregulation of turbot in the context of cortisol suppression. Oral administration of metyrapone at low concentration depressed the plasma cortisol level in turbot, as described by Marcolongo et al. [30] in which metyrapone administered at low micromolar concentrations enhanced the oxidation of cortisol. The previous result also suggested that oral administration of metyrapone decreased serum cortisol level in a dose-dependent fashion [16]. Although high concentrations of *myo*-inositol were suppled in the diets tested in this study, reduced cortisol levels resulted in decreased expression of ion channel genes and Na^+-K^+-ATPase activity, which in turn led to reduced survival time of turbot under low salinity. *Myo*-inositol also failed to increase the survival time of turbot under low salinity in the context of cortisol suppression. However, significant or strong correlations were identified between cortisol concentration and Na^+-K^+-ATPase activity, expression of partial ion channel genes, and survival time of turbot. Therefore, cortisol is likely to be an intermediate substance on which inositol acts during osmotic regulation in turbot.

5. Conclusion

Dietary *myo*-inositol resulted in regular changes in gene expression and Na^+-K^+-ATPase activity and prolonged the survival time of turbot under low salinity. Cortisol level regulated by dietary *myo*-inositol also was correlated with these outcomes. The optimal concentrations of dietary cortisol stimulated gill Na^+-K^+-ATPase activity and increased the expression of ion channel genes to enhance low salinity tolerance as indicated by longer survival time under low salinity. In the context of cortisol suppression, *myo*-inositol failed to increase the survival time of turbot under low salinity. Significant or strong correlations between cortisol concentration and Na^+-K^+-ATPase activity, expression of partial ion channel genes, and survival time of turbot were detected. These results showed that *myo*-inositol enhanced the low salinity tolerance of turbot by modulating cortisol synthesis.

Declaration of competing interest

The all authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2020.04.004.

References

[1] F. Shirmohammad, M. Mehri, S. Joezy-Shekalgorabi, A review on the role of inositol in aquaculture, Iran. J. Fish. Sci. 15 (2016) 1388–1409.
[2] F.R. Michael, S. Koshio, Biochemical studies on the interactive effects of dietary choline and inositol in juvenile Kuruma shrimp, *Marsupenaeus japonicus* Bate, Aquaculture 285 (2008) 179183.
[3] M.B. Burg, J.D. Ferraris, Intracellular organic osmolytes: function and regulation, J. Biol. Chem. 283 (2008) 7309–7313.
[4] H. Sakaguchi, T. Katafuchi, H. Hagiwara, et al., High-density localization of ANP receptors in chondrocytes of eel gill cartilage, Am. J. Physiol. Regul. Integr. Comp. Physiol. 265 (2) (1993) R474–R479.
[5] M. Deuschle, F. Hendlmeier, S. Witt, et al., Cortisol, cortisone, and bdnf in amniotic fluid in the second trimester of pregnancy: effect of early life and current maternal stress and socioeconomic status, Dev. Psychopathol. 30 (3) (2018) 1–10.
[6] G.A. Bisbal, J.L. Specker, Cortisol stimulates hypo-osmoregulatory ability in atlantic salmon, *salmo salar* l, J. Fish. Biol. 39 (3) (1991) 421–432.
[7] P. Kiilerich, K. Kristiansen, S.S. Madsen, Cortisol regulation of ion transporter mRNA in atlantic salmon gill and the effect of salinity on the signaling pathway, J. Endocrinol. 194 (2) (2007) 417–427.
[8] S.D. McCormick, Endocrine control of osmoregulation in teleost fish, Integr. Comp. Biol. 41 (4) (2001) 781–794.
[9] S. Watanabe, K. Itoh, T. Kaneko, Prolactin and cortisol mediate the maintenance of hyperosmoregulatory ionocytes in gills of Mozambique tilapia: exploring with an improved gill incubation system, Gen. Comp. Endocr. S0016648016300995. 232 (2016) 151–159.
[10] S.A. Cruz, C.H. Lin, P.L. Chao, et al., Glucocorticoid receptor, but not mineralocorticoid receptor, mediates cortisol regulation of epidermal ionocyte development and ion transport in zebrafish (*Danio rerio*), Electronic Science & Technology 8 (10) (2017), e77997.
[11] S.A. Cruz, P.L. Chao, P.P. Hwang, Cortisol promotes differentiation of epidermal ionocytes through Foxi 3 transcription factors in zebrafish (*Danio rerio*), Comp. Biochem. Physiol. 164 (2013) 249–257.
[12] P. Pereiro, P. Balseiro, A. Romero, et al., Highthroughput sequence analysis of turbot (*Scophthalmus maximus*) transcriptome using 454-pyrosequencing for the discovery of antiviral immune genes, PloS One 7 (5) (2012), e35369.
[13] A.K. Imsland, A. Foss, S. Gunnarsson, et al., The interaction of temperature and salinity on growth and food conversion in juvenile turbot (*Scophthalmus maximus*), Aquaculture 198 (3–4) (2001), 353367.
[14] A.j. Ma, W.X. Cui, X.A. Wang, et al., Osmoregulation by the *myo*-inositol biosynthesis pathway in turbot *Scophthalmus maximus* and its regulation by anabolite and c-Myc, Comp. Biochem. Physiol. A 242 (2020).
[15] S.A. Li, W.D. Jiang, L. Feng, et al., Dietary, myo -inositol deficiency decreased the growth performances and impaired intestinal physical barrier function partly relating to, nrf 2, jnk, e2f4, and, mlck, signaling in young grass carp (*Ctenopharyngodon idella*), Fish Shellfish Immunol. 67 (2017) 475–492. S1050464817303558.
[16] R.O. Bennett, R.C. Rhodes, Evaluation of oral administration of cortisol and metyrapone: the effects on serum cortisol in rainbow trout (*Salmo gairdneri*), Comp. Biochem. Physiol. A Comp. Physiol. 83 (4) (1986) 727–730.
[17] H. Huang, Y.M. Chen, F. Zhu, et al., Down-regulated Na^+/K^+-ATPase activity in ischemic penumbra after focal cerebral ischemia/reperfusion in rats, Int. J. Clin. Exp. Pathol. 8 (10) (2015) 12708–12717.
[18] J. Zhang, Z. Liu, A. Ma, et al., Response of aquaporin (AQP1, AQP3) and ion channel protein (CFTR, NHE1) of turbot (*Scophthalmus maximus*) to low-salinity stress, Prog. Fish. Sci. (2019) 1–9, https://doi.org/10.19663/j.issn2095-9869.20190410003.
[19] R.H. Michell, Inositol derivatives: evolution and functions, Nat. Rev. Mol. Cell Biol. 9 (2008) 151–161.
[20] F.D. Villarreal, D. Kültz, Direct ionic regulation of the activity of myo-inositol biosynthesis enzymes in Mozambique tilapia, PloS One 10 (2015).
[21] S.M. Silver, B.M. Schroeder, R.H. Sterns, et al., Myoinositol administration improves survival and reduces myelinolysis after rapid correction of chronic hyponatremia in rats, J. Neuropathol. Exp. Neurol. 65 (1) (2006) 37–44.
[22] H.U. Shetty, W. Huang, Measurement of myo-inositol in biological systems by mass spectrometric and in vivo 1h magnetic resonance spectroscopic techniques, Curr. Org. Chem. 4 (1) (2000) 1–17.
[23] H.H. Cui, B. Liu, X.P. Ge, et al., Effects of dietary inositol on immune function of juvenile wuchang bream under ammonia stress, J. Fish. China 38 (2014) 228–236.
[24] G.R. Lin, C.F. Weng, J.I. Wang, et al., Effects of cortisol on ion regulation in developing tilapia (*Oreochromis mossambicus*) larvae on seawater adaptation, Physiol. Biochem. Zool. 72 (4) (1999) 8.
[25] S.S. Madsen, Cortisol treatment improves the development of hypo-osmoregulatory mechanisms in the euryhaline rainbow trout, Salmo gairdneri, Fish Physiol. Biochem. 8 (1990) 45–52.
[26] C.P. Cutler, C. Phillips, N. Hazon, et al., Cortisol regulates eel (*Anguilla anguilla*) aquaporin 3 (aqp3) mRNA expression levels in gill, Gen. Comp. Endocrinol. 152 (2–3) (2007) 310–313.
[27] L. Gerber, S.M. Steffen, B.J. Jensen, Cortisol regulates nitric oxide synthase in freshwater and seawater acclimated rainbow trout, *Oncorhynchus mykiss*, Comp. Biochem. Physiol. A 204 (2017) 1–8.
[28] S.D. McCormick, Cortisol directly stimulates differentiation of chloride cells in

tilapia opercular membrane, Am. J. Physiol. 259 (1990) R857–R863.
[29] S.D. McCormick, Hormonal control of gill Na^+-K^+-ATPase and chloride cell function, in: C.M. Wood, T.J. Shuttleworth (Eds.), Cellular and Molecular Approaches to Fish Ionic Regulation (Fish Physiology, XIV), Academic Press, San Diego, CA, 1995, pp. 285–315.
[30] P. Marcolongo, S. Senesi, B. Gava, et al., Metyrapone prevents cortisone-induced preadipocyte differentiation by depleting luminal NADPH of the endoplasmic reticulum, Biochem. Pharmacol. 76 (2008) 382–390.

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