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ABSTRACTS

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STRESS RESPONSE AND CHANGES OF LIVER METABOLISM IN RAINBOW TROUT DEPEND ON THE STRESS DURATION

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Introduction

Stress has been described as a disruption of most fish functions, such as grow, food intake, reproduction and immune system (Wendelaar Bonga, 1997). Typical procedures in aquaculture might be potential stressors. In this way, our previous studies carried out in rainbow trout have evaluated the dynamics of the stress response (Gesto et al., 2013; López-Patiño et al., 2014). However the duration necessary for a stressor to initiate the stress response has not been determined yet. The aim of the present study was to evaluate in rainbow trout the time period necessary for the stress response to initiate and to test how the dynamic of a metabolic tissue such as the liver, by assessing the expression of enzymes related to carbohydrates and lipids metabolism.

Material and Methods

Following two weeks acclimation three sets of trout (*Oncorhynchus mykiss*) were subjected to 5 sec, 15 sec, and 3 min of handling disturbance each, sacrificed and sampled at different post-stress onset time periods (3 to 240 min; N=15 fish/time period). Blood was collected and the plasma obtained, dry-ice frozen and stored at -80ºC until assayed for cortisol, glucose and lactate levels. Individual liver samples were also collected and stored at -80ºC until assayed for mRNA abundance of GK, PK, PEPCK, G6Pase, GLUT2 and FAS. A two way ANOVA test was applied to assess the presence of interactions among both main factors: time and stress duration.

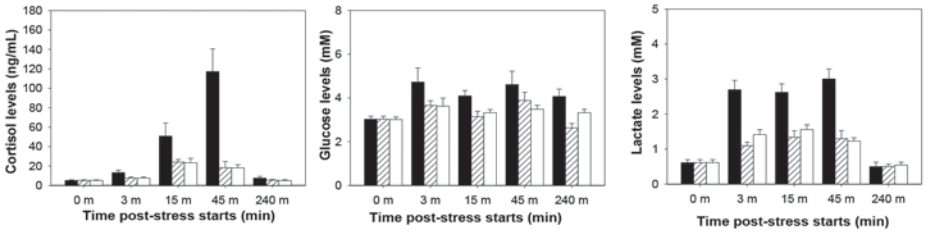

Figure 1. Plasma cortisol (a), glucose (b) and lactate (c) levels in fish handled by 3 min (black barrs), 15 sec. (dotted barrs) and 5 sec (white barrs) at 0 min (control non stressed group), and 3 to 240 min post stress onset. Data represent the average ± S.E.M. (N=10 fish / time point).

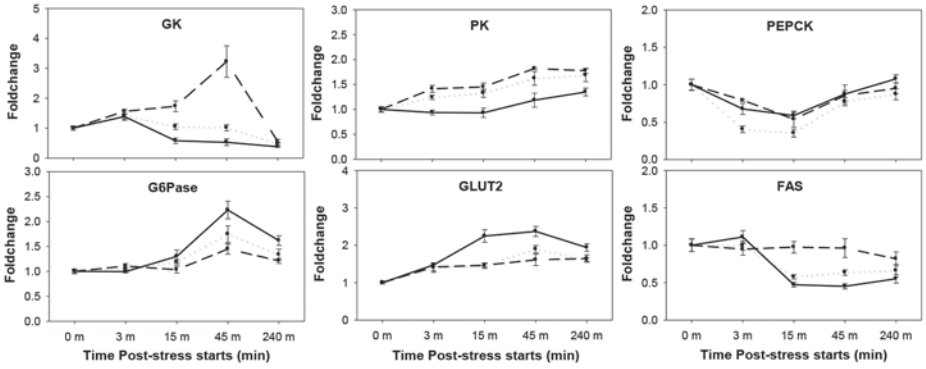

Figure 2. Liver GK, PK, PEPCK, G6Pase, GLUT2 and FAS mRNA abundance in liver of trout handled by 3 min (solid line), 15 sec. (dotted line) and 5 sec (dashed line) at 0 min (control non stressed group), and 3-240 min post stress to initiate. Data represent the average ± S.E.M. (N=5 fish / time point).

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Results

Plasma cortisol, glucose and lactate are shown in Figure 1. Cortisol levels were enhanced by any handling procedure, with the most prominent effect being observed in trout handled for 3 min. Highest cortisol levels were measured after 45 min of stress to initiate, and then turned back to basal levels at 240 min. Glucose increased only in trout disturbed for 3 min and remained high afterwards. Lactate levels rapidly increased, and maximal levels were observed at 15-45 min, then turning back to basal levels at 240 min. The highest increase was observed in those fish handled by 3 min.

Figure 2 shows the mRNA abundance of liver metabolism-related genes. The response was stress- duration-dependent, with the higher response being observed in fish handled for 3 min. GK, PK and FAS mRNA expression was inhibited by stress from 15-45 min and then recovered to normal levels at 240 min. In contrast, increased mRNA expression was detected for G6Pase and GLUT2 (15-45 min), with both genes turning back to basal levels at 240 min. No significant variation was noted for PEPCK expression.

Discussion and Conclusion

Cortisol, glucose and lactate levels show typical stress-related dynamics with higher increases being observed in fish subjected to longer stress duration. These changes are very similar to those previously observed in our laboratory (Gesto et al., 2013). Available references regarding short-term acute responses to stress in parameters related to liver energy metabolism are limited. Similarly to that previously reported by our laboratory (López-Patiño et al., 2014), our results point to increased mobilization of liver glycogen and reduced lipogenic potential, followed by the increased capacity of liver for releasing glucose, and the afterwards recovery of the lipogenic capacity. Such metabolic responses could associate with the differential post-stress temporal changes in plasma hormone levels: fast and short-term rise in catecholamine levels (Gesto et al., 2013) and the more sustained increase in cortisol levels (Pickering and Pottinger, 1995; Wendelaar Bonga, 1997; Gesto et al., 2013). In addition, our data point to the fact that handling procedures longer than 15 seconds might be necessary for a significant liver metabolic response to initiate after stress exposure. Such result may help understanding the nature of the physiological response of farmed fish to aquaculture related stressors, which is of major importance in improving animal welfare and increasing yields from farms.

Acknowledgements

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