UNIVERSIDADE DO ALGARVE
Faculdade de Engenharia de Recursos Naturais

Biotechnology in Aquaculture: polyploidy versus transgenic technology

Dissertação de Mestrado Integrado em Engenharia Biológica

Ângela Alexandra Martinho Ramos

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Orientadora: Prof Dr. Deborah M. Power

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Declaração: O conteúdo, e execução do trabalho experimental e interpretação de resultados é da exclusiva responsabilidade da autora

Ângela Alexandra Martinho Ramos

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LIST OF ABREVIATIONS

CA – Caudal

Ca – Calcium

DA – Diploid diet A

DC – Diploid diet C

GH – Growth Hormone

GMO's – Genetic modified organisms

OSC - Osteocalcin

OSN - Osteonectin

OSP- Osteopontin

P- Phosphorous

TA – Triploid diet A

TC – Triploid diet C

TCR-Trunco Cranial

TCA – Trunco caudal

VCR – Vizcero cranial

ABSTRACT

Triploidy is the condition in which somatic cells contain three sets of chromosomes which might be a consequence of environmental changes or hybrid stabilization, though in the present work the ploidy induction was done by hyperbare pressure. Diploid and triploid Rainbow trout, *Onchorynchus mykiss* (Walbaum, 1792) were the species characterized in this thesis. Besides ploidy a modification in the mineral availability of phosphorous, was applied influencing the whole body mineral homeostasis in diploid and triploid trout.

The skeletogenesis in triploids animal is different from the diploid, having a general delay, the mineral deficient diet has also an impact on the ossification retreating. It allowed studying the development through triploids, which at the end of this trial they grew less than diploids. Vertebra area is also affected varying according to their regions, total number of vertebra differs, and in average triploids have less one vertebra than diploid groups. And there are also histological differences at a muscle level, having triploid Diet A less myotomes in average.

Animals present a high capacity in recovering, once they start eating a normal diet in mineral composition, the restore their mineral content in bone structures in some situation an over calcification is observe (Diploid A). In a molecular level, there were any molecular differences observed only a high variability at the OSN levels.

Key words: Diploids, Triploids, Skeletogenesis, Ontogeny, mineral content.

RESUMO

Triploidia é um estado, em que as células somáticas contém três conjuntos de cromossomas (3n). Pode ocorre naturalmente ou ser induzido experimentalmente com este estudo em que foi usada uma câmara hiperbárica. A espécie modelo utilizada foi a truta (*Onchorynchus mykiss* Walbaum, 1792) Diplóides e Triplóides. Para além da manipulação na ploidia efectuou-se uma alteração na disponibilidade do mineral fósforo e foi avaliado o seu efeito no desenvolvimento do sistema musculo-esquelético, usando parametros morfométricos, biométricos, bioquímicos e moleculares.

Verifica-se uma diferença na esqueletogénese em trutas triplóides comparativamente às diplóides, denotando-se um atraso na ossificação em ambos os grupos triplóides (dieta controlo e dieta restrita em fósforo). A acumulação corporal total de cálcio em ambos os grupos diploides e triploides de alevinos alimentados com dieta pobre em fósforo, é significativamente mais baixa ($P<0,05$) comparativamente a alevinos diplóides ou triplóides alimentados com dieta controlo. Constata-se uma alteração significativa nas vertebras de trutas cujo esqueleto estava completamente formado, este efeito varia consoante a região da coluna vertebral. De um modo geral trutas triploides têm menos vertebras. Nas quatro regiões identificadas e estudadas da coluna vertebral, encontraram-se diferenças significativas ($p<0,01$-$0,001$) na área das vertebras. Os marcadores moleculares avaliados por *PCR* em tempo real, Osteocalcina, Osteonectina e Osteopontina mostraram baixa variabilidade entre nos indivíduos analisados, e não se obtiveram diferenças conclusivas entre os grupos experimentais a 64 dpf de idade.

Em conclusão, a triploidização afecta o esqueleto de truta. Um fornecimento deficiente em fósforo afecta significativamente a ontogenia esquelética, morfometria e parâmetros bioquímicos de trutas diplóides e triplóides.

Palavras Chave: Diplóide, Triplóide, esqueletogénese, conteúdo mineral.

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