EXPRESSION OF GENES RELATED TO LIPID METABOLISM IN ATLANTIC BLUEFIN TUNA (*Thunnus thynnus* L.) LARVAE FED ROTIFERS AND COPEPODS

M. B. Betancor[a*], A. Ortega[b], F. de la Gándara[b], D. R. Tocher[a] and G. Mourente[c]

[a] Institute of Aquaculture, School of Natural Sciences, University of Stirling, FK9 4LA Stirling, Scotland, UK
[b] Planta Experimental de Cultivos Marinos, Instituto Español de Oceanografía (IEO), 30860 Puerto de Mazarrón (Murcia), Spain
[c] Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, 11510 Puerto Real, Cádiz, Spain

Introduction

The aim of the present study was to investigate the effect of dietary lipid and fatty acid compositions on the expression of key genes involved in lipid metabolism in Atlantic bluefin tuna (ABT) larvae fed different live prey. To this end, the study investigated the expression of genes of lipid metabolism and regulation at 14 days after hatch (dah) in ABT larvae fed enriched rotifers *B. plicatilis* and *Acartia sp*. copepod nauplii and copepodites. Specific objectives were first to clone cDNAs of ABT genes involved in major lipid pathways and their control and regulation, including fatty acid and long-chain polyunsaturated fatty acid (LC-PUFA) biosynthesis, lipid deposition and β-oxidation, for the evaluation of gene expression. Second, the expression of this gene set was determined in first feeding ABT larvae 14 dah and, third, to determine expression of the major lipid pathways in tissues of adult broostock ABT. Our overarching hypothesis is that understanding the molecular basis of lipid metabolism and regulation will provide insight to enable diet formulations to be optimised, and the effective use of sustainable dietary lipid sources in ABT aquaculture.

Materials and methods

The ABT larval feeding trials were carried out in two consecutive years, 2013 and 2014. In the trial 1, rotifers enriched with Origreen ® and *Acartia granii* nauplii were used as live prey up to 14 dah. In trial 2, enriched rotifers, *A. tonsa* nauplii and co-feeding of rotifers and copepods were investigated. Tissue samples (brain, gills, heart, kidney, spleen, liver, intestine, white and red muscle, adipose tissue and gonads) of adult ABT were collected to provide material for cDNA cloning and tissue expression of lipid metabolism genes. Larval growth and survival were determined along with total lipid content, lipid class and fatty acid compositions of both live prey and ABT larvae (Table 1). In addition, key genes related to lipid and fatty acid metabolism and its regulation including LC-PUFA biosynthesis genes *fads2d6* and *elovl5*, fatty acid metabolism genes *fas*, *cpt1*, *aco*, *fabp2*, *fabp4*, *fabp7*, *lpl* and *hmgcl*, and transcription factors (TFs) *pparα*, *pparγ*, *lxr*, *rxr*, *srebp1* and *srebp2* were evaluated by quantitative real-time PCR (qPCR).

Results

Differing results were observed in expression of *fads2d6* between the two experiments. So that larvae fed copepods in 1st trial showed higher expression compared to larvae fed rotifers, whereas expression of *fads2d6* was lowest in copepod-fed larvae in the 2nd trial. No differences were observed in *elovl5* expression. Regarding TFs, no differences in expression were observed between larvae fed copepods or rotifers in 1st trial, whereas some genes showed differing expression among larvae fed the three dietary treatments in the 2nd trial. Thus, *srebp1* expression was highest in co-fed larvae, with larvae fed rotifers showing the lowest one. Differences between larvae fed the three dietary treatments in 2nd trial were observed for *pparγ* with lowest expression by co-fed larvae and highest in larvae fed rotifers. Although no significant differences were found in *lxr*

or *rxr* mRNA levels, the same expression patterns were observed in larvae from the two trials, with low expression of these TFs in larvae fed rotifers compared to larvae fed copepod, with co-fed larvae showing intermediate levels in the 2nd trial. The expression profiles of lipid homeostasis genes; *fabp*s showed relatively conserved patterns in both trials, with higher levels in larvae fed rotifers than in larvae fed copepods, or co-fed ones. However, significant differences were only observed in *fabp4* expression for 1st trial larvae and in *fabp4* and *2* in 2nd trial larvae. The expression of *fas* showed differences between treatments in both trials, with highest expression in larvae fed copepods, and no difference between larvae fed solely on rotifer or co-fed rotifers and copepods in 2nd trial. In contrast, to 1st trial larvae, *aco* showed differences in expression among dietary treatments in larvae in the 2nd trial. In this case, larvae fed rotifers showed the highest expression with no differences between copepod or co-fed larvae. Larvae fed rotifers showed the lowest expression of *lpl* in 1st trial with a similar but non-significant tendency observed in 2nd trial. No differences among treatments in either trial were found in the expression of *fabp7, cpt1* and *hmgcl*.

Table 1. EPA, DHA (% total fatty acids) and DHA/EPA ratio of live feeds and ABT larvae fed them, and length (mm), dry mass (mg) and survival (%) of the larvae at 14 dph.

	2013		2014		
	Rotifer	Copepod	Rotifer	Copepod	Rot+Cop
Live feeds					
EPA	4.5 ± 0.1	8.9 ± 0.1*	4.5 ± 0.1	3.8 ± 0.1*	
DHA	12.1 ± 0.5	24.9 ± 0.9*	17.5 ± 3.1	26.0 ± 0.9*	
DHA/EPA	2.7 ± 0.1	2.8 ± 0.1	3.8 ± 0.6	6.9 ± 0.6*	
ABT Larvae					
EPA	5.4 ± 0.1	5.9 ± 0.4	6.6 ± 0.4^{a}	5.5 ± 0.1^{b}	4.8 ± 0.1^{c}
DHA	17.5 ± 0.9	29.6 ± 1.0*	17.6 ± 0.5^{b}	28.2 ± 0.3^{a}	24.4 ± 0.5^{a}
DHA/EPA	3.2 ± 0.2	5.0 ± 0.1*	2.7 ± 0.2^{b}	5.1 ± 0.2^{a}	5.0 ± 0.2^{a}
Total length	7.75 ± 0.61	7.26 ± 0.51*	7.02 ± 0.17^{c}	8.29 ± 0.08^{a}	7.50 ± 0.22^{b}
Dry mass	0.66 ± 0.13	0.61 ± 0.13	0.35 ± 0.03^{c}	0.77 ± 0.01^{a}	0.51 ± 0.06^{b}
Survival	3.18 ± 1.12	5.91 ± 0.93*	2.88 ± 1.02^{c}	7.49 ± 1.17^{b}	10.24 ± 3.50^{a}

Results are means ± SD (n = 3). Values bearing an asterisk (2013 and live feeds 2014) or different superscript letters (ABT larvae 2014) are significantly different ($P<0.05$).
ABT, Atlantic bluefin tuna; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Discussion and conclusions

The study demonstrated that copepods were a better live prey for first feeding ABT larvae based on growth and survival. Differences in the expression patterns of lipid metabolism genes were observed between the two trials. Some of the responses in lipid gene expression could be due to dietary lipid and fatty acid composition, but there was no obvious direct correlation between gene expression patterns and growth or survival. Differences in performance and metabolism among larval groups between the trials could also partly reflect differences in broodstock nutrition in the two trials. Hence, further studies are required to investigate lipid requirements, lipid accumulation and metabolism during development of ABT larvae. Special importance should be given to the expression analysis of genes related to lipid metabolism and its regulation, combined with biochemical studies of tuna lipid metabolism in order to develop optimal feeds to facilitate the commercial culture of this iconic species.

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