

Book of Abstracts



FEEDING OUR FOOD

XV International Symposium on Fish Nutrition and Feeding
Molde, Norway • 4–7 June 2012

ORGANIZERS



Norwegian School of Veterinary Science



www.isfnf.org

Effect of dietary vitamin K supplementation on larval proteome and bone marker gene expression in Senegalese sole

Nadège Richard*, Ignacio Fernández, Luis E.C. Conceição, Paulo J. Gavaia

CCMAR, Centro de Ciências do Mar do Algarve, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

Email: nrichard@ualg.pt

Several studies have identified rearing environment, nutrition and genetics as causative factors of skeletal deformities (Boglione et al., 2003; Cahu et al., 2003). Within nutrition, vitamin K (VK) is a liposoluble vitamin classically involved in blood coagulation, and until recently largely disregarded in aquaculture. This vitamin is known to play an important role in bone metabolism, acting as a coenzyme for VK-dependant gamma carboxylation of Gla proteins as Osteocalcin and Matrix Gla Protein, and regulating Msx gene expression, through the recently identified VK activation of Pregnane X receptor (PXR). Since the mechanisms underlying the association of VK with the changes of skeletal microanatomy are still not fully understood (Hamre et al., 2010), the aim of the present study was to investigate the effects of dietary VK supplementation on skeleton deformities, larval proteome and the expression of selected genes involved in bone metabolism in Senegalese sole larvae.

Quadruplicate groups of Senegalese sole larvae were fed from first-feeding onwards with rotifers and *Artemia* enriched with different levels of VK₁ (0 to 250 mg of VK₁/kg of Selco). Larvae were sampled at 40 DAH. Mineral content, larval growth and incidence in skeletal deformities were measured in order to evaluate the effects of increasing dietary VK levels in larval performance. Gene expression of VK related pathway was analysed by quantitative PCR. In addition, a comparative proteome analysis was performed by two-dimensional electrophoresis on entire larvae after removing the head and the gut. Larval skeleton quality analyses revealed that dietary VK supplementation in sole larvae leads to an improvement in larval quality, decreasing the incidence of skeletal deformities, as well as increasing mineral content. Skeletal quality has been correlated with gene expression levels of selected genes, which showed an altered gene expression under dietary VK supplementation. Regarding proteome analysis, among protein spots detected as differentially expressed between the two experimental groups, some were identified as being involved in clotting process, energy metabolism, protein folding, cellular contractile system and cytoskeleton proteins. Interestingly, type VI collagen, which is known to have a role in skeletal metabolism (Hall 2005) was also identified. The potential benefits of increasing dietary VK levels in Senegalese sole diets during larval development will be discussed.

References

- Boglione, C., Costa, C., Di Dato, P., Ferzini, G., Scardi, M., Cataudella, S. (2003) *Aquaculture* 227:373-394
Cahu, C., Zambonino Infante, J., Takeuchi, T. (2003). *Aquaculture* 227:245-258
Hall, B.K. (2005) *Bones and cartilage*; Elsevier academic press, California USA. 760 pp.
Hamre, K., Krossøy, C., Lock, E.-J., Moren, M. (2010) *Aquaculture Research* 41:745-750

Acknowledgements

NR and IF acknowledge financial support from Fundação para a Ciência e Tecnologia, Portugal, through grants SFRH/BDP/65578/2009 and SFRH/BDP/82049/2011 respectively. This work was partly funded by project PDTC-MAR-105152-2008 (SPECIAL_K) from the Portuguese Foundation of Science and Technology.

Effect of variable levels of dietary cholesterol and plant sterols on the growth performance and bone metabolism in gilthead seabream (*Sparus aurata*) juveniles

Jorge Dias, Rita Colen, Vera Rodrigues, Cláudia Aragão, Sofia Engrola, Michael Viegas, Vincent Laizé, Paulo Gavaia, Leonor Cancela

CIMAR/CCMAR, University of Algarve, Campus Gambelas, Faro, Portugal.
E-mail: jorgedias@ualg.pt

Cholesterol is found in all animal tissues and is an important component of biological cell membranes with functions such as precursor to bile acids, hormones and vitamins. Fish meal and fish oil are cholesterol-rich ingredients. Replacement of these marine-derived ingredients by plant proteins and vegetable oils tends to reduce dietary cholesterol levels. Additionally, the utilization of vegetable oils will increase dietary supply of phytosterols, which are known to compete with cholesterol for binding sites thereby lowering the intestinal cholesterol absorption and serum LDL-cholesterol levels. In rodent models, elevated levels of plasma LDL cholesterol is regarded as an osteopenic factor. On the other hand, plant sterols (beta-sitosterol, campesterol, stigmasterol, and brassicasterol) either through its direct action on cholesterol absorption or in association to its potent anti-inflammatory effect have been shown to effectively reduce bone losses. Knowledge on the effect of such dietary variables in fish bone is limited.

A trial was undertaken to evaluate the effect of variable dietary cholesterol:phytosterols (C:P) ratios on the growth performance and bone metabolism of gilthead seabream juveniles. Five isoproteic (crude protein, 55% DM) and isolipidic (18% DM) diets were formulated with purified ingredients to fulfill the known nutritional requirements of juvenile seabream. By using concentrated sources of cholesterol and phytosterols, the dietary C:P ratios tested were 2.2:0 (diet CHOL 2.2), 0.7:0 (diet CHOL 0.7), 0.7:2 (diet PHYTO 2), 0.7:4 (diet PHYTO 4), 0:4 (diet CHOL 0). Each diet was tested in duplicate groups of 30 seabream (IBW: 6.7 ± 0.8 g) stocked in 100 L circular tanks, supplied with gravel-filtered aerated seawater (temperature: $24 \pm 1^\circ\text{C}$). Fish were fed to apparent satiety, by hand, three times a day, during 56 days.

At the end of the trial, fish had a 4-fold increase of their initial weight. Dietary changes on the cholesterol and phytosterols levels had little effect on weight gain and specific growth rate. However, for a constant dietary cholesterol level of 0.7%, the increase of phytosterols significantly reduced feed efficiency and protein efficiency ratio. Analysis of hepatic lipid metabolic enzymes and plasma LDL-cholesterol levels is currently underway. Additionally, lipid composition, phosphorus content, activities of alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) will be assess in vertebral bone homogenates.

Acknowledgements: this research was supported by Fundação para a Ciência e Tecnologia of Portugal (Project *PTDC/MAR/70855/2006*).

Effect of variable levels of dietary cholesterol and plant sterols on the growth performance and bone metabolism in gilthead seabream (*Sparus aurata*) juveniles

Jorge Dias, Rita Colen, Vera Rodrigues, Cláudia Aragão, Sofia Engrola, Michael Viegas, Vincent Laizé, Paulo Gavaia, Leonor Cancela

CIMAR/CCMAR, University of Algarve, Campus Gambelas, Faro, Portugal.
E-mail: jorgedias@ualg.pt

Cholesterol is found in all animal tissues and is an important component of biological cell membranes with functions such as precursor to bile acids, hormones and vitamins. Fish meal and fish oil are cholesterol-rich ingredients. Replacement of these marine-derived ingredients by plant proteins and vegetable oils tends to reduce dietary cholesterol levels. Additionally, the utilization of vegetable oils will increase dietary supply of phytosterols, which are known to compete with cholesterol for binding sites thereby lowering the intestinal cholesterol absorption and serum LDL-cholesterol levels. In rodent models, elevated levels of plasma LDL cholesterol is regarded as an osteopenic factor. On the other hand, plant sterols (beta-sitosterol, campesterol, stigmasterol, and brassicasterol) either through its direct action on cholesterol absorption or in association to its potent anti-inflammatory effect have been shown to effectively reduce bone losses. Knowledge on the effect of such dietary variables in fish bone is limited.

A trial was undertaken to evaluate the effect of variable dietary cholesterol:phytosterols (C:P) ratios on the growth performance and bone metabolism of gilthead seabream juveniles. Five isoproteic (crude protein, 55% DM) and isolipidic (18% DM) diets were formulated with purified ingredients to fulfill the known nutritional requirements of juvenile seabream. By using concentrated sources of cholesterol and phytosterols, the dietary C:P ratios tested were 2.2:0 (diet CHOL 2.2), 0.7:0 (diet CHOL 0.7), 0.7:2 (diet PHYTO 2), 0.7:4 (diet PHYTO 4), 0:4 (diet CHOL 0). Each diet was tested in duplicate groups of 30 seabream (IBW: 6.7 ± 0.8 g) stocked in 100 L circular tanks, supplied with gravel-filtered aerated seawater (temperature: 24 ± 1 °C). Fish were fed to apparent satiety, by hand, three times a day, during 56 days.

At the end of the trial, fish had a 4-fold increase of their initial weight. Dietary changes on the cholesterol and phytosterols levels had little effect on weight gain and specific growth rate. However, for a constant dietary cholesterol level of 0.7%, the increase of phytosterols significantly reduced feed efficiency and protein efficiency ratio. Analysis of hepatic lipid metabolic enzymes and plasma LDL-cholesterol levels is currently underway. Additionally, lipid composition, phosphorus content, activities of alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) will be assess in vertebral bone homogenates.

Acknowledgements: this research was supported by Fundação para a Ciência e Tecnologia of Portugal (Project *PTDC/MAR/70855/2006*).

Tolerance of zebrafish embryos to supra-physiologic yolk levels of glucose

Filipa Rocha^{1*}, Jorge Dias¹, Sofia Engrola¹, Paulo Gavaia¹, Maria Teresa Dinis¹, Stéphane Panserat²

¹ CIMAR/CCMAR, University of Algarve, Campus Gambelas, Faro, Portugal.

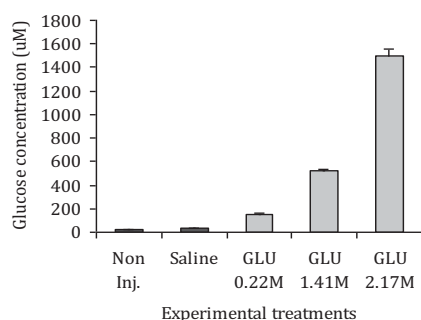
² INRA UR 1067 NuMeA (Nutrition, Métabolisme, Aquaculture), Pôle d'Hydrobiologie, St-Pée-sur-Nivelle, France.

E-mail: fsrocha@ualg.pt

Once spawned and fertilized the oviparous fish eggs operate as closed systems; only respiratory gases, heat and negligible amounts of solutes and water are exchanged freely, as a result of an extremely low permeability of the egg surface membranes. Currently, the opportunities to exert a nutritional stimulus during a stage of high metabolic plasticity, such as fish embryogenesis, are restricted to maternal transfer and the onset of exogenous feeding.

Batches of fertilized zebrafish eggs were used to assess the utilization pattern of energy-yielding nutrients during embryogenesis and first feeding phases. Changes on total N, free amino acids, free fatty acids and glucose are observed throughout the various developmental stages. This information on nutrient usage was cross-referenced with available gene expression data (ZFIN database) during embryogenesis. This info clear shows that major metabolic pathways of nutritional relevance seem to be already active at the blastula stage. Analysis of gene expression (RT-PCR) data at 6 dpf, a critical period of transition between endogenous and exogenous feeding will be presented.

Moreover, knowledge about the effects of a nutritional supplementation strategy during fish embryogenesis is extremely scarce. Therefore, a set of preliminary experiments was undertaken to assess the technical and biological feasibility of fortifying zebrafish eggs with glucose by a microinjection approach. The yolk of zebrafish viable eggs, at 30% epiboly stage (5 hpf), was enriched with various glucose solutions (0.22, 1.41 and 2.17M) by means of microinjection. Additional treatments comprised non injected eggs and saline solution injected eggs. Following microinjection, eggs and yolk-feeding larvae were incubated and reared under identical conditions and following standardized protocols.



In comparison to non-injected eggs, we could achieve a 4, 15 and 43-fold increase of the glucose concentration of eggs, showing that microinjection is an effective way to fortify yolk reserves in zebrafish eggs.

At 6 dpf, survival was high (>92%) for eggs injected with saline and 0.22M glucose, and slightly lower (>76%) for those injected with the highest glucose concentration .

These developments are the preliminary steps to a better insight on the nutritional regulation of key biological and metabolic processes occurring in early developmental phases of fish.

Acknowledgements: this research was supported by Fundação para a Ciência e Tecnologia of Portugal (*Project PTDC/CVT/102481/2008*). Filipa Rocha is recipient of FCT Grant *SFRH/BD/74921/2010*.