A multifactorial approach for investigating the effects of dietary fatty acids, vitamins and minerals on early development of pikeperch (*Sander lucioperca*)

WP 10 – Pikeperch – Nutrition

WP 10.1: Nutrition - Pikeperch

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WP 10.1: Nutrition - Pikeperch

Objective: Screening of selected nutrients (fatty acids, vitamins and minerals) for development of specific formulated diets for pikeperch larvae

8 nutritional variables

2 modalities: high and low dietary levels

↓

8 factors * 2 modalities/factors

2^8 = 256 combinations

Factorial fractional design ↓

16 different combinations

Dietary variables	Low level	High level
Ca/P	0.6	1.2
DHA+EPA	1.25%	3.5%
ARA	0.8%	1.6%
Vitamin E	1000 mg/kg	3000 mg/kg
Vitamin C	2000 mg/kg	3600 mg/kg
Vitamin A	8000 IU/kg	30000 IU/kg
Vitamin D	2800 IU/kg	28000 IU/kg
Se	3 mg/kg	12 mg/kg

Multifactorial experiment : rationale for the selected variables

High levels were chosen to be below the anticipated toxic level while low levels were slightly above the requirements reported in previous studies on pikeperch or based on literature for other species

Dietary variables	Low level	High level	References
Ca/P	0.6	1.2	Good performance at Ca/P: **0.5-0.6** (Kestemont et al. 2007)
DHA+EPA	1.25%	3.5%	Best growth and development in pikeperch larvae fed **1.25%** EPA+DHA (Hamza et al. 2008) Significant improvement in growth at **3.8%** (Lund et al. 2015).
ARA	0.8%	1.6%	Best growth and development in pikeperch at **0.8%** ARA (Hamza et al.2008)
Vitamin E	1000 mg/kg	3000 mg/Kg	High requirement (**3000mg/kg**) due to high HUFA needs during larval stages (Atalah et al. 2012; Izquierdo et al. 2013).
Vitamin C	2000 mg/kg	3600 mg/kg	**3600 mg/kg** improved the protection against peroxidation, decreased TBARS contents, spared vitamin E, and reduced the incidence of muscular lesions (Betancor et al. 2012).
Vitamin A	8000 IU/kg	30000 IU/kg	Optimum dietary VA content: **8000–9000** IU VA/kg for atlantic halibut and japanese flounder juveniles (Moren et al. 2004; Hernandez et al.2005). **Toxic effect: maximum dose 45,000 IU VA/kg** dry diet (Cahu et al. 2003)
Vitamin D	2800 IU/kg	28000 IU/kg	**27,600 IU VD_3/kg** : Best result of larval morphogenesis, earlier maturation of digestive function (Darias et al. 2010) VD content in commercial larval diets: **2500-2800 IU VD_3/kg** diet (NRC recommendation)
Se	3 mg/kg	12 mg/kg	Up to **11.65 mg/kg**: improved survival rate, stress resistance and promoted the expression of bone formation and mineralization genes in seabream larvae (Saleh et al.2014). Se concentration in fish around **2-3 mg/kg** (Prabhu et al.2014)

Experimental design using Planor (Kobilinsky)

	Dietary Variables							
Diet	Ca/P	DHA+EPA (%dw)	ARA (%dw)	Vit E (mg/kg)	Vit D (IU/kg)	Vit C (mg/kg)	Vit A (IU/kg)	Se (mg/kg)
D.1	0.6	1.25	0.8	1000	2800	2000	8000	3
D.2	1.2	1.25	0.8	1000	28000	3600	8000	12
D.3	0.6	3.5	0.8	1000	2800	3600	30000	12
D.4	1.2	3.5	0.8	1000	28000	2000	30000	3
D.5	0.6	1.25	1.6	1000	28000	2000	30000	12
D.6	1.2	1.25	1.6	1000	2800	3600	30000	3
D.7	0.6	3.5	1.6	1000	28000	3600	8000	3
D.8	1.2	3.5	1.6	1000	2800	2000	8000	12
D.9	0.6	1.25	0.8	3000	28000	3600	30000	3
D.10	1.2	1.25	0.8	3000	2800	2000	30000	12
D.11	0.6	3.5	0.8	3000	28000	2000	8000	12
D.12	1.2	3.5	0.8	3000	2800	3600	8000	3
D.13	0.6	1.25	1.6	3000	2800	3600	8000	12
D.14	1.2	1.25	1.6	3000	28000	2000	8000	3
D.15	0.6	3.5	1.6	3000	2800	2000	30000	3
D.16	1.2	3.5	1.6	3000	28000	3600	30000	12

No replicates in this experiment : 1 tank per treatment

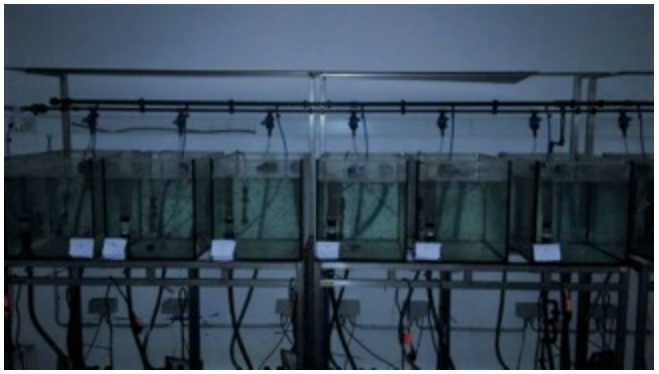
❖ Materials and methods

Diet formulation 16 isoenergetic and isolipidic diets formulated and fabricated by SPAROS as cold extruded feeds

Ingredients (%)	D1	D3	D5	D6	D7	D9	D11	D16
MicroNorse	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
CPSP 90	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Squid meal	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00
Krill meal (Aker Biomarine)	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Fish gelatin	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Wheat Gluten	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Potat starch gelatinised (Pregeflo)	10.45	9.15	8.95	13.27	9.49	8.68	9.14	12.08
Fish oil - SAVINOR	**1.20**	**0.80**	**1.15**	**1.15**	**0.00**	**1.20**	**0.80**	**0.00**
Incromega DHA 500TG	**0.00**	**3.40**	**0.00**	**0.00**	**3.58**	**0.00**	**3.40**	**3.58**
VEVODAR	**2.10**	**2.10**	**4.25**	**4.25**	**4.25**	**2.10**	**2.10**	**4.25**
Soybean oil	**3.00**	**0.00**	**1.05**	**1.05**	**0.00**	**3.00**	**0.00**	**0.00**
Vit & Min Premix PV02	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lutavit C35	**0.58**	**1.02**	**0.58**	**1.02**	**1.02**	**1.02**	**0.58**	**1.02**
Lutavit E50	**0.20**	**0.20**	**0.20**	**0.20**	**0.20**	**0.60**	**0.60**	**0.60**
Rovimix A (5000000 IU/kg)	**0.00**	**0.44**	**0.44**	**0.44**	**0.00**	**0.44**	**0.00**	**0.44**
Rovimix D3 (5000000 IU/kg)	**0.015**	**0.015**	**0.51**	**0.015**	**0.51**	**0.51**	**0.51**	**0.51**
Brewer's yeast	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Soy lecithin - Powder	**6.00**	**6.00**	**6.00**	**6.00**	**4.50**	**6.00**	**6.00**	**4.50**
Binder (sodium alginate)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
NaH2PO4	**4.20**	**4.20**	**4.20**	**0.35**	**4.20**	**4.20**	**4.20**	**0.35**
SelPlex - Se yeast	**0.05**	**0.47**	**0.47**	**0.05**	**0.05**	**0.05**	**0.47**	**0.47**
L-Taurine	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

❖ Materials and methods

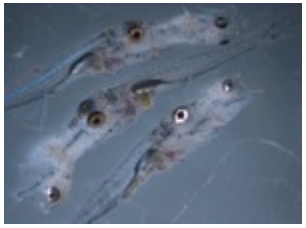
The experiment was conducted at URBE facilities, University of Namur, in 16 experimental tanks (90 l)



Experimental set up at URBE, UNAMUR

Experiment timeline: May- June 2016

- 1st feeding with Artemia nauplii (non enriched and enriched with HUFA)
- Co-feeding period from 18 to 24 dph using Artemia and mixture of the 16 diets



D_{-24} → D_0 → D_{14}

Daily mortality counting

Initial sampling

25 dph larvae (9.44 mg) randomly distributed at a density of 770 larvae $tank^{-1}$

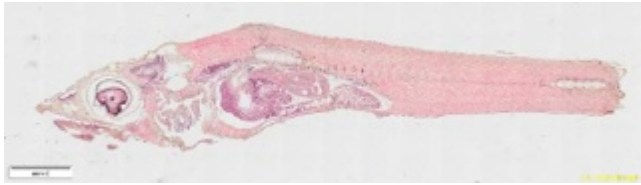
Final sampling

- Growth and survival
- Biochemical composition
- Digestive enzymatic assays
- Deformities evaluation
- Histology
- Gene expression

❖ Materials and methods

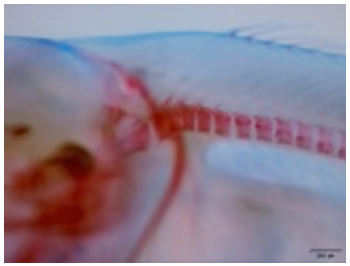
Measured variables

- **Husbandry variables:** survival, body weight, SGR, cannibalism (UNamur)
- **Biochemical assays:** proximate composition and fatty acid content (DTU AQUA)
- **Digestive enzymatic activities:** Trypsin, Pepsin, Amylase, Leu-Ala, Aminopeptidase, Alkaline phosphatase (UNamur)
- **Deformities:** specific staining procedure for pikeperch larvae has been developed using Alcian Blue- Alizarin Red Skeletal Staining (ULPGC & UNamur)
- **Candidate gene expression** (ULPGC)
- **Organ development and tissue morphology:** Hematoxilin and eosin staining (UNamur)
- **Statistics:** Planor software (UL & UNamur)

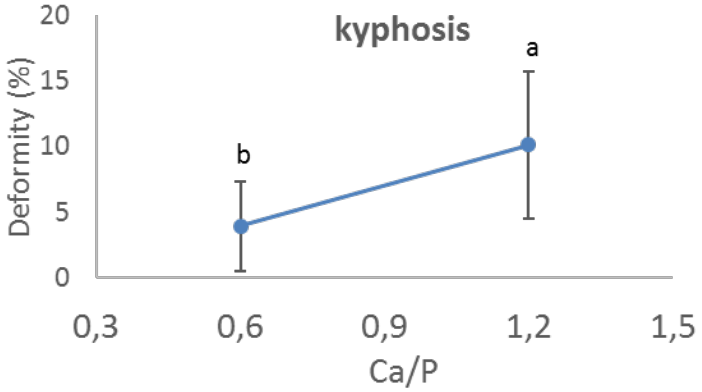


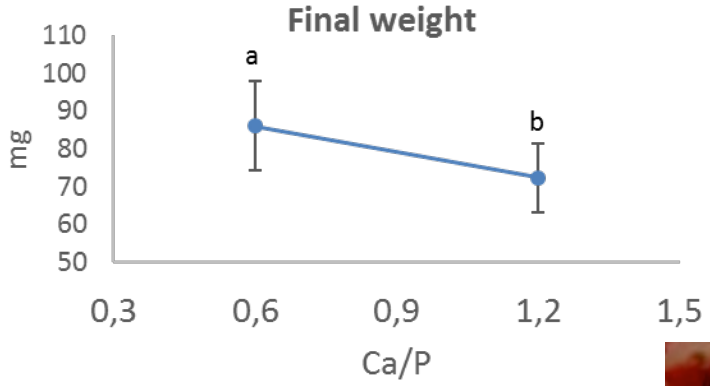
❖ Results

Ca/P effect

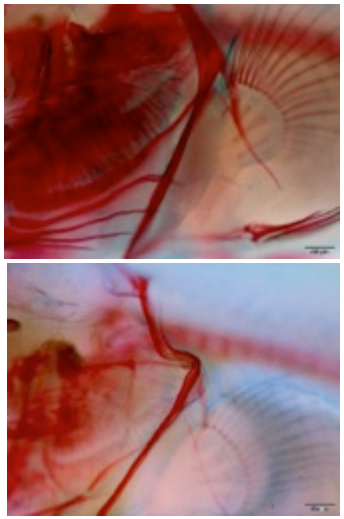


Cephalo-prehaemal kyphosis

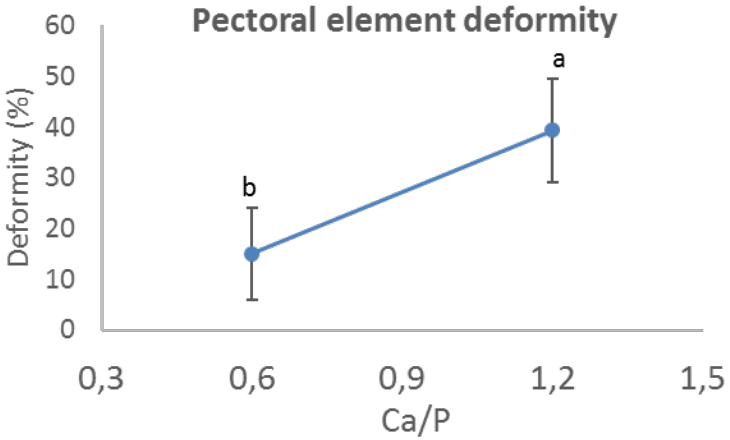




✓ Kyphosis and pectoral deformities significantly higher in larvae fed high Ca/P diet

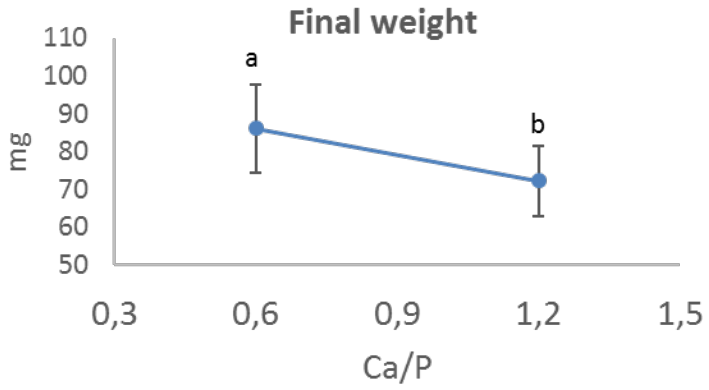


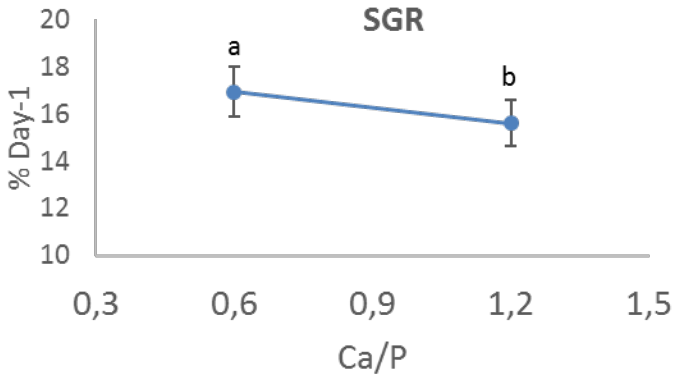
Pectoral elements

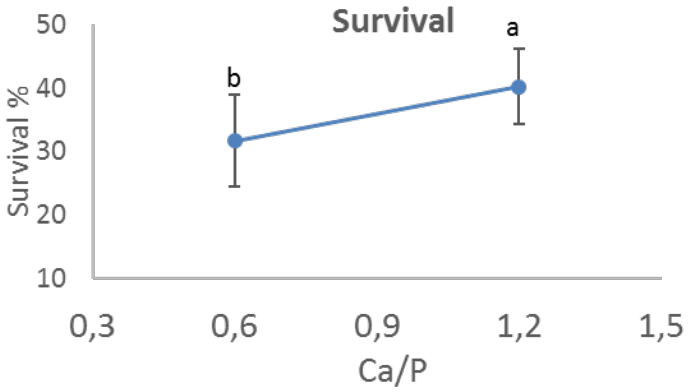


❖ Results

Ca/P effect





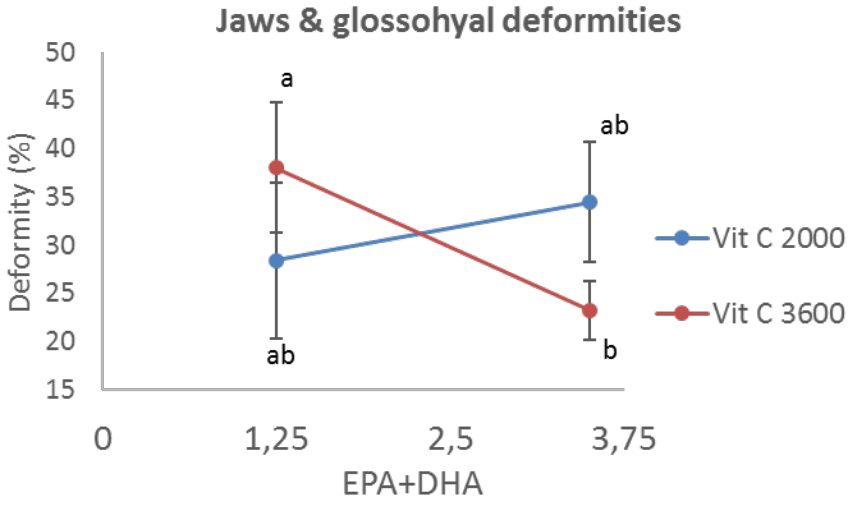


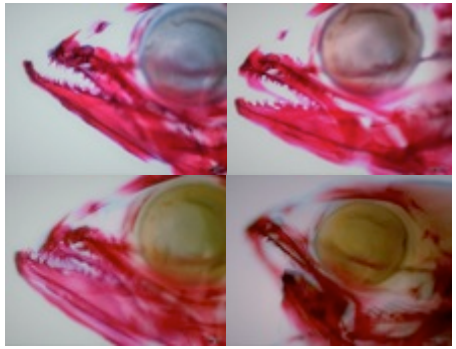
✓ Growth significantly lower in larvae fed high Ca/P diet, while high Ca/P improved survival rate

❖ Results

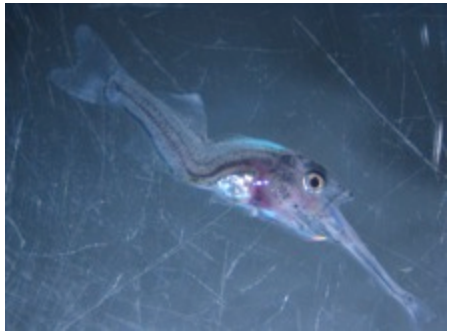
Larval deformities and cannibalism

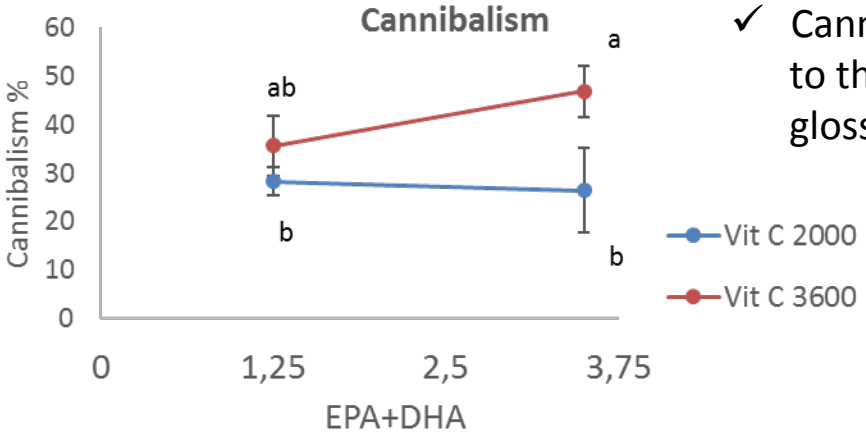
- ✓ Reduction of deformities at high EPA+DHA and high Vit C level
- ✓ Interaction with Vit C involved in collagen synthesis?





Jaws deformities

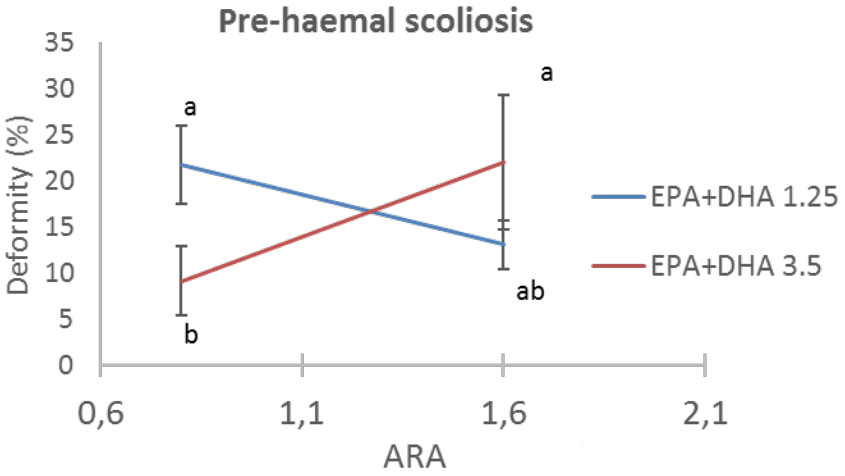




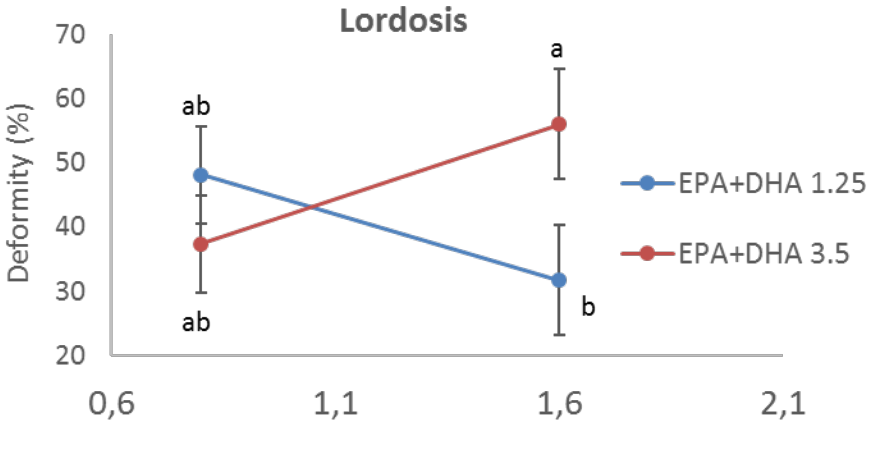
- ✓ Cannibalism decrease linked to the prevalence of jaws and glossohyal deformities?

❖ Results

Effects of fatty acids on skeletal deformities



Scoliosis and kyphosis

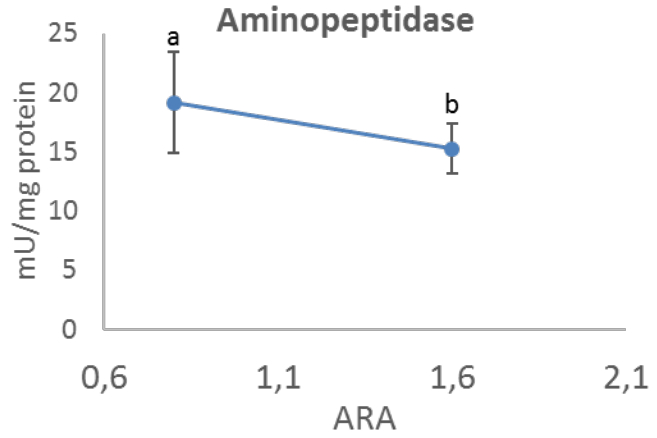


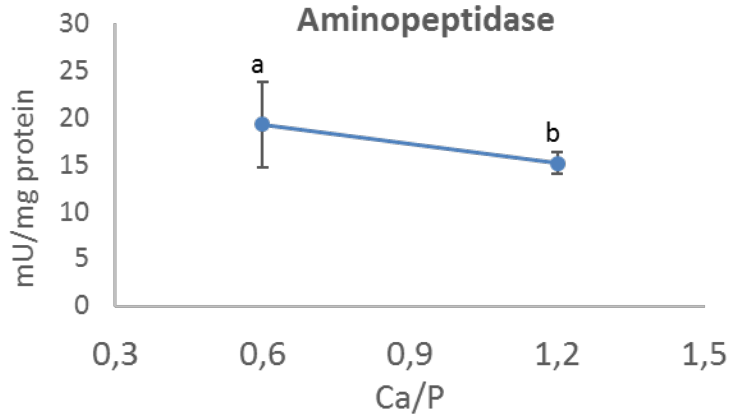
Lordosis

- ✓ At 0.8% ARA, increase of EPA+DHA seems reducing prevalence scoliosis, while high levels of ARA had the opposite effect
- ✓ High EPA+DHA with high levels of ARA seems increasing prevalence of lordosis
- ✓ EPA/DHA/ARA to be investigated

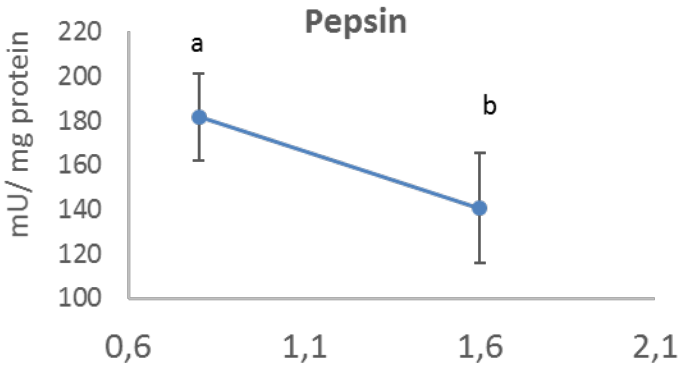
❖ Results

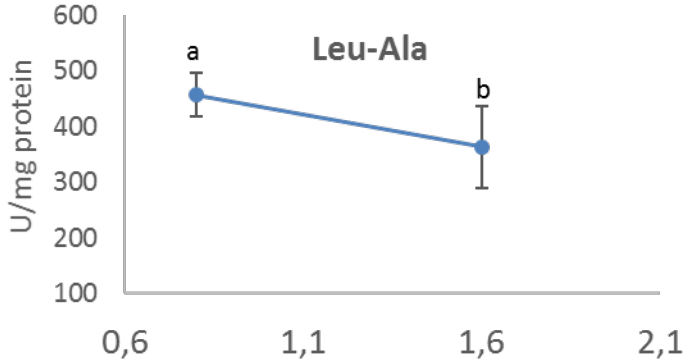
Activity of digestive enzymes

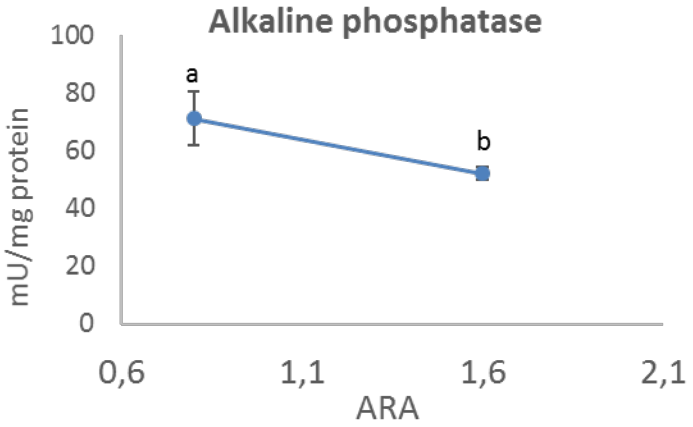




✓ Aminopeptidase activity negatively correlated with ARA and Ca/P levels



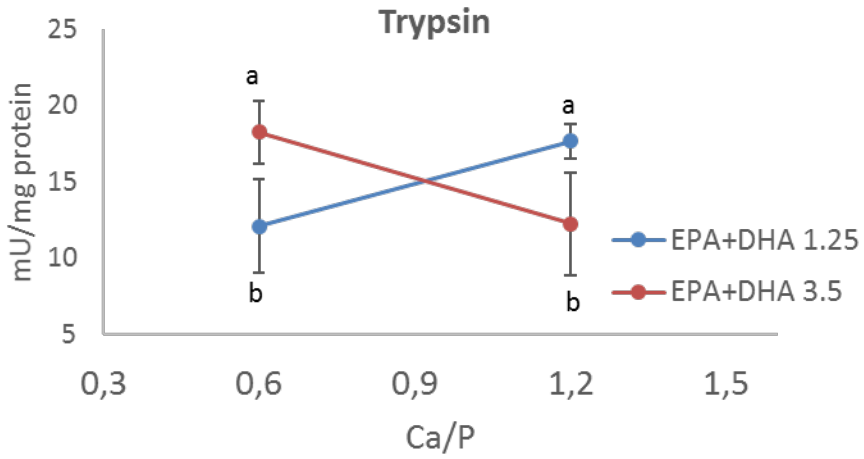


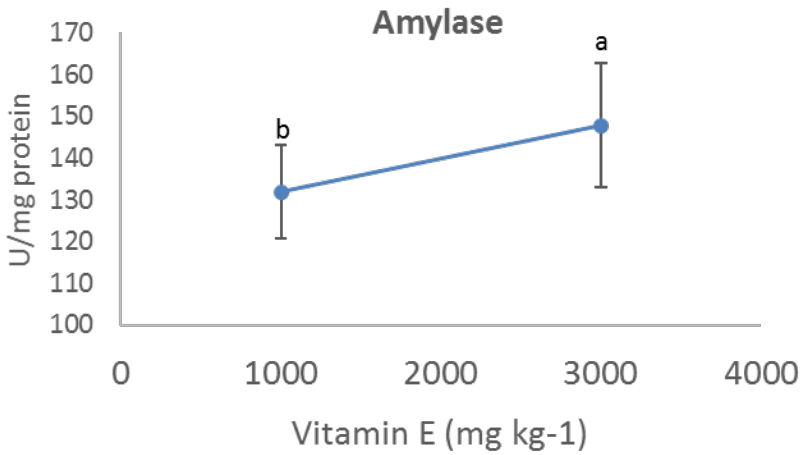


✓ High ARA level decreased enzyme activities

❖ Results

Activity of digestive enzymes



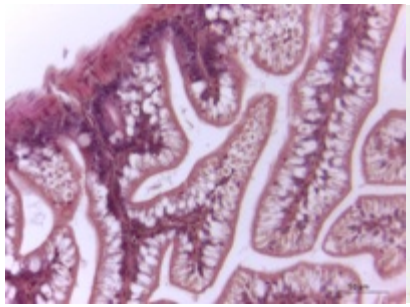


- ✓ At low EPA+DHA, increase of Ca/P enhanced trypsin activity, while opposite effect at high EPA+DHA (interaction with growth?)
- ✓ Trypsin secreted as a trypsinogen (inactive form), then activated by an enterokinase requiring Ca

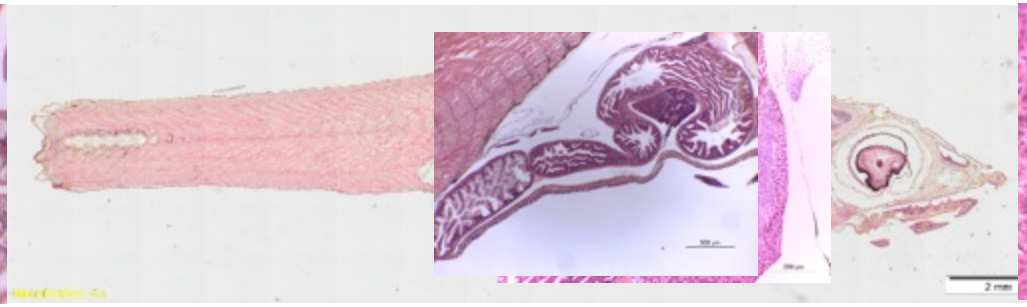
- ✓ Amylase activity significantly affected by vitamin E dietary content

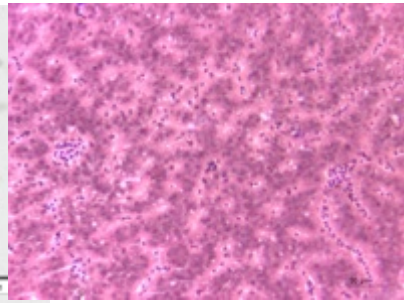
❖ Workplan for the coming months

✓ Organ development and tissue morphology under analysis

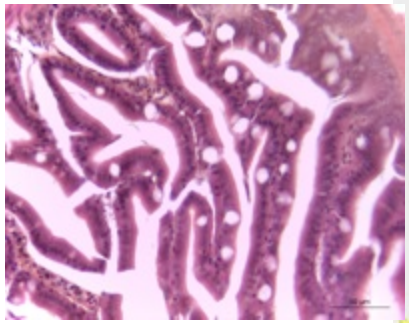


Posterior intestine (X40)

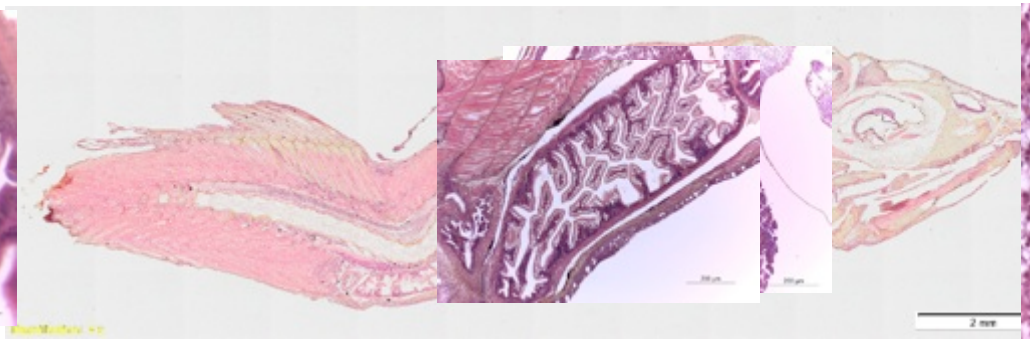


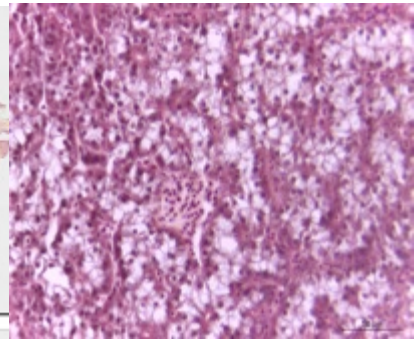


Condensed hepatocytes with centred nucleus and marked cytoplasm staining



Lipid vacuoles accumulation in the enterocytes





Hepatocytes with high lipid vacuoles deposition

❖ Workplan for the coming months…and later…

Larval FA composition and gene expression under analysis in DTU and ULPGC respectively

Based on the whole set of results, a confirmatory experiment will be carried out in May – June 2017

Getting an increased knowledge on the effects of **the most influential nutrients and their interactions** :

- ✓ Feeding trial testing gradual levels of different dietary Ca/P ratios
- ✓ Investigating the interactions between Vit C and EPA+DHA level with respect to oxidative stress
- ✓ Possible explanation related to the dietary ratios of n-3/n-6 (ARA/DHA/EPA)
- ✓ Identification of possible non-monotonic responses


Thanks for your attention