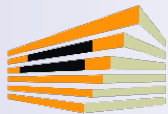




**Grupo de Investigación
en Acuicultura**

Nutrition of Meagre (*Argyrosomus regius*): Advances in larval and juvenile nutrition.

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Universidad de Las Palmas de Gran Canaria (ULPGC)



WP 8. Nutrition in Meagre



Objective: to better define the nutritional needs of meagre during both pre-growing and on-growing phases to improve growth consistency and fish health and welfare

Task 8.1. Improvement of larval weaning feeds (Led by FCPCT-ULPGC)

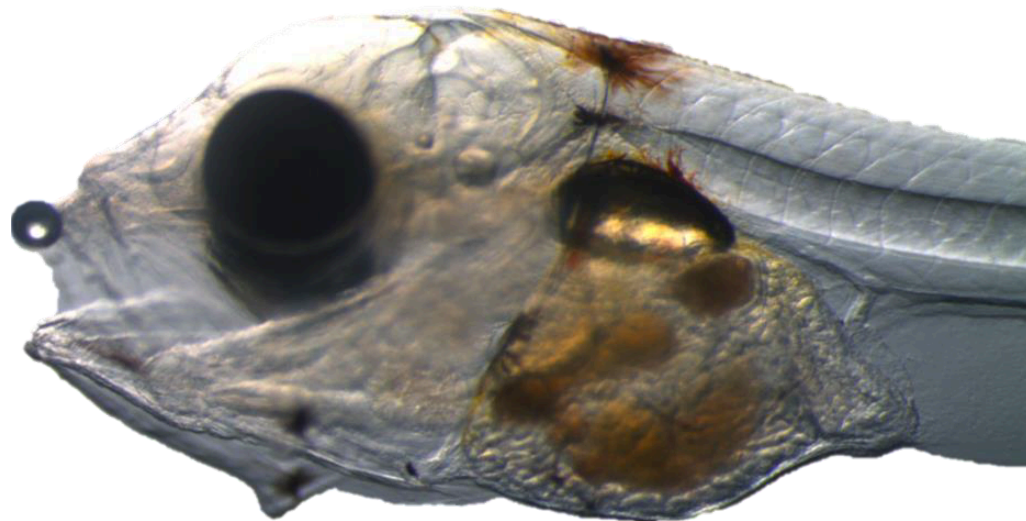
Task 8.2. Determination of nutritional requirements to promote feed utilization, growth and welfare (Led by FCPCT- ULPGC)



WP 8. Nutrition in Meagre

■ **Task 8.1. Improvement of larval weaning diets**

1. Optimum essential fatty acids and related micronutrient levels in weaning diets for meagre
2. Importance of dietary vitamins A, K and D in weaning diets for meagre
3. The importance of Vitamin K in quality of meagre fry



- **Task 8.1.** Improvement of larval weaning diets.
 1. **essential fatty acids + micronutrients**

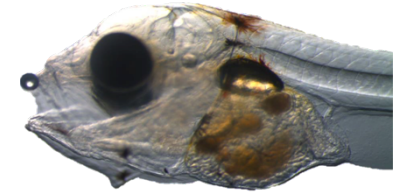


Table 1. Variable ingredients and proximate composition (g 100 g⁻¹dw) of early weaning diets containing several n-3 HUFA, vitamin E and vitamin C levels fed to meagre (*A. regius*) larvae from 14 to 28 dah.

	Diets					
	0.4/150/180	0.4/300/180	0.4/300/360	3/150/180	3/300/180	3/300/360
<i>Ingredients</i>						
Peruvian anchovy oil	0.00	0.00	0.00	10.00	10.00	10.00
Oleic acid^a	10.00	10.00	10.00	0.00	0.00	0.00
Vitamin E*	150.00	300.00	300.00	150.00	300.00	300.00
Vitamin C*	180.00	180.00	360.00	180.00	180.00	360.00
<i>Proximate composition</i>						
Lipid	16.01	17.09	17.06	17.52	17.34	17.44
Protein	65.14	64.72	64.97	65.43	65.45	64.88
Moisture	10.32	10.59	9.38	9.67	9.39	9.35
Ash	5.47	5.55	5.70	5.88	5.73	5.81

Experimental design

Material & Methods

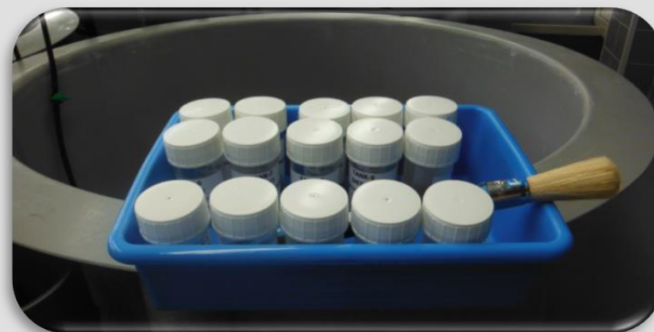
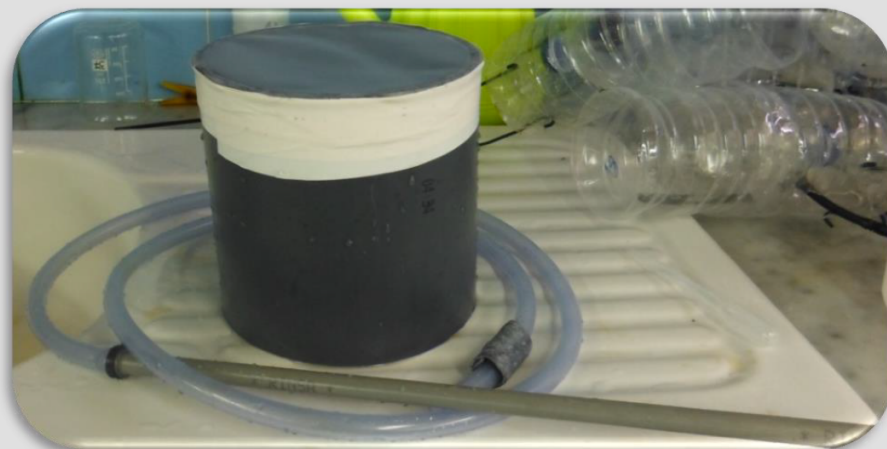
3000 larvae in 15 tanks (200l)
17 dph-33 dph
16 days of feeding



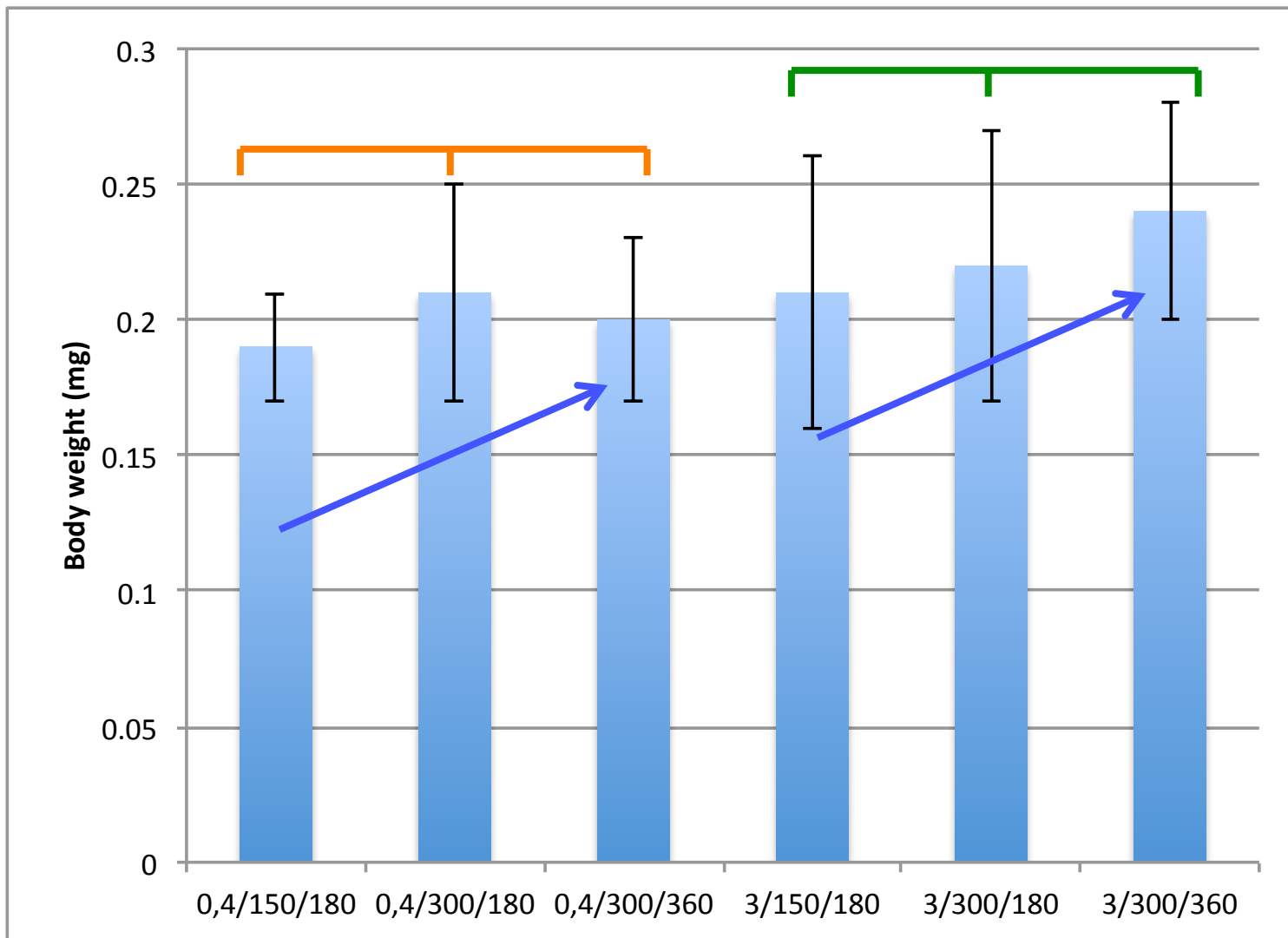
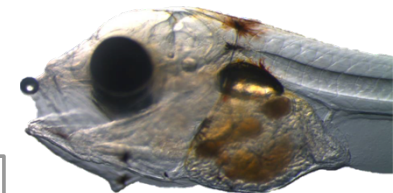
Feeding routine:
Every 45 min
From 8 am to 7pm

Daily tasks:

T^a , O_2 , Saturation, Siphoning, Skimmer
Daily Mortality

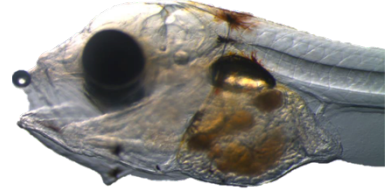


- **Task 8.1.** Improvement of larval weaning diets.
1. essential fatty acids + micronutrients



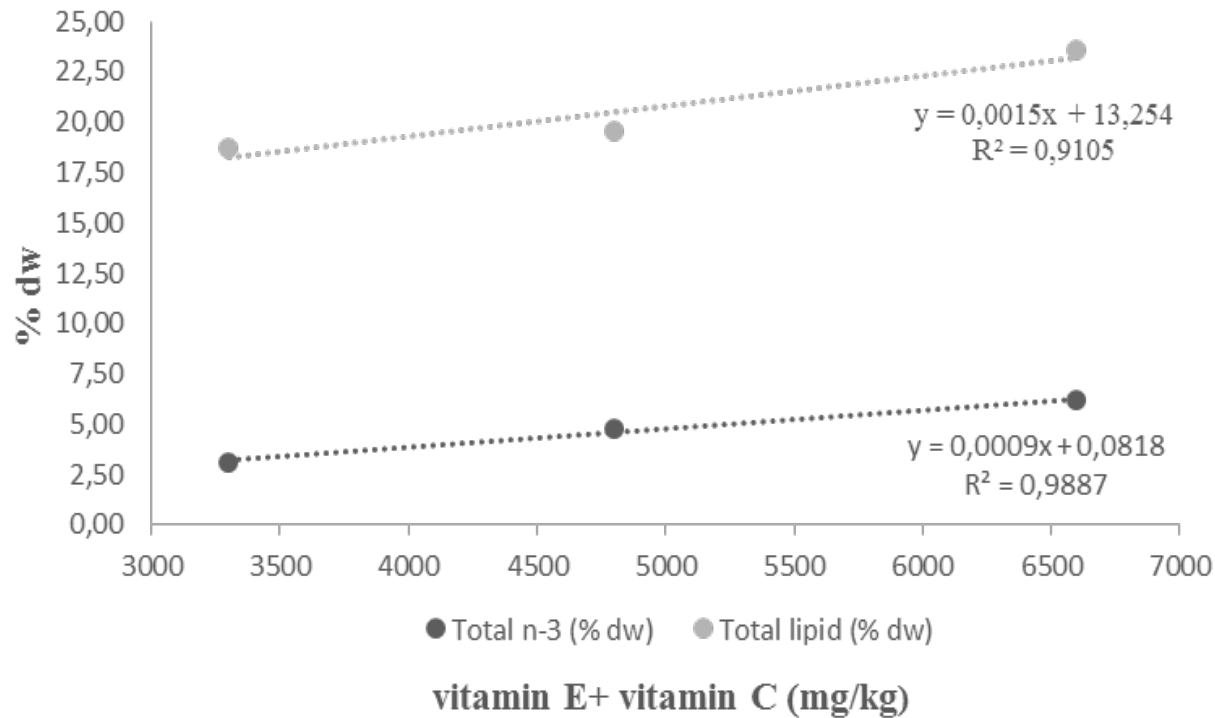
— Low HUFA
— High HUFA

→
Increases of vitamins

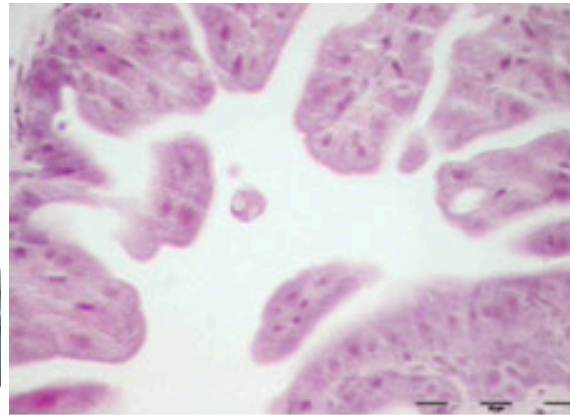
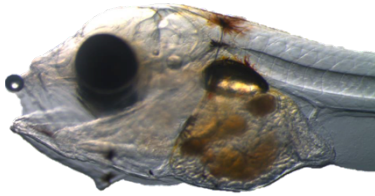


- **Task 8.1. Improvement of larval weaning diets.**
 1. essential fatty acids + micronutrients

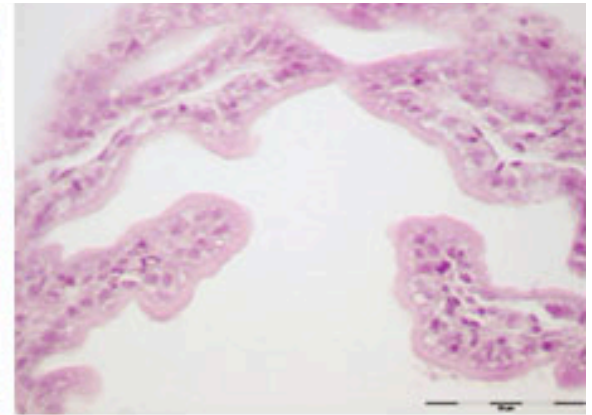
Effect of dietary vitamin E and C on lipid and n-3 contents (dw) in meagre (*A. regius*) larvae after 14 days of feeding 3% HUFA diets



**Morphological study
H&E stain**

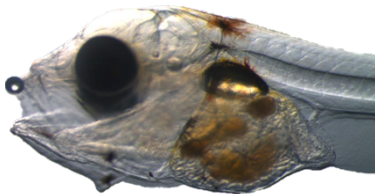


Intestine of larvae 0.4/150/180 (40X) scarce lipid vacuoles accumulation in the enterocytes;

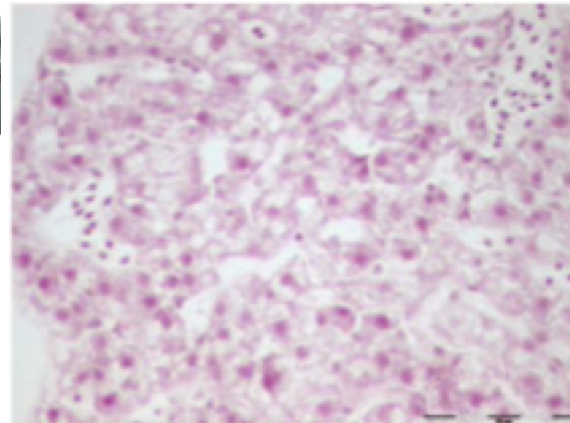


Intestine larvae 3/150/180 (40X) large lipid vacuoles around the nucleus and in the basal part of the enterocytes;

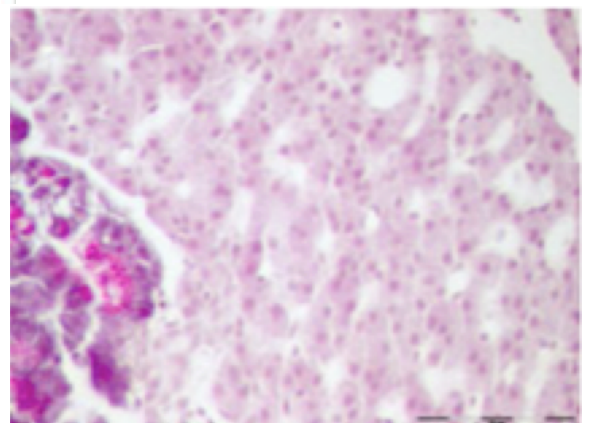
**Increased lipid
mobilization**

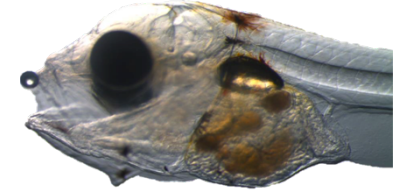


liver larvae 0.4/ 150/180 (40X) hepatocytes with high lipid vacuoles deposition



liver larvae 3/150/180 (40X) condensed hepatocytes with centred nucleus and marked cytoplasm staining;



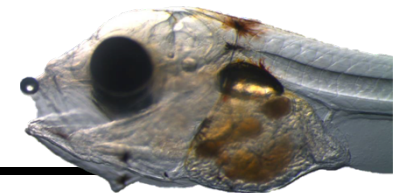


- **Task 8.1. Improvement of larval weaning diets.**

1. essential fatty acids + micronutrients

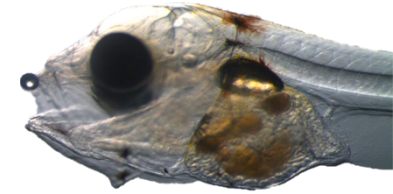
- ✓ 0.4% dietary HUFA is not enough to cover the essential fatty acid requirements of larval meagre and, since their elevation up to 3% markedly improved lipid absorption, essential fatty acids levels and growth, a high HUFA requirement in weaning diets is foreseen for this species.
- ✓ pointed out the importance of dietary vitamin E and vitamin C to protect these essential fatty acids from oxidation, increase their contents in larval tissues and promote growth, suggesting as well high vitamin E and vitamin C requirements in meagre larvae (higher than 1500 and 1800 mg kg⁻¹ for vitamin E and vitamin C, respectively).

■ **Task 8.1.** Improvement of larval weaning diets.
2. Vitamins A, K & D



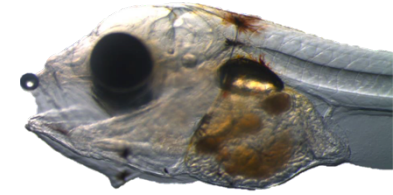
	Diets				
	C	C+Taurine	C-Vit K	C-Vit D	C-Vit A
<i>Ingredients</i>					
Taurine ⁱ	0.0	200.0	0.0	0.0	0.0
Vit K ^j	17.3	17.3	0.0	17.3	17.3
Vit D ^k	3.7	3.7	3.7	0.0	3.7
Vit A ^l	0.3	0.3	0.3	0.3	0.0
<i>Proximate composition (%)</i>					
Crude lipids	16.4	16.2	16.5	17.1	17.9
Crude protein	76.0	75.9	76.4	76.4	76.1
Moisture	13.7	13.6	13.6	13.8	13.8
Ash	6.5	6.5	6.5	6.6	6.5
Taurine ¹	4.0	5.8	4.0	4.0	4.0
Vitamin K ²	2.4	2.4	0.0	2.6	2.2
Vitamin D ³	28.9	29.0	30.4	2.3	27.4
Vitamin A ⁴	4.2	4.3	4.2	4.3	4.1

- **Task 8.1.** Improvement of larval weaning diets.
2. Vitamins A, K & D



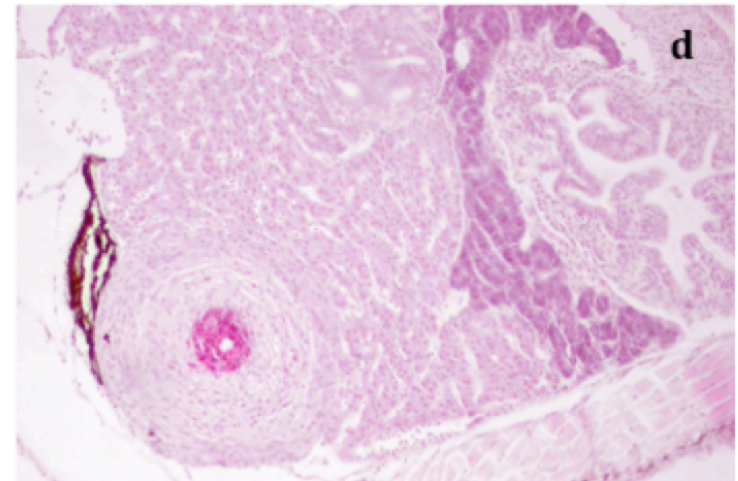
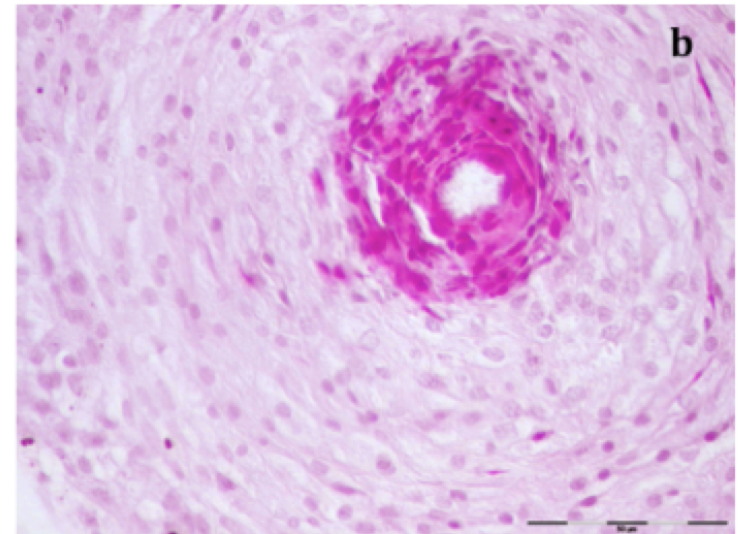
		Diets				
		C	C+Taurine	C-Vit K	C-Vit D	C-Vit A
Total length	26 dah	8.3±1.0 ^a	8.5±0.9 ^a	8.5±1.0 ^a	8.9±1.0 ^b	8.6±1.0 ^a
	33 dah	11.5±1.7 ^a	11.7±1.3 ^a	12.8±1.6 ^{b*}	12.6±1.3 ^b	12.2±1.7 ^b
Body weight	26 dah	0.7±0.1 ^a	0.8±0.1 ^a	0.7±0.1 ^a	0.9±0.2 ^b	0.8±0.2 ^a
	33 dah	2.4±0.6 ^a	2.3±0.4 ^a	3.2±0.2 ^{b*}	3.3±0.2 ^b	2.5±0.3 ^a
Survival (%)		16.7±6.5	12.9±1.2	7.1*	17.7±12.3	19.0±0.5

- **Task 8.1.** Improvement of larval weaning diets.
2. Vitamins A, K & D



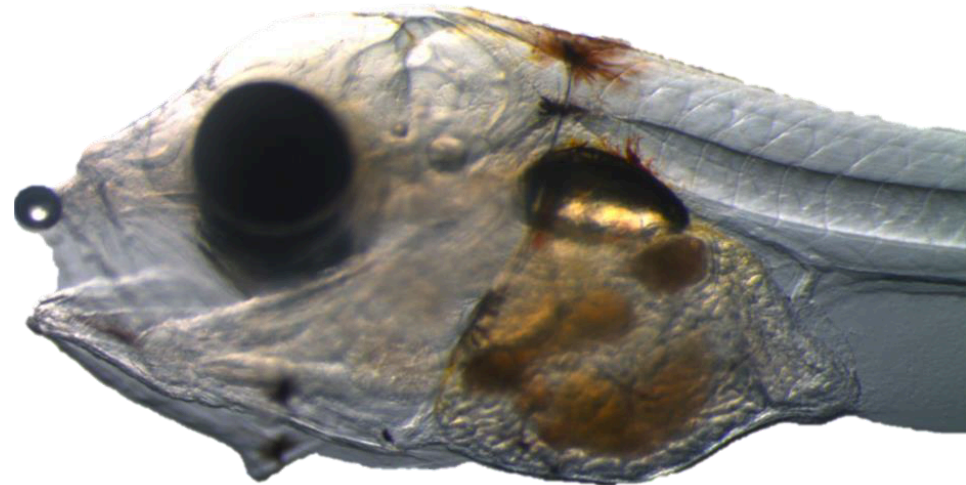
Effect on systemic granulomatosis

33 dah			
Diets	Total	Stage I	Stage II
C	0.0±0.0	0.0±0.0	0.0±0.0
C+Taurine	0.0±0.0	0.0±0.0	0.0±0.0
C-Vit K	12.5±10.6	5.0±7.1	7.5±3.5
C-Vit D	3.3±2.9	1.7±2.9	1.7±2.9
C-Vit A	8.3±10.4	6.7±7.6	1.7±2.9

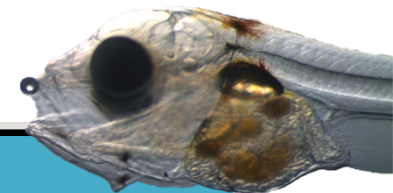


- **Task 8.1.** Improvement of larval weaning diets.
2. Vitamins A, K & D

- ✓ supplementation of meagre weaning diets with 2.4 mg/kg vit K, since the absence of this vitamin markedly reduced larval survival.
- ✓ very sensitive to hypervitaminosis D and, only mildly to hypervitaminosis A, since supplementation with these vitamins led to a growth reduction.
- ✓ taurine supplementation did not have any effect in meagre larvae performance

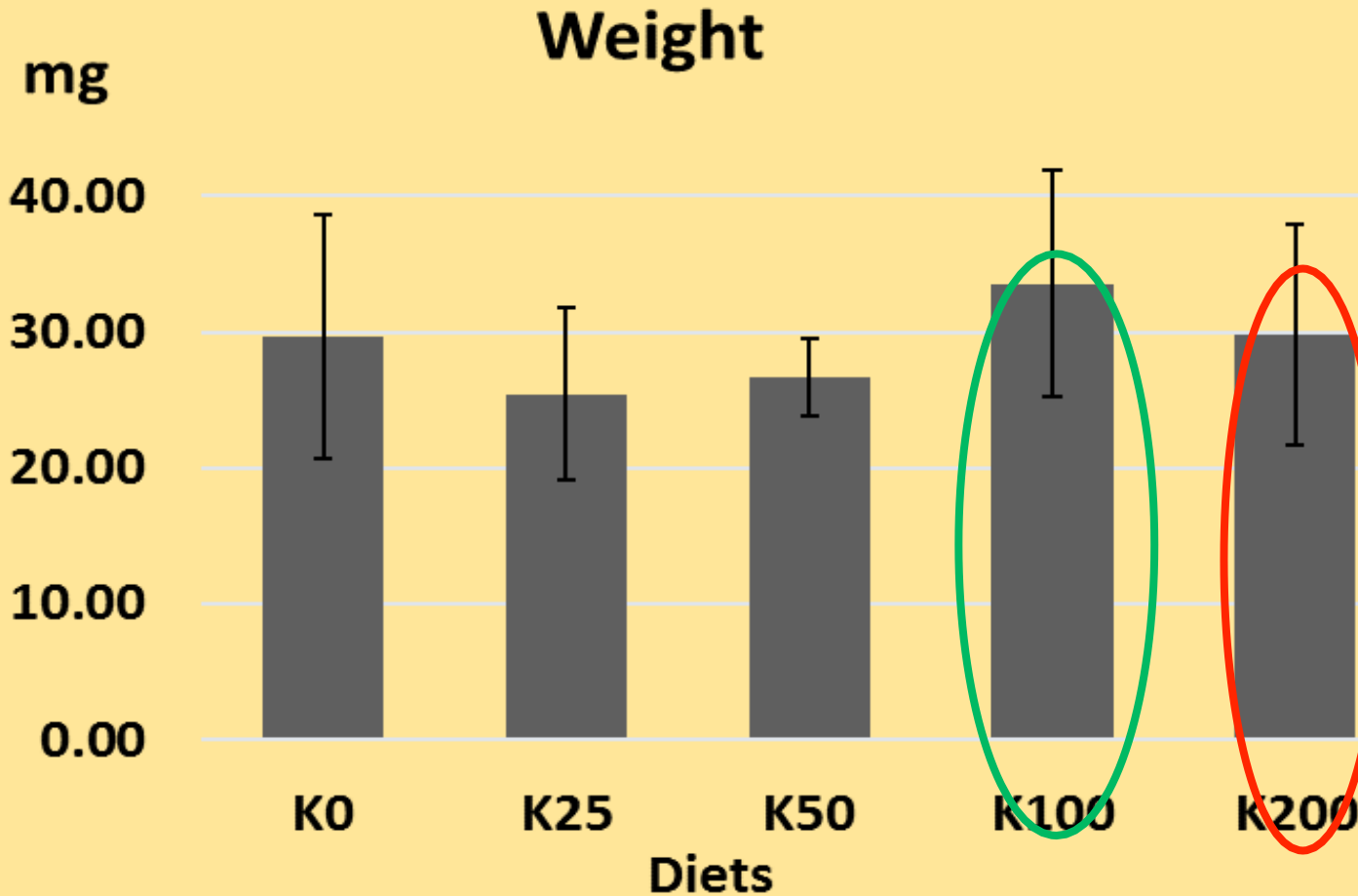
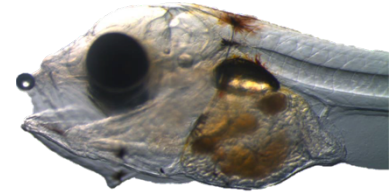


- **Task 8.1.** Improvement of larval weaning diets.
- 3. Vitamin K

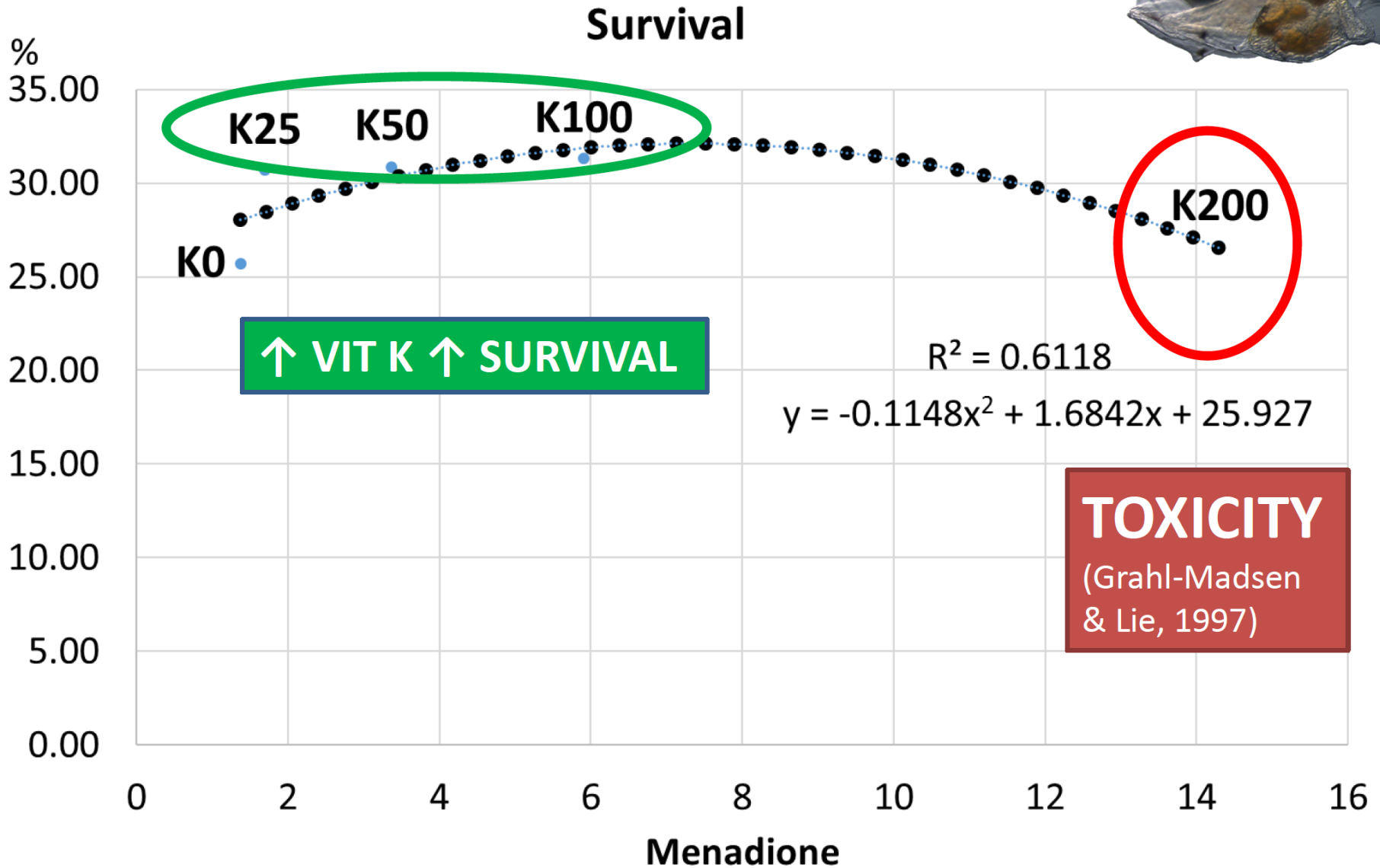
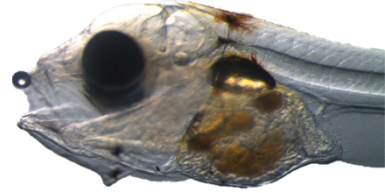


		Diets				
Ingredients (%)	K0	K25	K50	K100	K200	
Squeed meal	72.9	72.9	72.9	72.9	72.9	
Krill oil	10	10	10	10	10	
Menadione SB (mg/kg)	0	4.32	8.64	17.28	34.56	
Vit E (g/kg)	0.3	0.3	0.3	0.3	0.3	
Vit C (g/kg)	0.4	0.4	0.4	0.4	0.4	
Proximate composition (%)	K0	K25	K50	K100	K200	
Proteins	72.9	68.5	70.8	66.9	68.6	
Lipids	14.4	15.6	15.7	13.7	15.0	
Ash	6.5	6.1	5.8	6.1	6.1	
Moisture	9.9	9.3	11.3	9.6	10.5	
Menadione SB (mg/kg)	1.37	1.69	3.37	5.90	14.30	

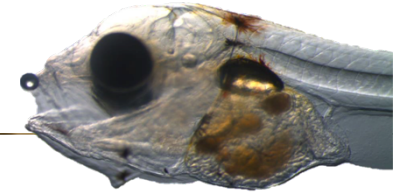
- **Task 8.1.** Improvement of larval weaning diets.
3. Vitamin K



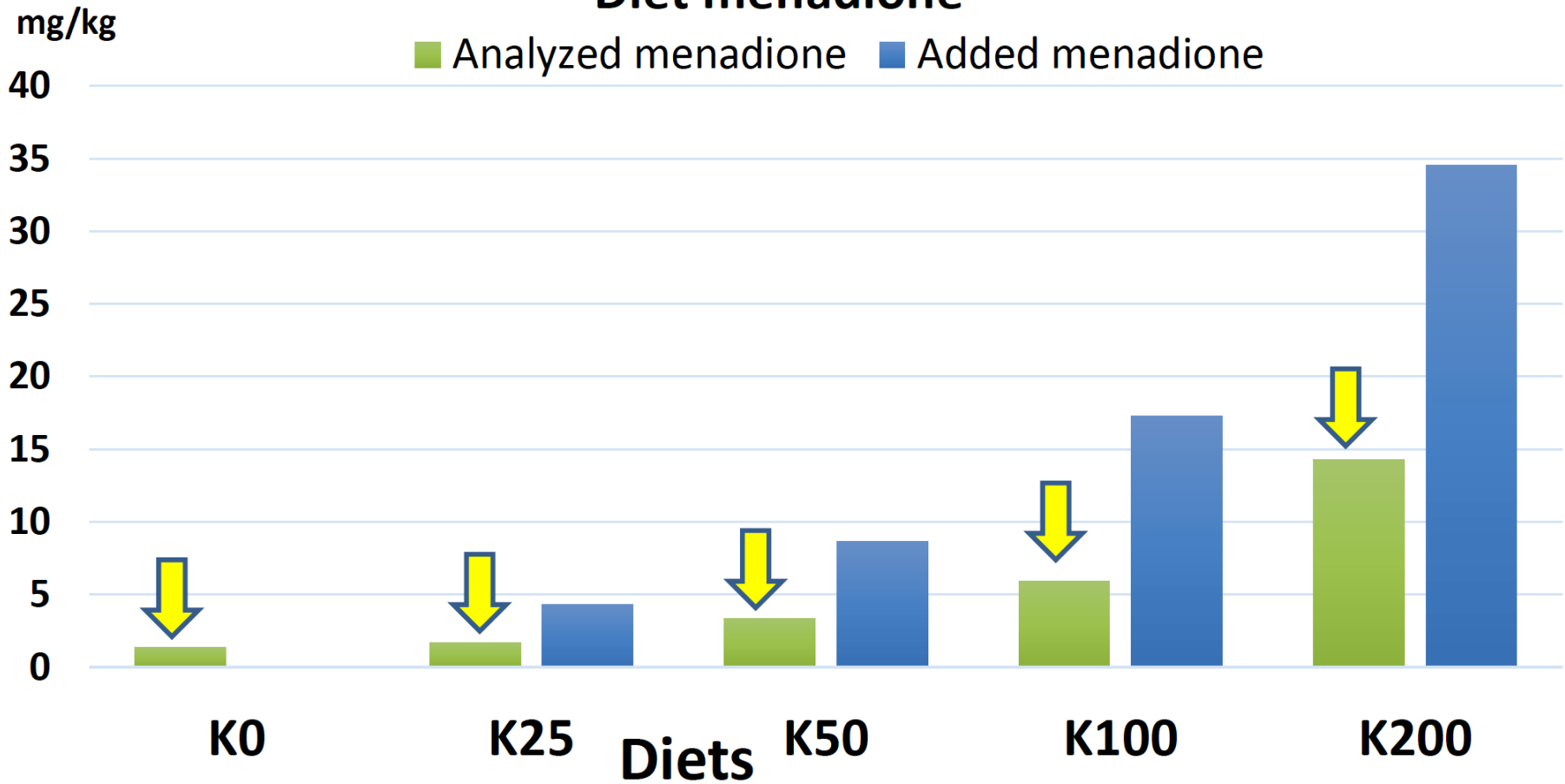
- **Task 8.1.** Improvement of larval weaning diets.
3. Vitamin K



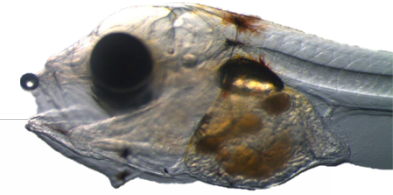
- **Task 8.1.** Improvement of larval weaning diets.
3. Vitamin K



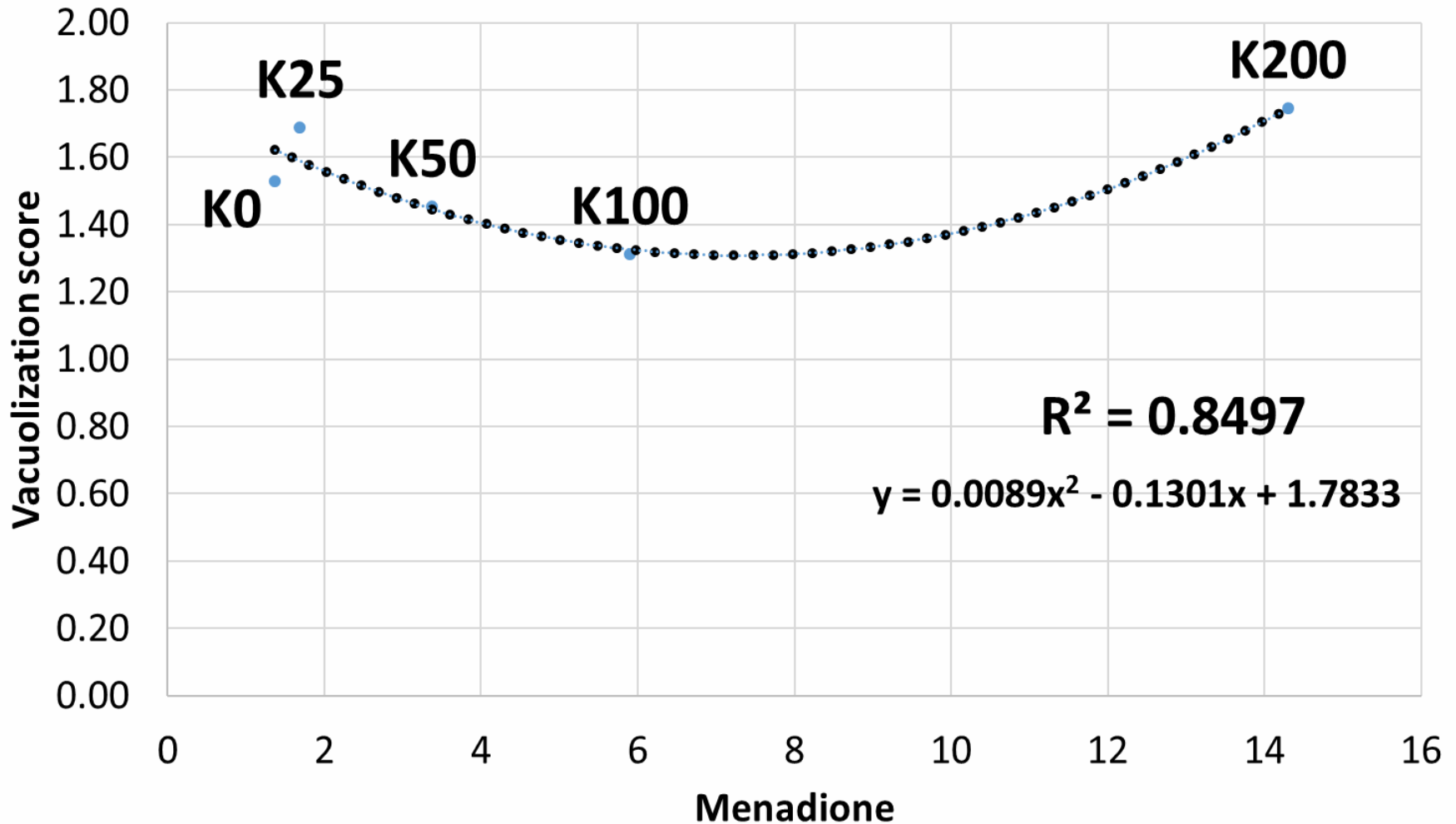
Diet menadione



- **Task 8.1.** Improvement of larval weaning diets.
3. Vitamin K



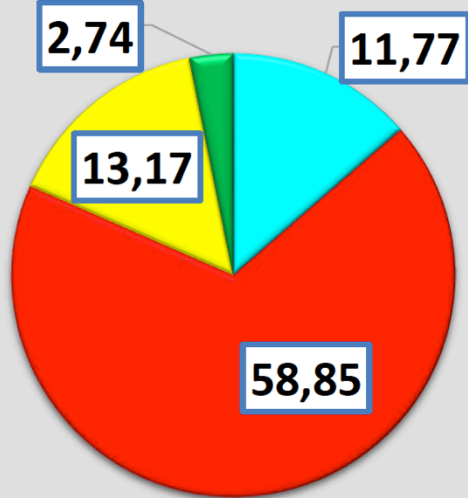
Liver vacuolization



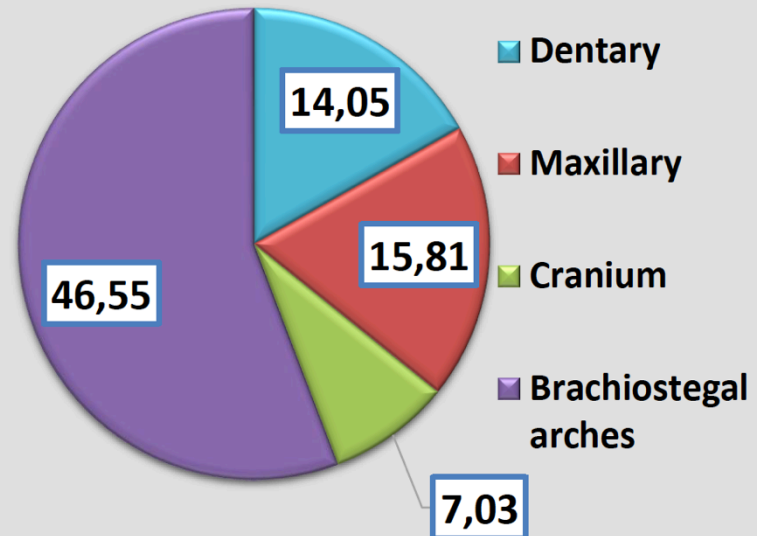
Osteology

Results & Discussion

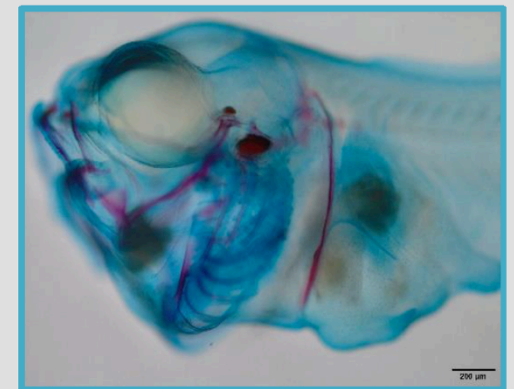
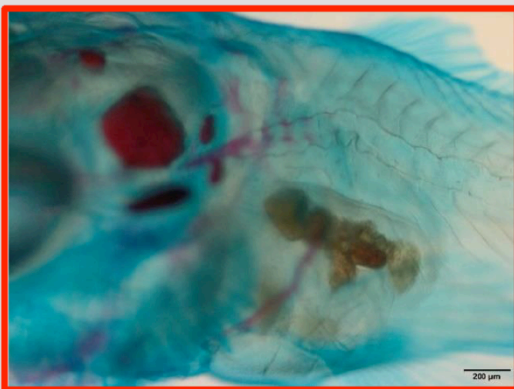
Vertebral regions anomalies



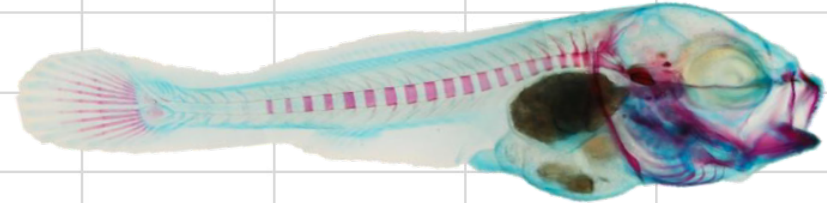
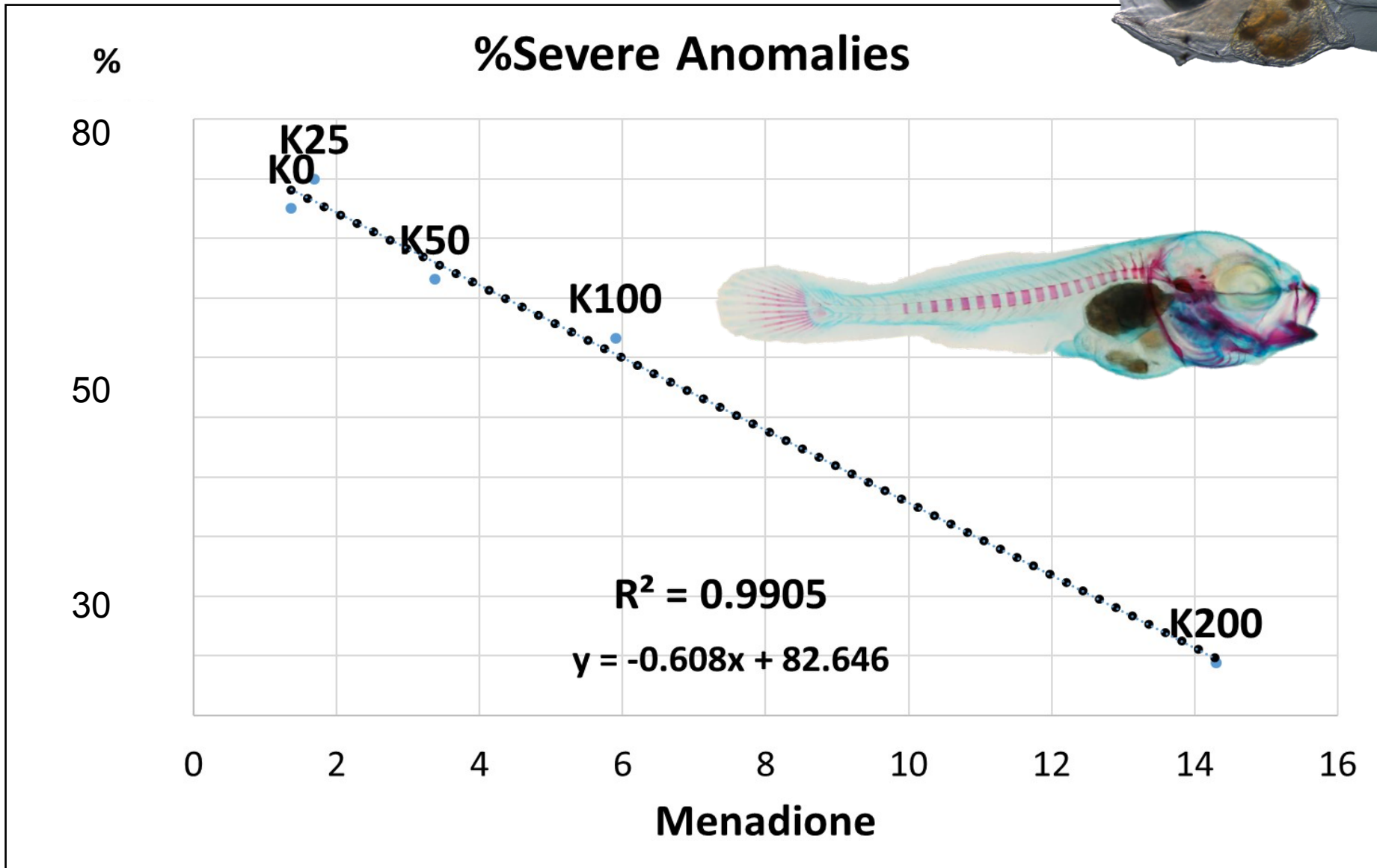
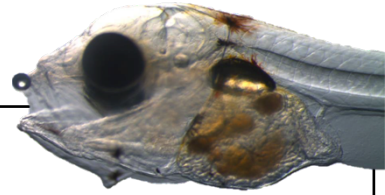
Cephalic anomalies



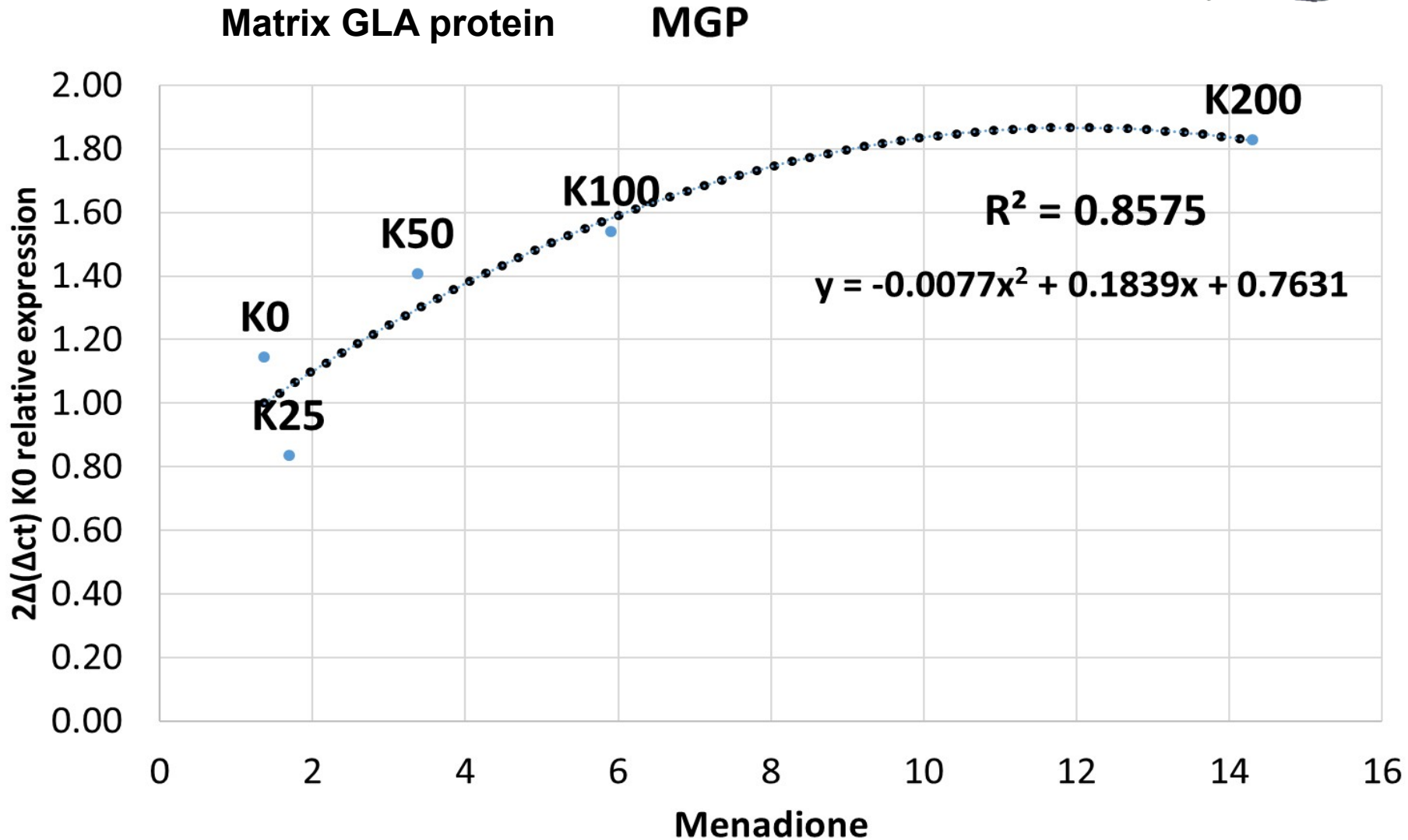
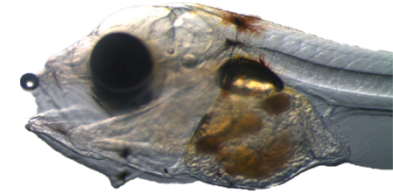
■ Cephalic ■ Pre-haemal ■ Haemal ■ Caudal



- **Task 8.1.** Improvement of larval weaning diets.
3. Vitamin K



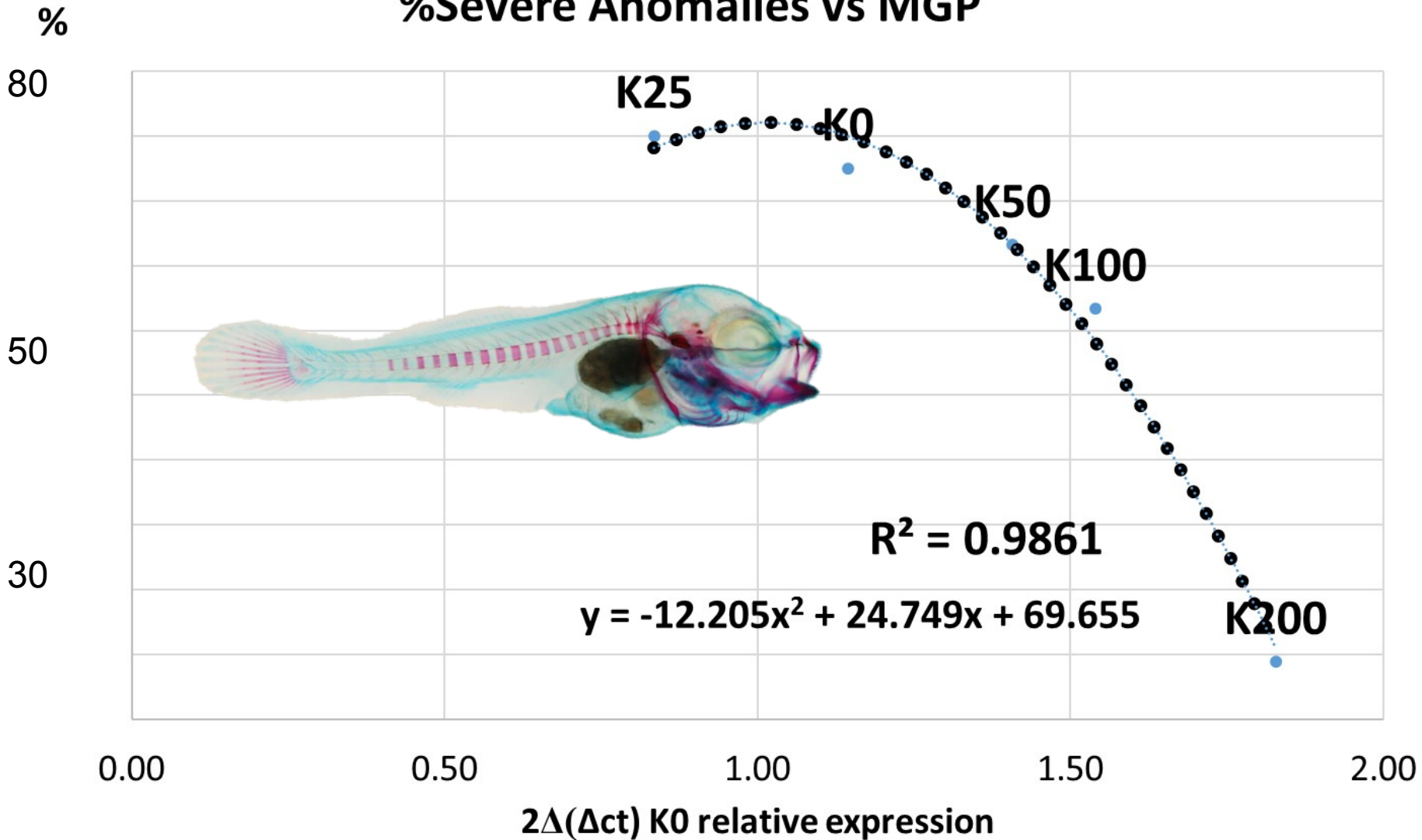
Relationship between menadione and MGP gene expression



Relationship between anomalies and MGP gene expression



%Severe Anomalies vs MGP



✓ 5.90 mg/kg of menadione in diet shows a trend to increase growth and survival of meagre larvae, however, large amount seems to decrease growth and survival, possibly due to its toxic potential.

✓ Low levels of vitamin K may interfere in lipids utilization, increasing intestinal and hepatic steatosis.

✓ **5.90 mg/kg of menadione may be an adequate level for meagre larvae good performance.**



- **Task 8.2** Determination of nutritional requirements to promote feed utilization, consistent growth rates and fish welfare.



Implementation: FCPCT, SARC



Objective

- ➔
 - ✓ Evaluate the effect of 5 increasing dietary n-3 HUFA levels on:
 - ✓ Growth performance
 - ✓ Feed utilization
 - ✓ Whole-body composition
 - ✓ Desaturase and elongase gene expression

- ➔
 - ✓ Estimate the effect of vitamins for meagre fingerlings health



Methodological approach

⇒ Dietary treatments



16.5% DM lipids
0.9 EPA/DHA
0.1-0.7% ARA (of total FA)
Feeding: 3x/day until
apparent satiety during 30
days.

Composition (%) and proximate analysis of the experimental diets for meagre fingerlings.

	Dietary n-3 HUFA level (%DM)				
	0.8	1.4	2.0	2.6	3.6
Ingredients (%)					
Fish meal, N. Atlantic ^a	15.0	15.0	15.0	15.0	15.0
Corn gluten ^b	10.0	10.0	10.0	10.0	10.0
Faba beans ^a	10.0	10.0	10.0	10.0	10.0
Wheat ^a	8.0	8.0	8.0	8.0	8.0
Wheat gluten ^a	18.4	18.4	18.4	18.4	18.4
Soy protein concentrate ^a	25.0	25.0	25.0	25.0	25.0
Fish oil, S. American ^a	0.0	2.7	5.4	8.2	10.9
Linseed oil ^c	1.6	1.2	0.8	0.4	0.0
Palm oil ³	3.3	2.5	1.7	0.8	0.0
Rapeseed oil ^a	6.0	4.5	3.0	1.5	0.0
Premix ^d	2.8	2.8	2.8	2.8	2.8
Proximate analysis (% DM)					
Protein	56.5	54.5	54.5	56.0	54.3
Lipids	16.2	17.0	16.5	16.9	16.2
Ash	4.9	5.0	5.1	5.2	5.0
Moisture	8.7	8.5	8.5	8.2	7.9

Methodological approach



⇒ Rearing conditions

- IBW: 2.8 g; IBL: 6.4 cm
- 200 L - tanks (3 x treatment)
- 45 fish/tank
- Temperature: 23 ± 0.2 °C



⇒ Final sampling

- Biological parameters: body weight, length and feed intake
- Biochemistry: whole-body composition and diets (protein, lipids, FA content, ash and moisture)
- Gene expression in liver: $\Delta 6$ desaturase and Elov15



Results- Growth performance

Dietary n-3 HUFA level (%DM)

	0.8	1.4	2.0	2.6	3.6
Survival (%)	93.3±0.7	97.8±1.3	99.3±0.7	94.8±1.5	97.8±2.2
Initial total length (cm)	6.4±0.0	6.3±0.1	6.3±0.0	6.4±0.0	6.2±0.0
Final total length (cm)	9.0±0.1 ^c	9.4±0.1 ^b	9.3±0.1 ^{ab}	9.6±0.1 ^a	9.3±0.1 ^{bc}
Initial body weight (g)	2.8±0.1	2.8±0.1	2.7±0.1	2.7±0.1	2.6±0.1
Final body weight (g)	9.5±0.3 ^b	10.4±0.3 ^a	10.2±0.4 ^{ab}	10.7±0.3 ^a	10.4±0.3 ^a
WG (g)	6.7±0.4	7.5±0.4	7.6±0.3	8.0±0.3	7.8±0.2
SGR (% day ⁻¹)	4.1±0.1	4.3±0.2	4.5±0.1	4.5±0.1	4.6±0.1
TGC	1.0±0.0	1.1±0.1	1.1±0.0	1.2±0.0	1.2±0.0
FI (g feed fish ⁻¹ day ⁻¹)	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
FCR	0.8±0.1	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0
K (%)	1.3±0.0	1.3±0.0	1.3±0.1	1.2±0.1	1.3±0.0
PER	2.4±0.2	2.6±0.1	2.5±0.1	2.6±0.1	2.6±0.1

Different letters are significantly different (P<0.05)

- No external signs of EFA-deficiency nor mortality
- **Higher growth rates and good feed conversion ratios**



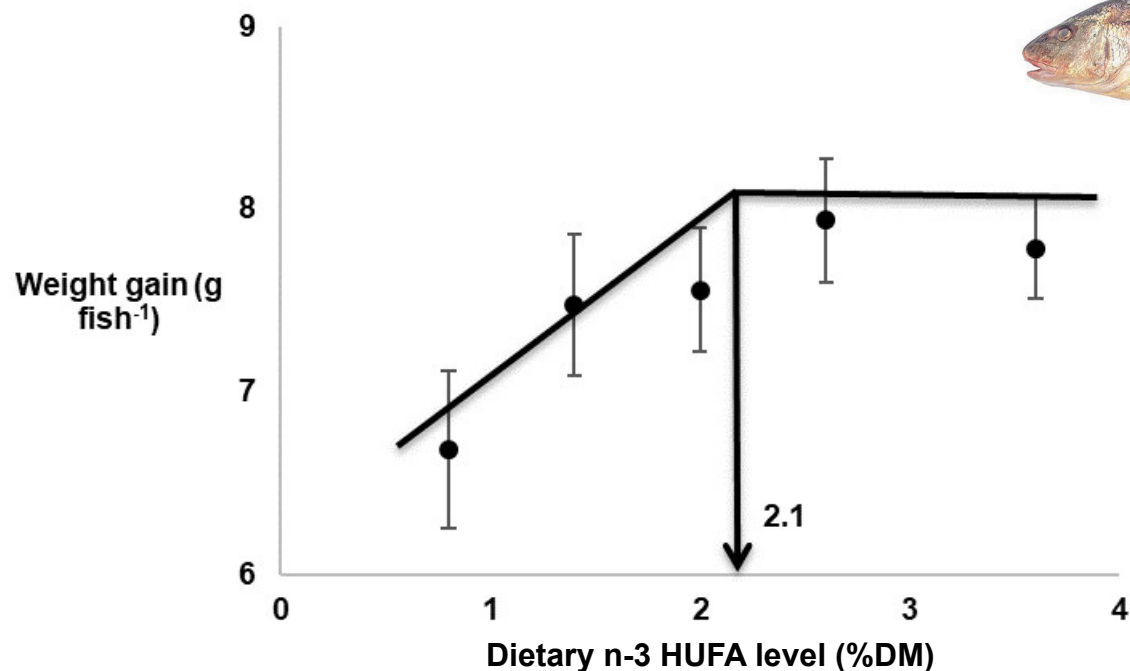
Results- Growth performance

	Dietary n-3 HUFA level (%DM)				
	0.8	1.4	2.0	2.6	3.6
Survival (%)	93.3±0.7	97.8±1.3	99.3±0.7	94.8±1.5	97.8±2.2
Initial total length (cm)	6.4±0.0	6.3±0.1	6.3±0.0	6.4±0.0	6.2±0.0
Final total length (cm)	9.0±0.1 ^c	9.4±0.1 ^b	9.3±0.1 ^{ab}	9.6±0.1 ^a	9.3±0.1 ^{bc}
Initial body weight (g)	2.8±0.1	2.8±0.1	2.7±0.1	2.7±0.1	2.6±0.1
Final body weight (g)	9.5±0.3 ^b	10.4±0.3 ^a	10.2±0.4 ^{ab}	10.7±0.3 ^a	10.4±0.3 ^a
WG (g)	6.7±0.4	7.5±0.4	7.6±0.3	8.0±0.3	7.8±0.2
SGR (% day ⁻¹)	4.1±0.1	4.3±0.2	4.5±0.1	4.5±0.1	4.6±0.1
TGC	1.0±0.0	1.1±0.1	1.1±0.0	1.2±0.0	1.2±0.0
FI (g feed fish ⁻¹ day ⁻¹)	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
FCR	0.8±0.1	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0
K (%)	1.3±0.0	1.3±0.0	1.3±0.1	1.2±0.1	1.3±0.0
PER	2.4±0.2	2.6±0.1	2.5±0.1	2.6±0.1	2.6±0.1

Different letters are significantly different (P<0.05)

Dietary n-3 HUFA increase was **significantly correlated** to final body weight (P=0.09, r²=0.82), WG (P=0.05, r²=0.86), SGR (P=0.01, r²=0.94) and TGC (P=0.01, r²=0.94).

Results- Growth performance



Requirement: **2.1% n-3 HUFA**

Similar to what found for red seabream (Takeuchi et al., 1992a) and yellowtail (Takeuchi et al., 1992b) , but higher than red drum (Lochman and Gatlin, 1993), seabream (Ibeas et al., 1996) and seabass (Skalli and Robin, 2004).

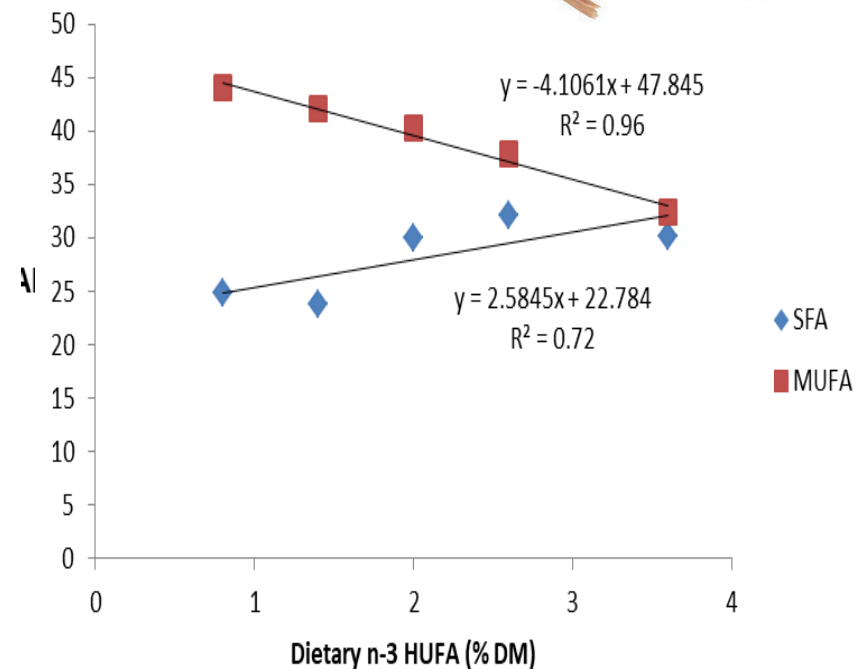
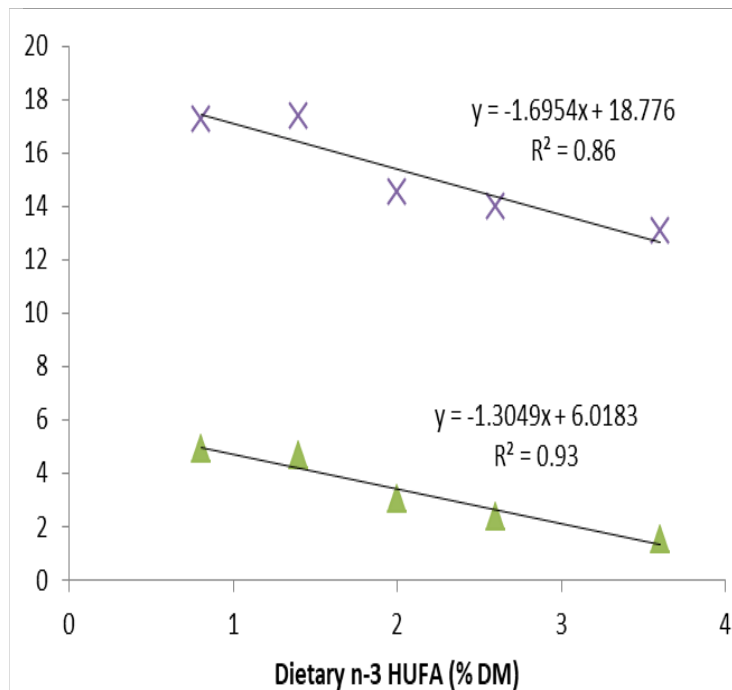


Results- Body composition (% wet weight)

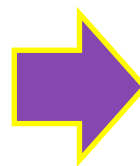
	Dietary n-3 HUFA level (% DM)					
	Initial	0.8	1.4	2.0	2.6	3.6
Protein	15.6±0.49	15.8±0.17	16.1±0.12	15.6±0.28	16.3±0.50	16.0±0.61
Lipid	2.2±0.12	4.1±0.47	4.1±0.25	3.8±0.24	3.8±0.28	3.9±0.25
Ash	3.9±0.26	2.7±0.22	2.7±0.09	2.8±0.14	2.8±0.04	2.6±0.29
Moisture	78.6±0.28	78.1±0.82	77.7±0.03	78.0±0.60	78.5±0.13	78.7±0.20

No significant differences observed between fish fed different treatments.

Results- Whole-body FA content






Reflecting diet composition



According to the increase substitution of VO by FO

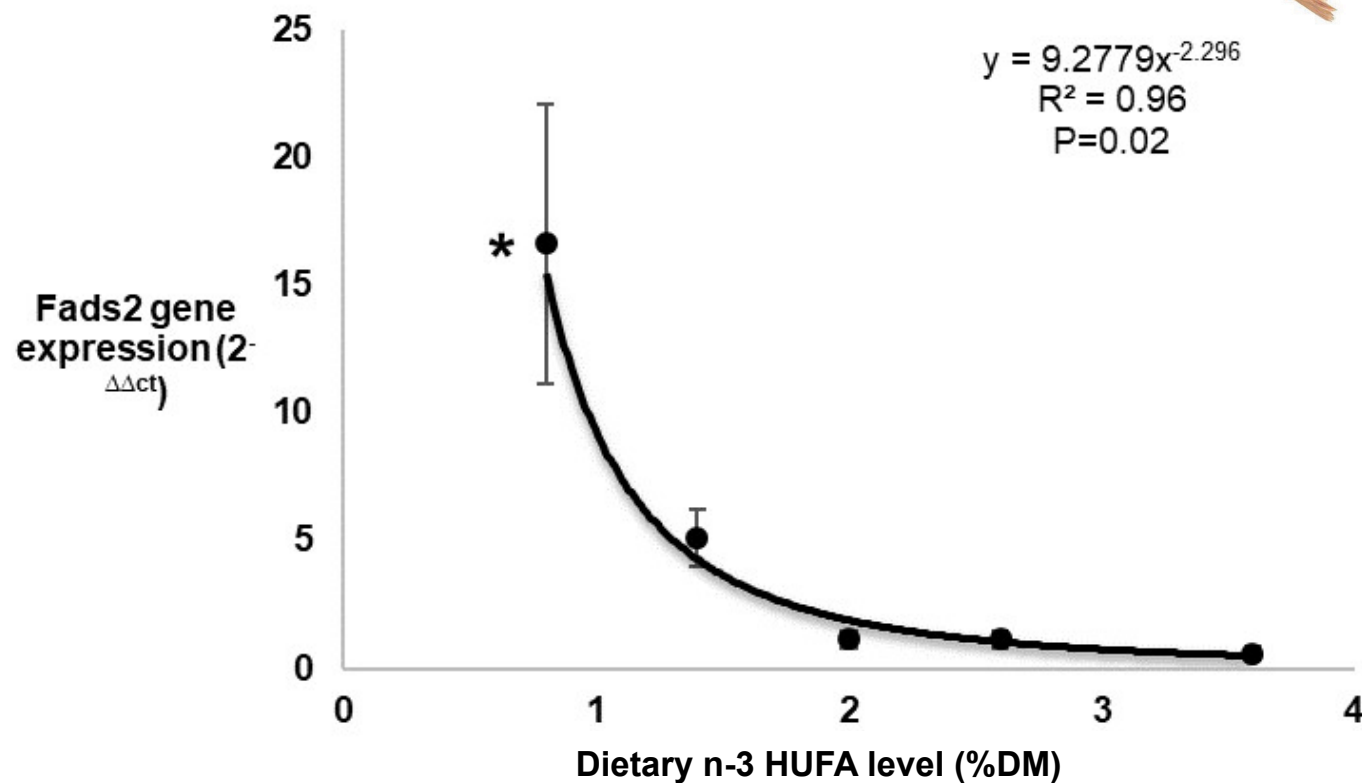
Results- FA retention efficiency (% FA intake)

Fatty acid	Dietary n-3 HUFA level (% DM)				
	0.8	1.4	2.0	2.6	3.6
18:2n-9	251.9±43.0 ^a	125.7±16.5 ^b	55.1±16.2 ^b	47.2±1.2 ^b	37.7±1.4 ^b
18:3n-6	190.8±44.8	107.8±14.7	48.2±13.7	40.9±2.4	65.5±12.6
18:4n-3	23.3±3.6	26.9±4.7	14.6±4.3	18.9±3.3	30.0±3.2
18:3n-3	25.7±5.3 ^b	38.7±4.1 ^{ab}	24.5±4.3 ^b	31.7±2.9 ^b	56.8±5.2 ^a
20:3n-3	315.0±88.6 ^a	256.1±24.6 ^{ab}	136.6±12.9 ^{abc}	50.8±0.6 ^b	68.2±10.7 ^b
20:2n-6	227.8±57.4 ^a	165.4±11.4 ^{ab}	114.2±12.8 ^{ab}	82.2±110.7 ^b	86.5±10.3 ^{ab}
20:4n-6	55.0±18.2 	57.2±2.8 	28.7±8.9	32.9±3.0	53.1±6.7
20:5n-3	25.0±6.7	31.3±4.4	13.3±4.7	17.9±4.1	30.3±4.3
22:6n-3	56.1±5.8 ^a 	41.4±3. ^b	41.5±4.7 ^b	37.5±0.7 ^b	39.9±3.7 ^b

 Δ6 desaturase product

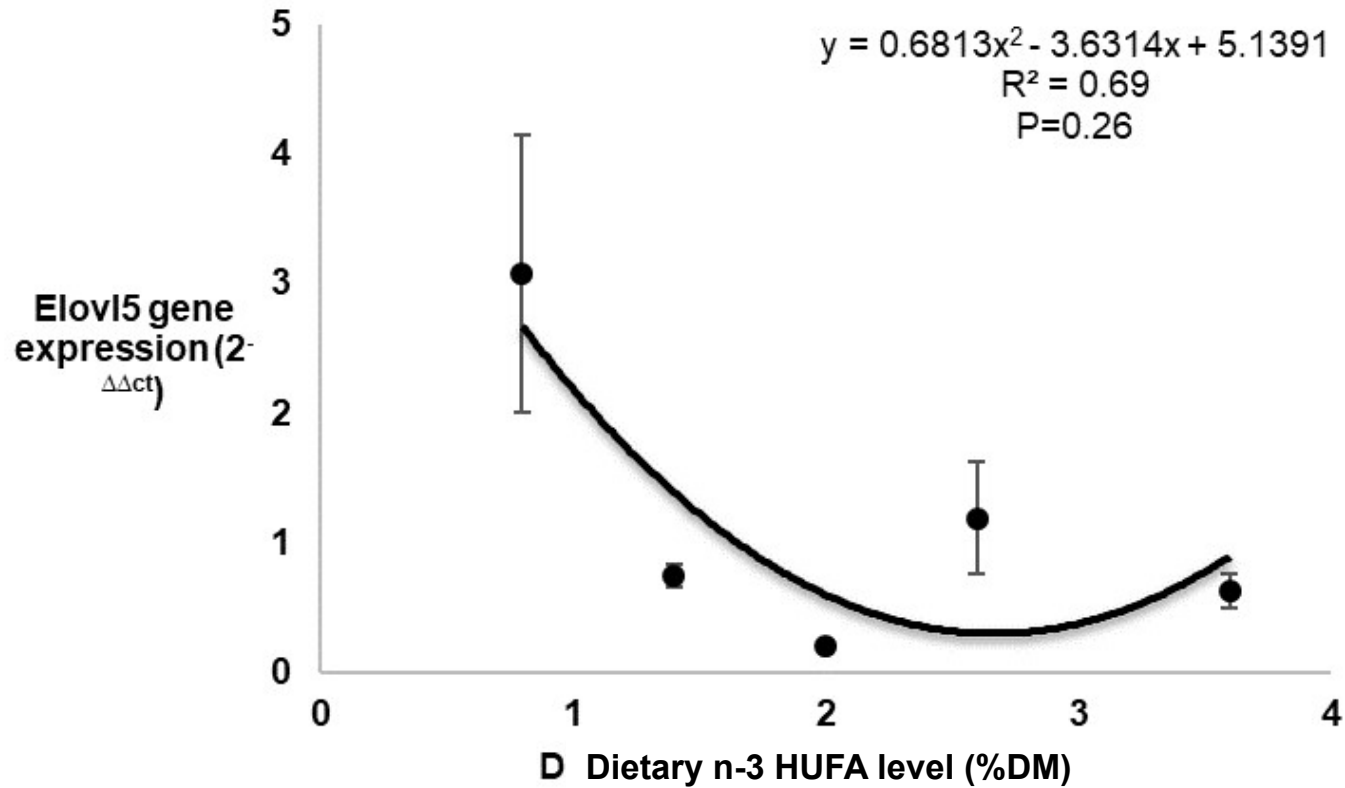
 Elovl5 product

Results- Gene expression



* indicates significance difference ($P=0.04$) compared to diet 3.6% n-3 HUFA
 Similar results found by Monroing et al., 2013

Results- Gene expression



Similar results found by Monroing et al., 2013

- ✓ Meagre showed the ability to selectively conserve key FA, particularly DHA and ARA over other FA, in response to EFA-deficiency.
- ✓ Meagre seems to have active $\Delta 6$ desaturase and Elovl5, but their activities being insufficient to produce enough DHA and EPA from PUFA precursors to sustain fast growth.
- ✓ Meagre fingerlings have n-3 HUFA requirement around 2.1% DM in diets containing 16.5% DM lipids, 0.9 EPA/DHA and 0.4% ARA of total FA contents.



Dietary combinations of vitamin E, C and K affect the incidence of systemic granulomatosis in on-growing meagre (*Argyrosomus regius*)



Systemic granulomatosis in Canary Islands meagre



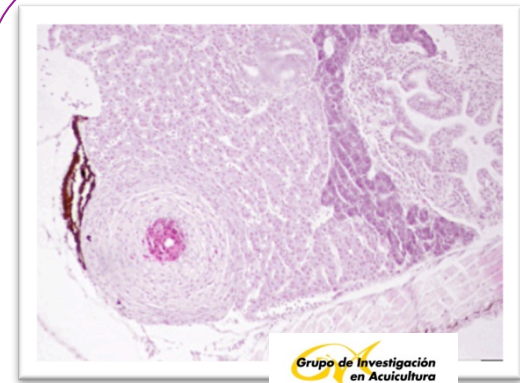
Fish Farm – sea cage

↓ **vitamin C in feeds**



Grupo de Investigación
en Acuicultura

**High incidence in
meagre broodstock
(F1>) and juveniles**



Grupo de Investigación
en Acuicultura

**First record of
granulomas in
meagre larvae**

**> Low vitamin K
levels in weaning
diets (D 8.1)**



Objective

- To evaluate the effect of vitamins E and C and K on granulomatosis occurrence in meagre (*Argyrosomus regius*)

WP 24. Meagre health



Initial weight 79.6 ± 0.3 g

Initial density tank) 7.9 kg/m³ (50 fish/


Tank volume 0.5 m³

Water temperature 17.6 to 21.6 °C

Feeding period 90 days

Experimental design



Ingredients	Diets					
	O	K	EC	KEC	EECC	KEECC
Lutavit E-50 	0.0	0.0	300.0	300.0	700.0	700.0
Lutavit C Aquastab 35%	0.0	0.0	100.0	100.0	600.0	600.0
Vitamin K	0.0	35.0	0.0	35.0	0.0	35.0
Proximate Composition						
Crude Protein (%dw)	48.8 ± 0.1	49.6 ± 0.1	48.7 ± 0.2	48.6 ± 0.1	48.7 ± 0.3	48.5 ± 0.3
Crude Lipid (%dw)	17.9 ± 0.2	17.6 ± 0.1	19.5 ± 0.4	17.4 ± 0.3	17.7 ± 0.1	16.8 ± 0.2
Vitamin E (mg/kg)	158.7	172.5	283.6	276.5	416.4	449.2
Vitamin C (mg/kg)	16.6	19.0	71.1	72.4	227	240

Growth performance

Weight, length, weight gain, K, FCR and SGR calculated at 45 and 90 days of feeding

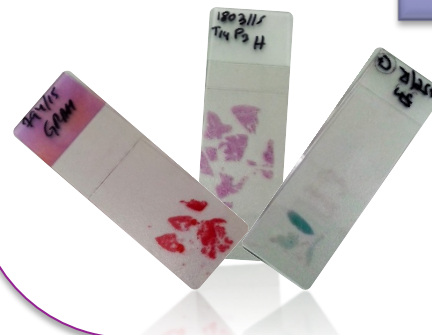


Histology

Samples of liver, kidney, heart and spleen

- 50 initial fish
- 23 fish / diet at 90 days

Kidney, heart and spleen were completely processed, liver was cut in several sections (5-6) to evaluate the largest tissue-surface possible.



Hematoxylin and Eosin

Ziel-Neelsen (ZN)

Fite-Faraco method

Gram stain

Analysis Nocardia culture

Samples of liver, kidney, heart and spleen

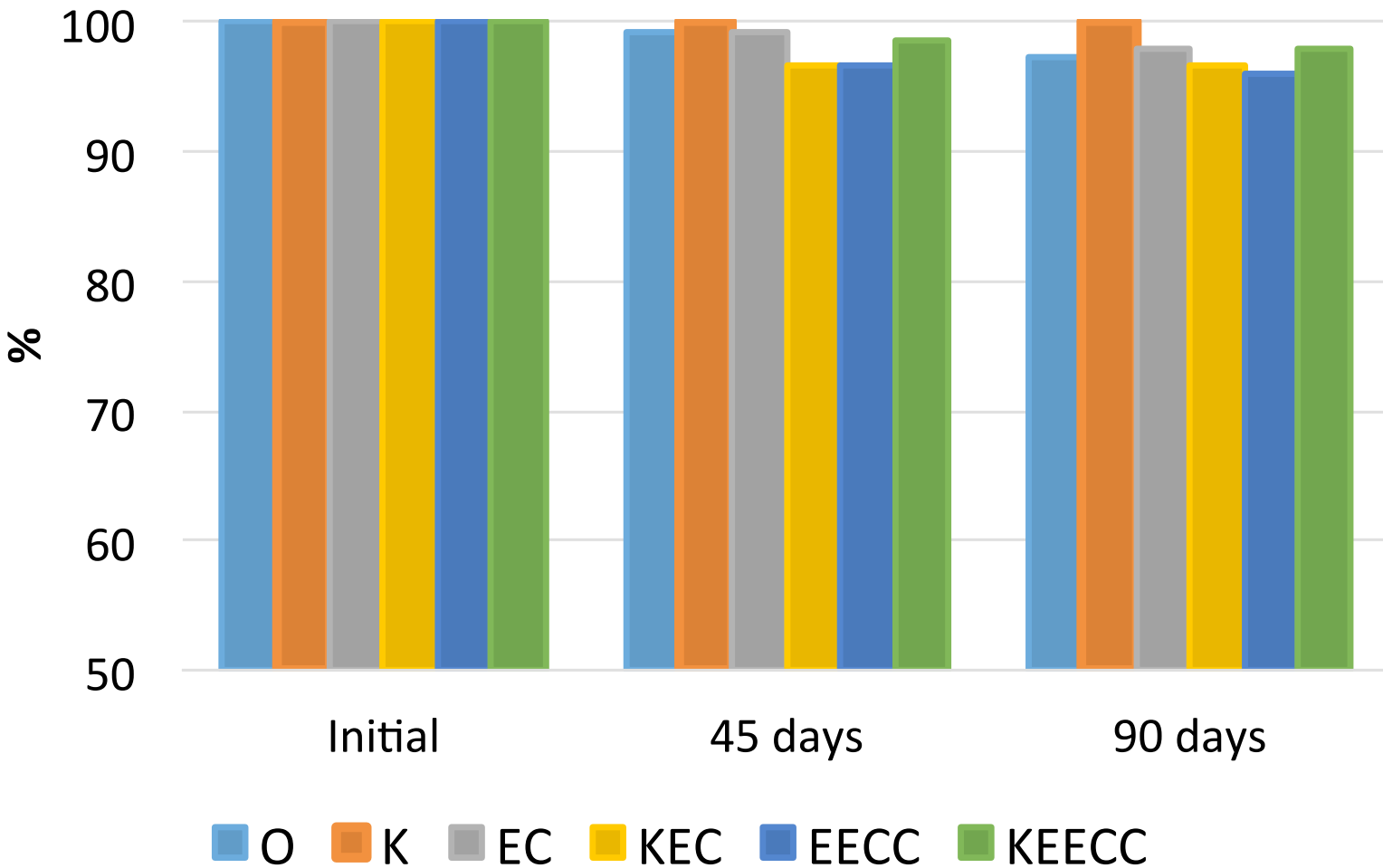
Culture medium: YEME

Gram Stain

Survival was high (96-100 %) and no significantly affected by dietary vitamin levels

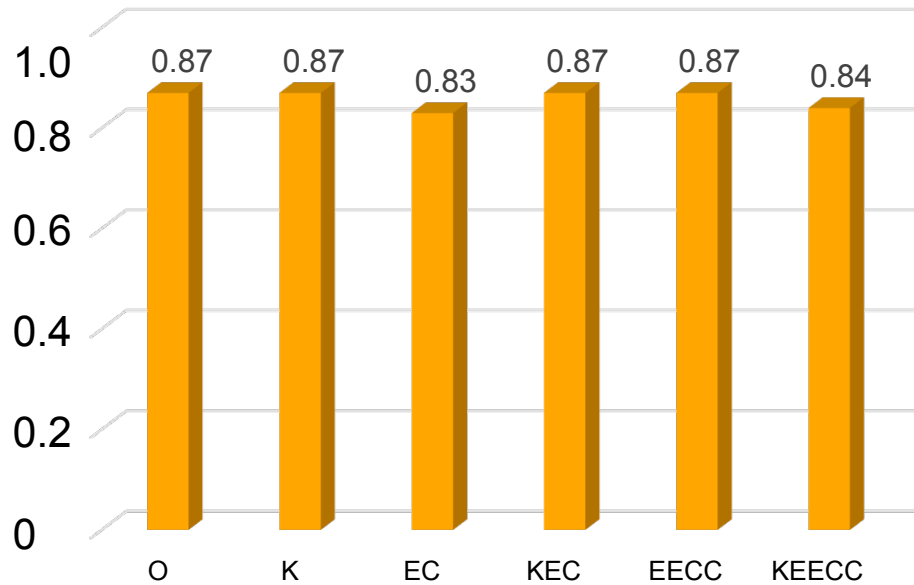


Survival

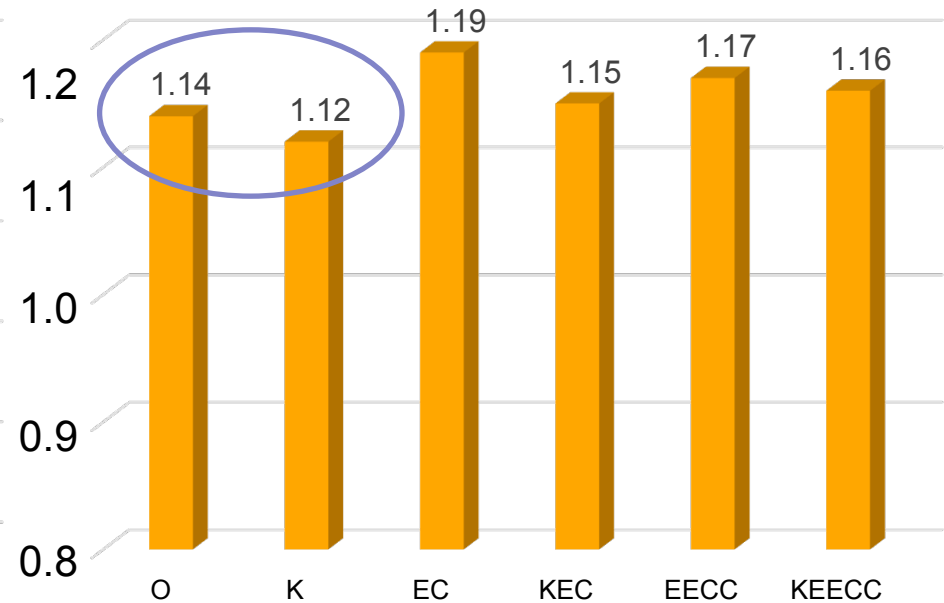




FCR

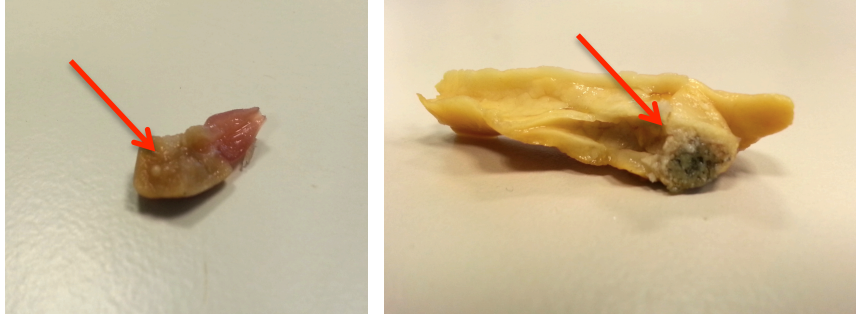


SGR



Growth was lower in meagre fed the lowest Vit E+Vit C levels

**Macroscopic lesions in heart and liver
only observed in 10 fish**



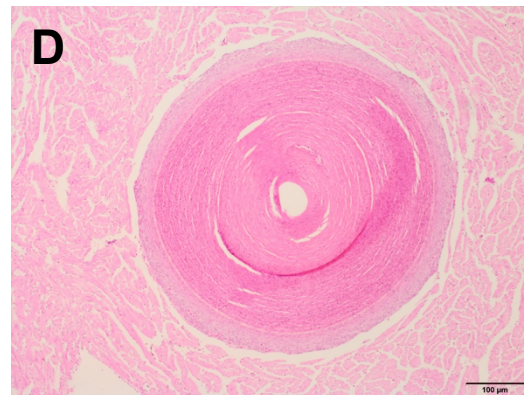
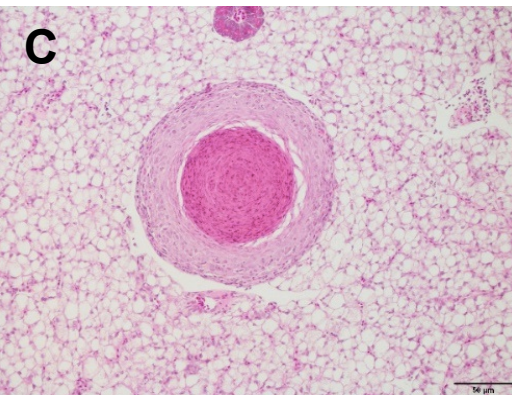
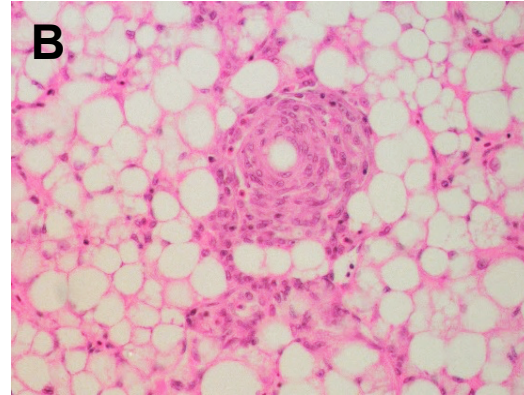
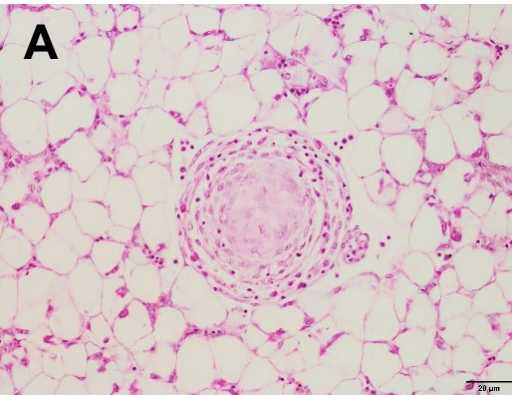
**Macroscopic and histological
description**

**(A & B) Concentric layers of
macrophages and
inflammatory cells around
vascular endothelial cells**

**(C) Necrotic center with
external fibroblast layer and
inflammatory cells**

**(D) Granuloma composed
completely of laminar material**

**No calcification was observed
in any of the granulomas
studied**

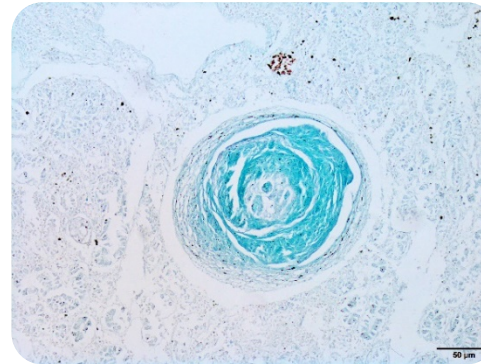
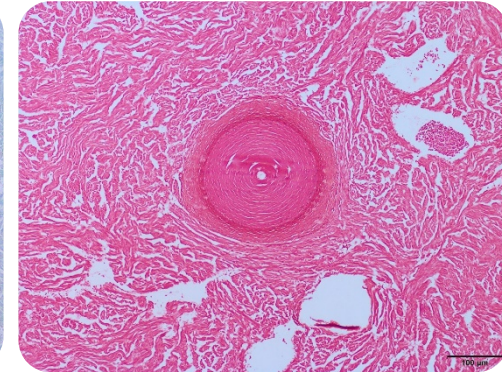
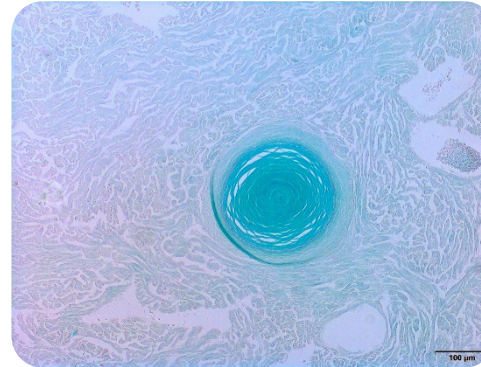


Ziehl-Neelsen / Fite-Faraco / Gram stain

- ✓ None of the specific stains revealed the presence of bacteria
- ✓ Nocardia culture was negative
- ✓ PCR negative to Nocardia performed in paraffin samples



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STIRLING

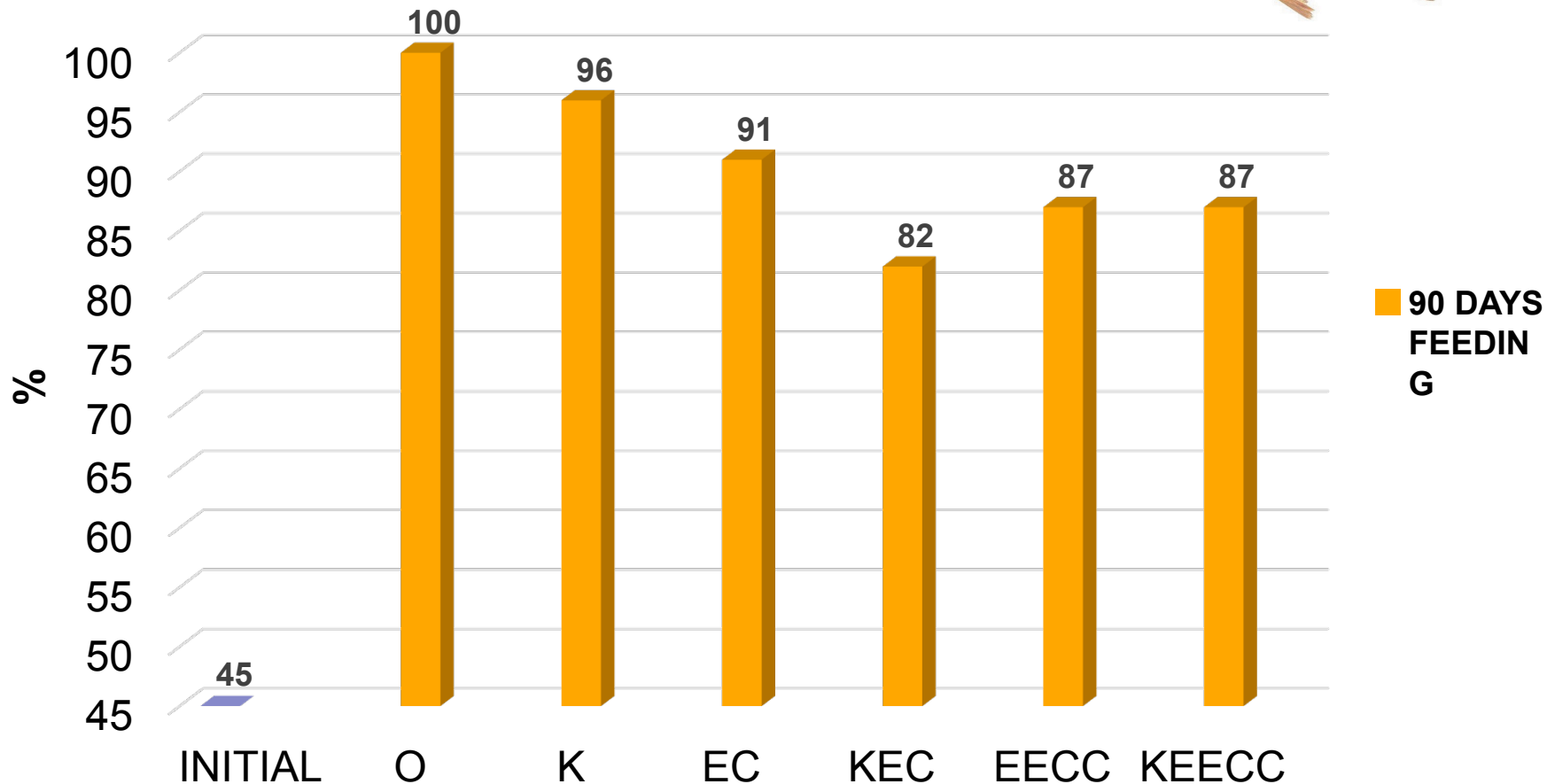


Negative results in Ziel-Neelsen, Fite-Faraco, Gram stain, bacterial cultures and PCR reinforce the non infectious character of the systemic granulomatosis in meagre (As was seen in larvae).

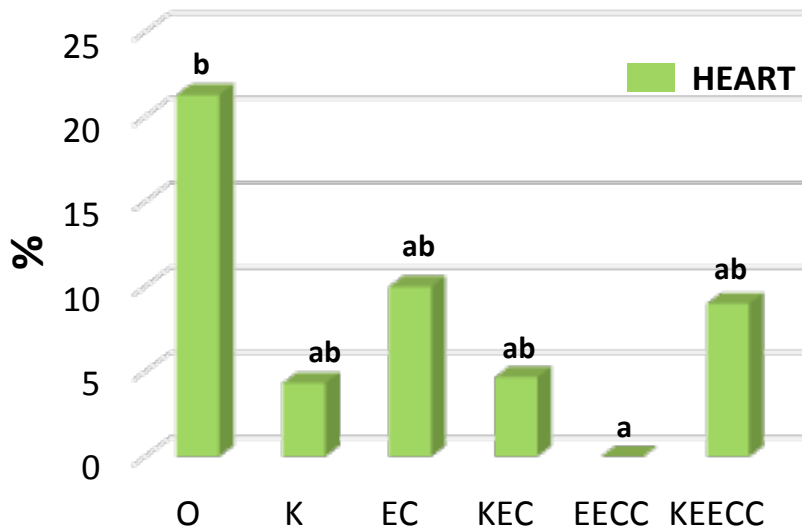
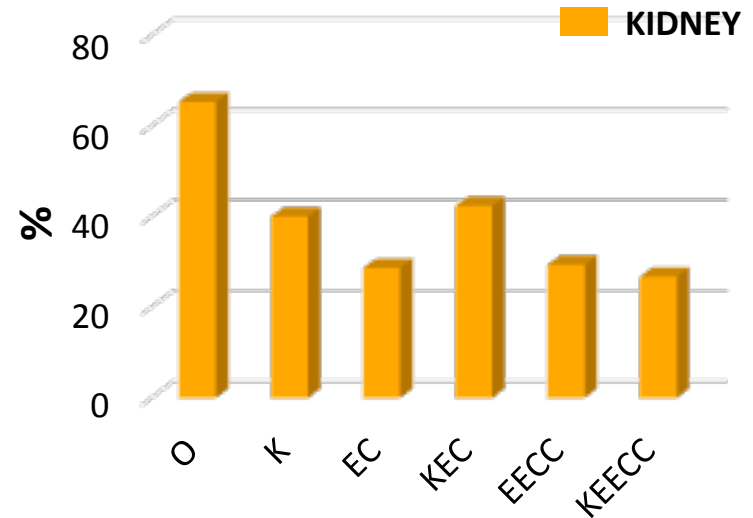
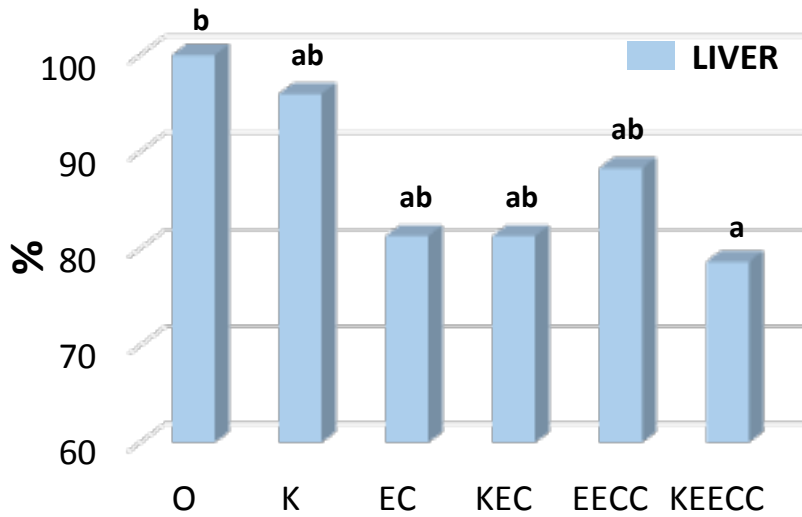
Effects of diets on granulomatosis



% FISH WITH GRANULOMAS



Effects of diets on granulomatosis incidence



% FISH WITH GRANULOMAS IN DIFFERENT TISSUES

Liver > kidney > heart
 Spleen was not affected

Score - granuloma severity

0

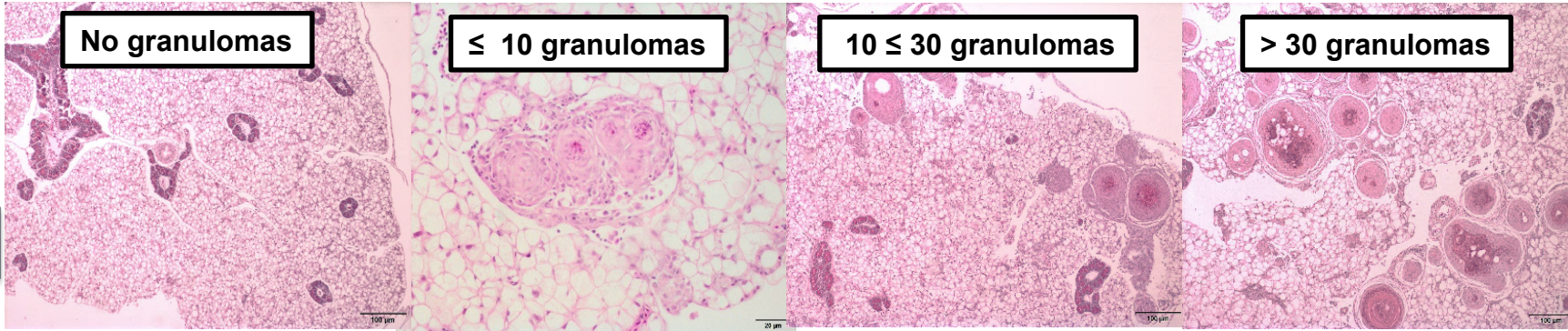
1

2

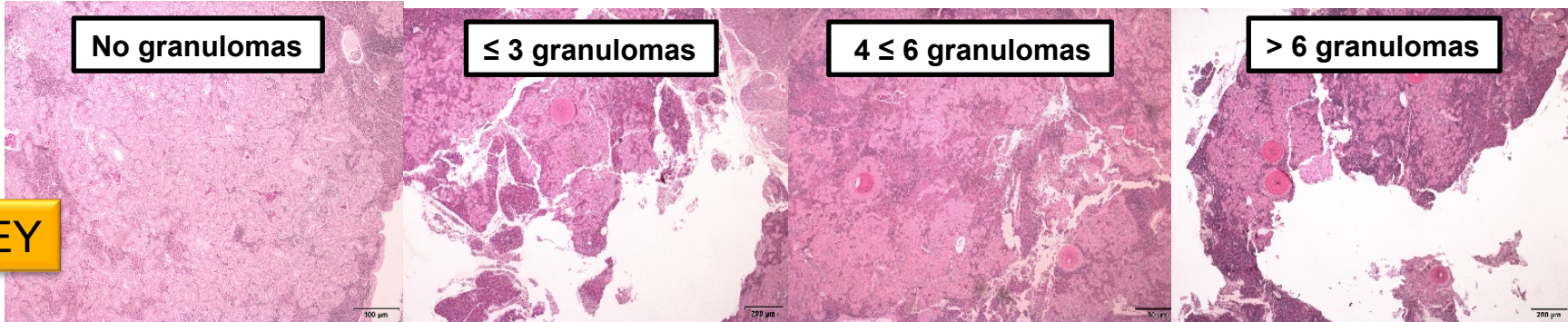
3



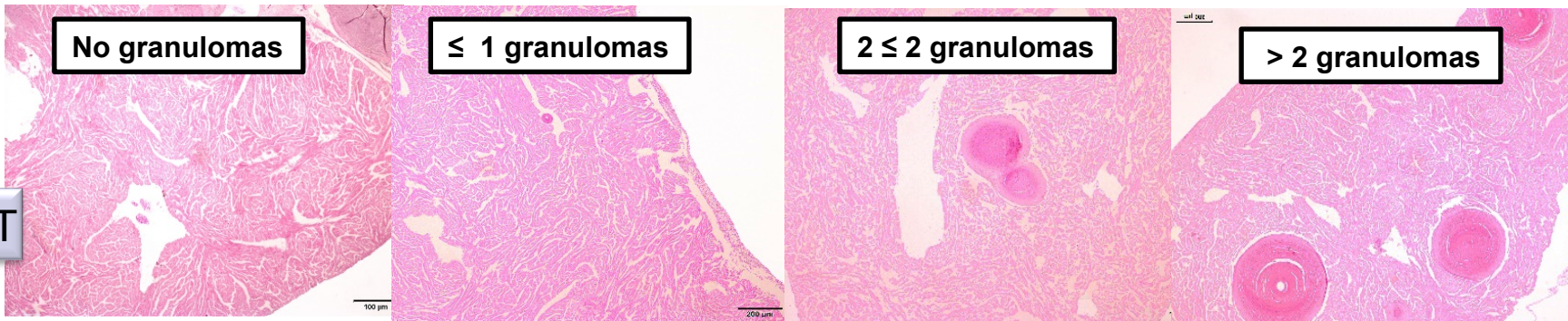
LIVER



KIDNEY



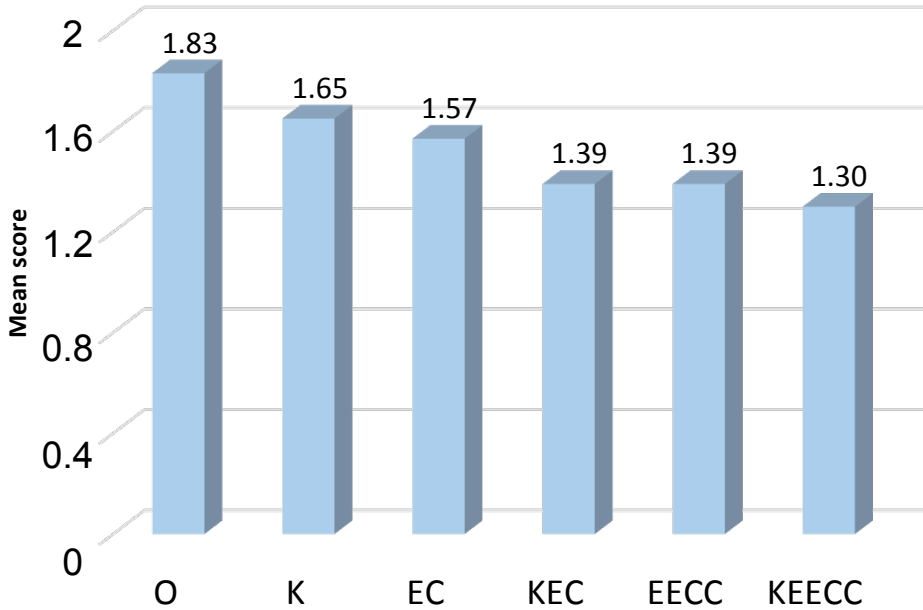
HEART



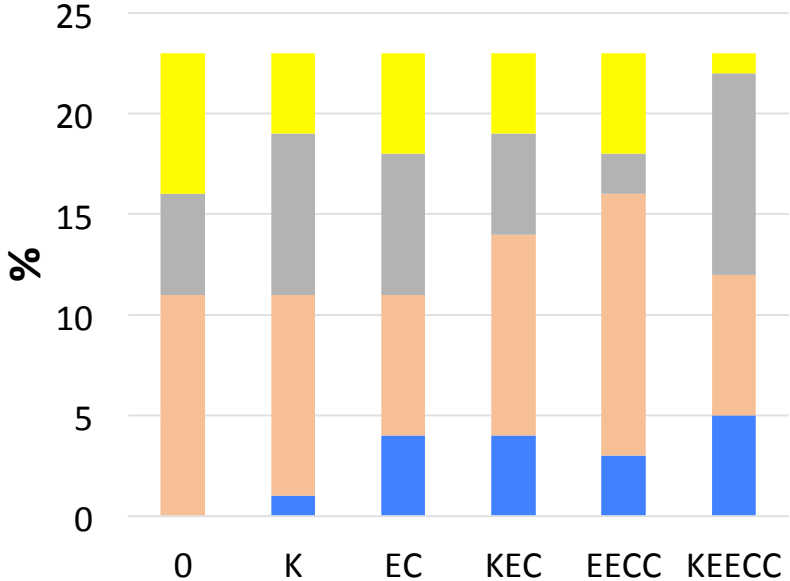
Effects of diets on granulomatosis severity



Granuloma severity in liver



% Fish with different severity of granuloma in liver



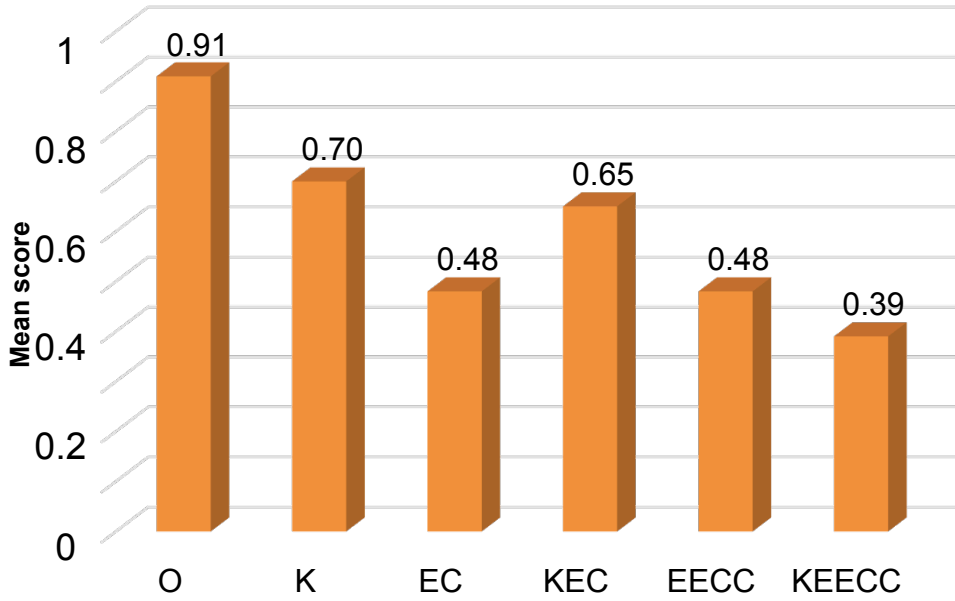
SCORING- SEVERITY



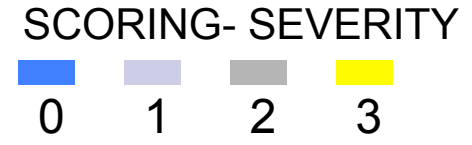
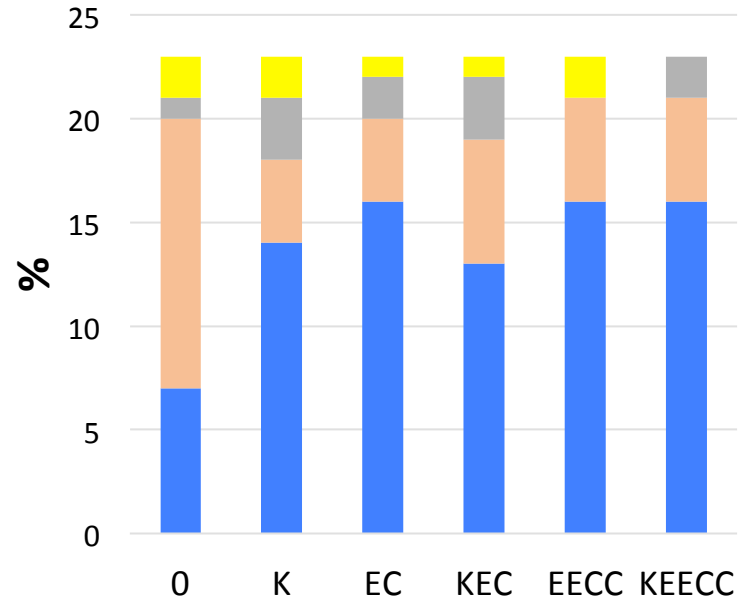
Effects of diets on granulomatosis severity



Granuloma severity in kidney



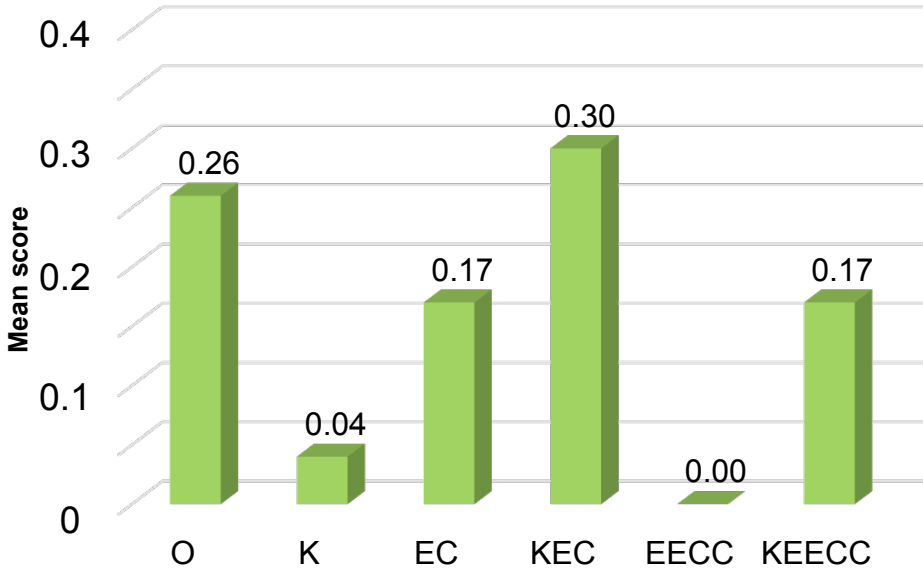
% Fish with different severity of granuloma in kidney



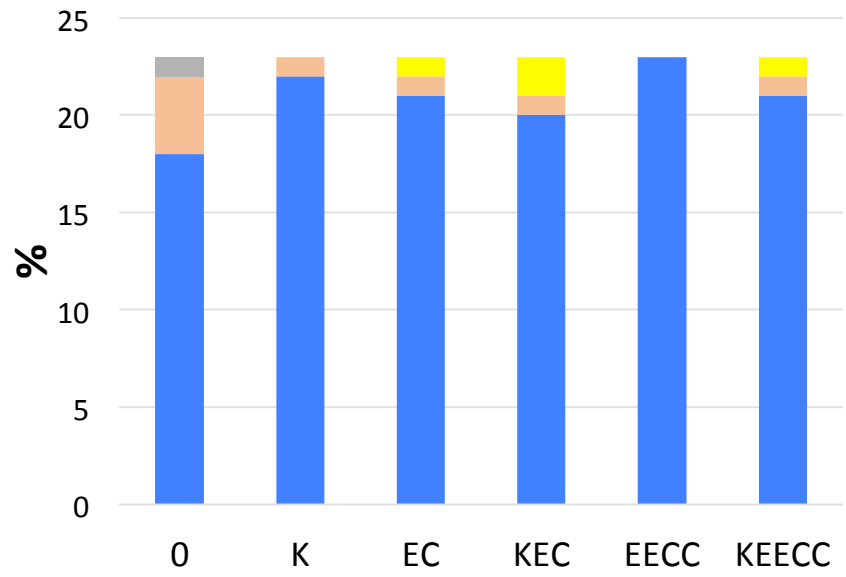
Effects of diets on granulomatosis



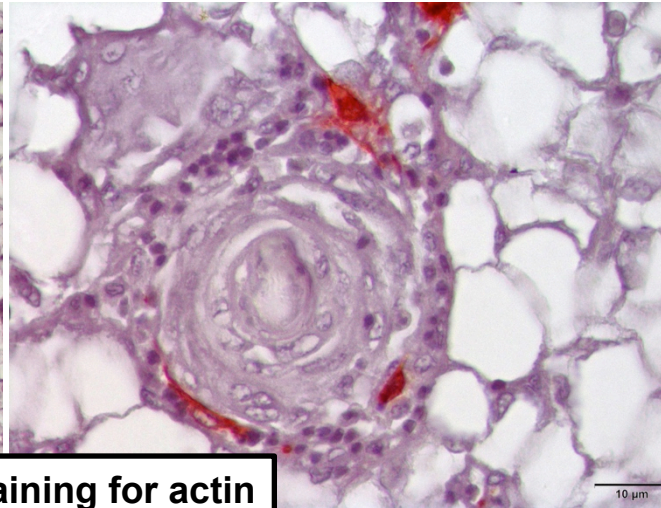
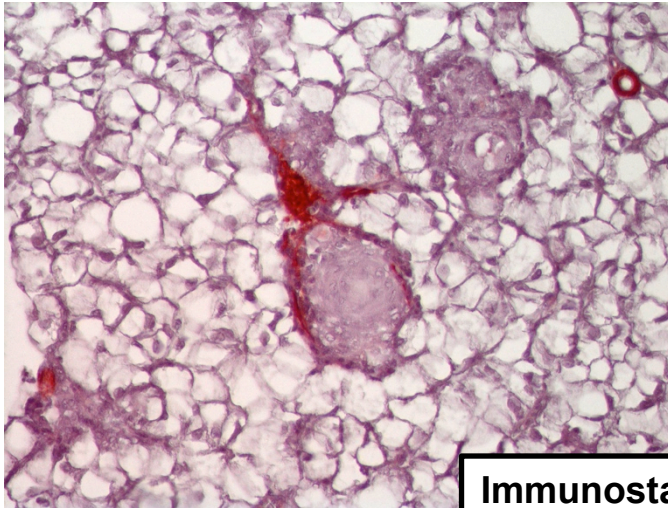
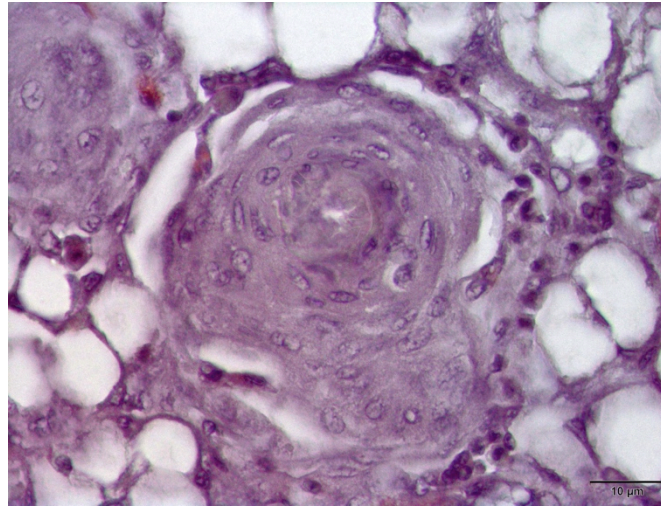
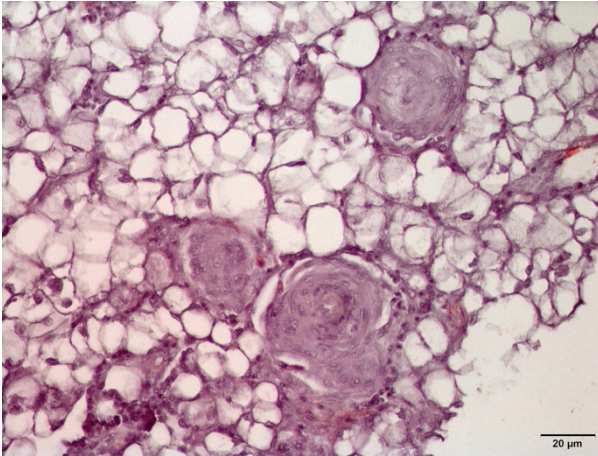
Granuloma severity in heart



% Fish with different severity of granuloma in heart



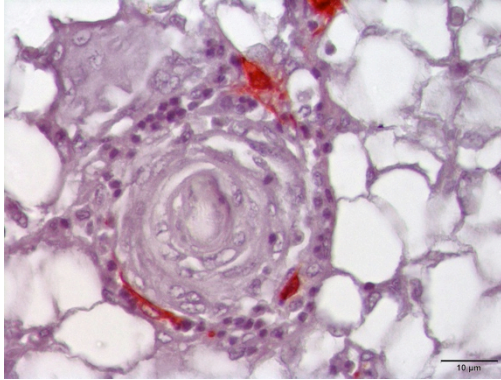
Granuloma formation



Immunostaining for actin

Granulomas were initiated always in the blood vessels, with inflammation of the endothelial cells, alteration of vascular permeability and causing vascular dysfunction, but severity was reduced by vit E & C supplementation. No calcification was observed.

Granuloma formation



Oxidative stress promotes vascular dysfunction, inducing altered vascular permeability and inflammation and the aberrant expression of inflammatory adhesion

Vitamin E and vitamin C counteract lipid peroxidative damage restoring vascular function and protecting endothelial tissue in blood vessels.

Vitamin K supplementation improves arterial stiffness in mammals and could contribute to reduce the severity of the lesions

- ✓ Dietary addition of vitamins E and C improved meagre growth performance.
- ✓ Increase in dietary vit E and C reduced granulomas incidence and severity, particularly with supplementation of vit K
- ✓ The granuloma formation was initiated in blood vessels where oxidative stress would affect endothelial tissue causing vascular dysfunction, altered vascular permeability and inflammation.



Thanks for your attention

The importance of dietary HUFA for meagre larvae (*Argyrosomus regius*; Asso, 1801) and its relation with antioxidant vitamins E and C

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¹Grupo de Investigación en Acuicultura (GIA), Universidad de Las Palmas de Gran Canaria, Las Palmas, Spain



ELSEVIER

Aquaculture

Volume 498, 1 January 2019, Pages 606-620



Dietary combination of vitamin E, C and K affects growth, antioxidant activity, and the incidence of systemic granulomatosis in meagre (*Argyrosomus regius*)

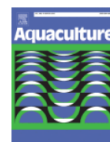
Ruiz, M.A. ^a ✉, Betancor, M.B. ^b, Robaina, L. ^a, Montero, D. ^a, Hernández-Cruz, C.M. ^a, Izquierdo, M.S. ^a, Rosenlund, G. ^c, Fontanillas, R. ^c, Caballero, M.J. ^a



ELSEVIER

Aquaculture

Volume 488, 10 March 2018, Pages 105-113



Dietary requirement for n-3 long-chain polyunsaturated fatty acids for fast growth of meagre (*Argyrosomus regius*, Asso 1801) fingerlings

Carvalho, M. ^{a, b, c} ✉, Peres, H. ^{b, c}, Saleh, R. ^{a, d}, Fontanillas, R. ^e, Rosenlund, G. ^e, Oliva-Teles, A. ^{b, c}, Izquierdo, M. ^a