



*Influence of dietary combinations of
vitamin E, C and K in the development
of systemic granulomatosis in meagre*



Barcelona, 17-19 January 2017





ISFNF
2016

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

Ruiz, M.A.¹, Izquierdo, M.S.¹, Robaina, L.¹, Hernández-Cruz, C.¹, Betancor, M.B.², Montero, D.¹, Fontanillas, R.³, Rosenlund, G.³ and Caballero M.J.¹

¹Aquaculture Research Group (GIA), IU-ECOQUA, Universidad de Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Spain

²Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, United Kingdom

³Skretting Aquaculture Research Centre, PO Box 48, 4001 Stavanger, Norway



Task 24.1. Systemic Granulomatosis

Subtask 24.1.1. Feeding trials

- Trial 4. Effects of vitamins



Meagre as an emerging species for aquaculture diversification



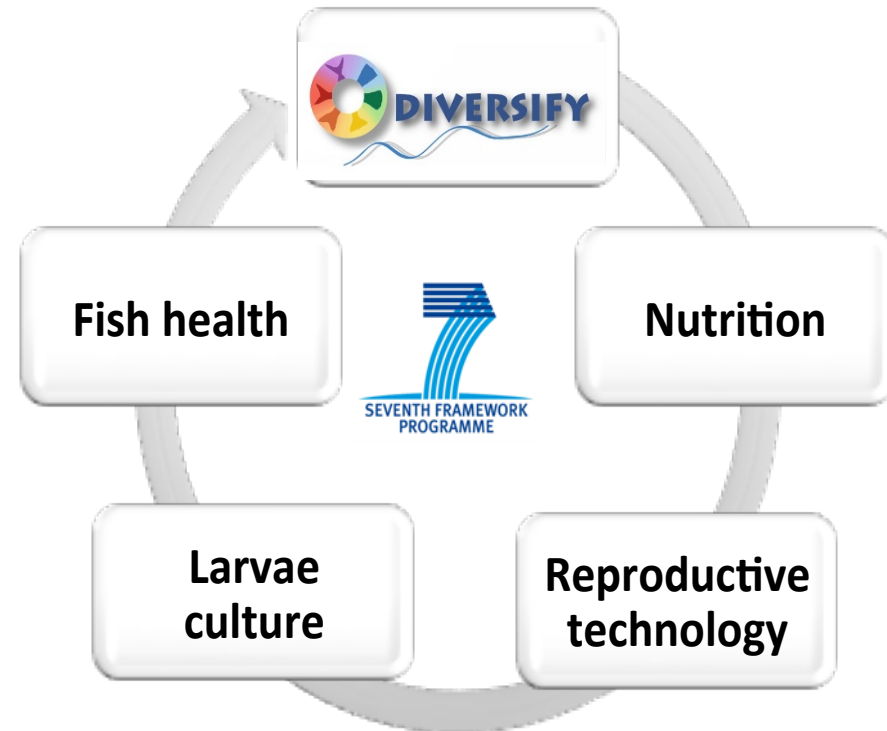
Fast growth

Good feed conversion

Easy adaptation to captivity

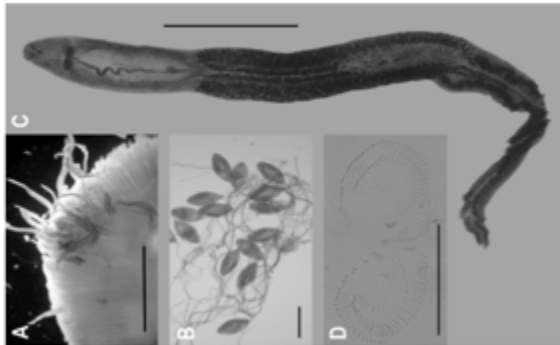
Eurihaline

Excellent consumer acceptance



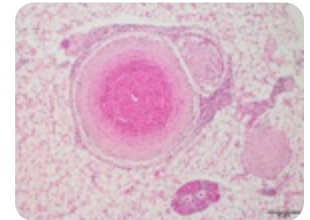
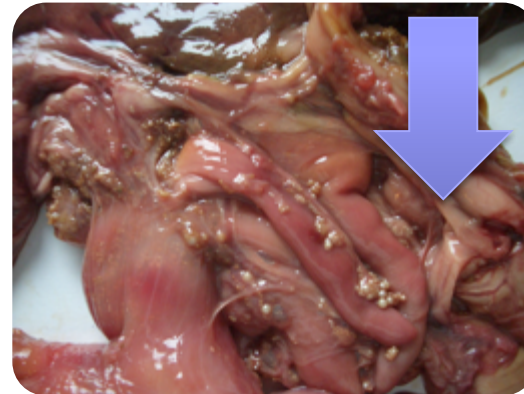
Main health problems found in meagre

Parasite: *Monogenea* / *Nematode*



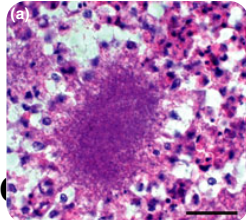
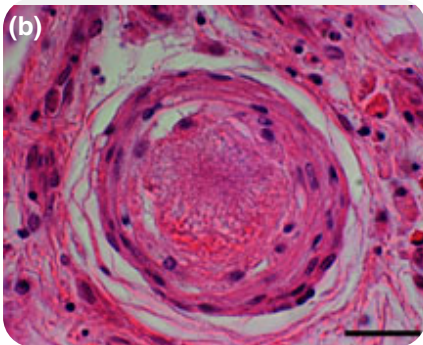
Moravec et al., 2007; Merella et al., 2009; Ternendo et al., 2010; Andree et al., 2016

Systemic granulomatosis



Grupo de Investigación en Acuicultura

Bacteria: *Nocardiosis*



Elkesh et al., 2013
Vega et al submitted

Grupo de Investigación en Acuicultura

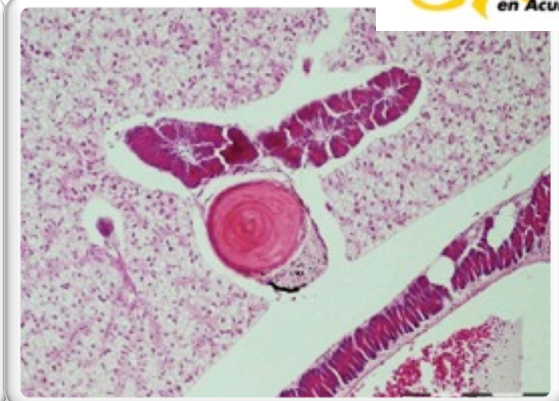
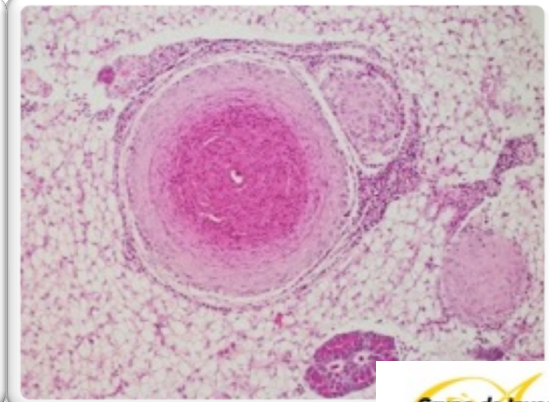
Mycobacterium

Timur et al., 2015

- Non infectious disease
- Wide impact on meagre population
- High prevalence but low mortalities
- Associated to poor growth
- Unacceptable for the consumer

Systemic granulomatosis

- Unknown and possibly diverse ethiology
- Could be related to nutritional unbalances
- For meagre only described in farmed fish



Multiple white to yellow lesions with hard consistency, progressively developing into necrotic focus that may be calcified with concentric layers of cells (endotelial, inflammatory and macrophages)

Systemic granulomatosis in other fish species

Aquaculture Research

Aquaculture Research, 2015, 1–5

doi:10.1111/are.12790

SHORT COMMUNICATION

**Systemic granuloma observed in Atlantic salmon
Salmo salar raised to market size in a freshwater
recirculation aquaculture system**

Christopher Good¹, David P Marancik^{2*}, Timothy J Welch², Travis May¹, John Davidson¹ & Steven Summerfelt¹

¹The Conservation Fund's Freshwater Institute, Shepherdstown, WV, USA

²National Center for Cool and Cold Water Aquaculture, USDA-ARS, Kearneysville, WV, USA



Granulomas formed by mineral deposits of unknown origin



**Visceral Granuloma in Brook Trout
(*Salvelinus fontinalis*)¹**

CLARENCE E. DUNBAR AND ROGER LEE HERMAN^{*}
Bureau of Sport Fisheries and Wildlife, Eastern Fish Disease Laboratory,
Kearneysville, West Virginia 25430

Journal of Fish Diseases 1980, 3, 213–221

**Pathology and histopathology of a systemic granuloma
in *Sparus aurata* (L.) cultured in the Gulf of Aqaba**

I. PAPERNA H. Steinitz Marine Biology Laboratory of the Hebrew University of Jerusalem, Eilat, Israel

J. G. HARRISON Unit of Aquatic Pathobiology, Institute of Aquaculture, University of Stirling, Scotland

G. W. KISSIL Mariculture Laboratory, Oceanographic and Limnological Research, Eilat, Israel



Fish with granulomatosis showed an excess of tyrosine



Granulomatous syndrome and tyrosine metabolism defect in turbot (*Scophthalmus maximus*) [1984]

Tixeront, G. (Ministère de l'Agriculture, Brest (France), Services Veterinaires, Laboratoire National de Pathologie des Animaux)

Aldrin, J.F.

Baudin Laurencin, F.

Messenger, J.L.

Granulomatosis hypertyrosinemia in turbot fed diets deficient in vitamin C

Systemic granulomatosis in Canary Islands meagre



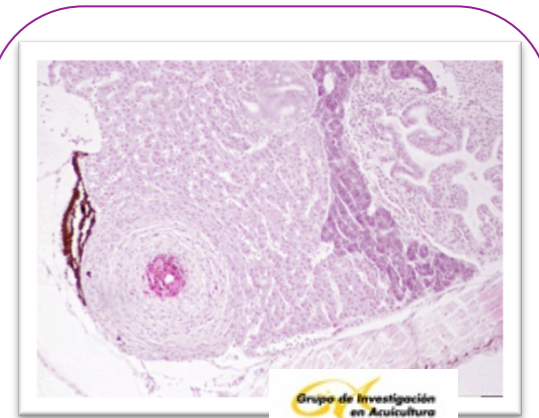
Fish Farm – sea cage

↓ 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*)

Experimental design

Initial weight 79.6 ± 0.3 g
Initial density (tank) 7.9 kg/m³ (50 fish/tank)
Tank volume 0.5 m³
Water temperature 17.6 to 21.6 °C
Feeding period 90 days



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



Growth performance

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

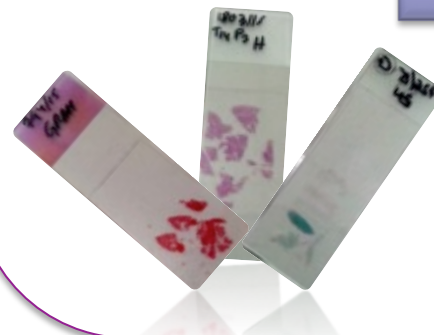


Histology

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

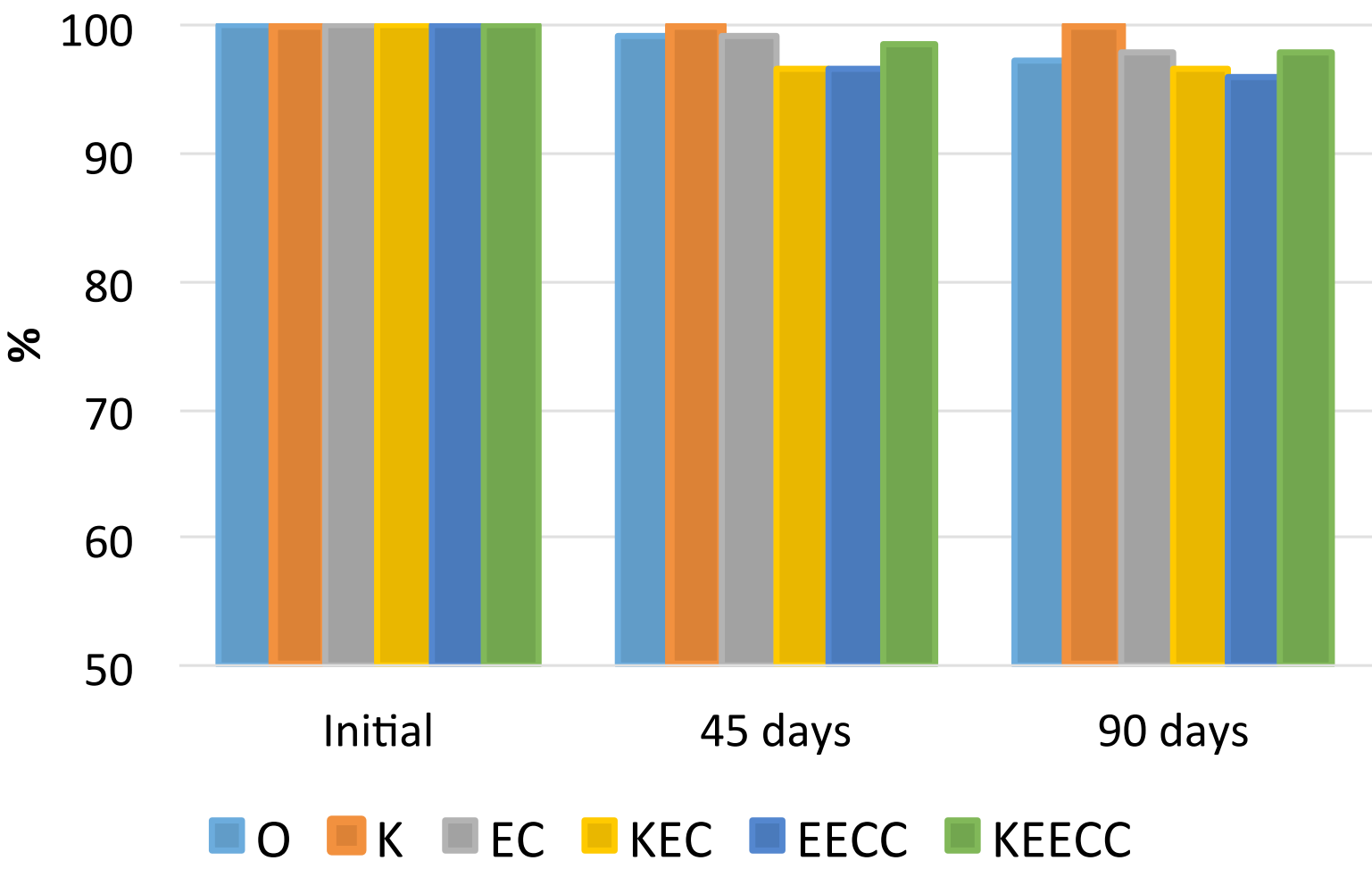
Culture medium: YEME

Gram Stain

Results

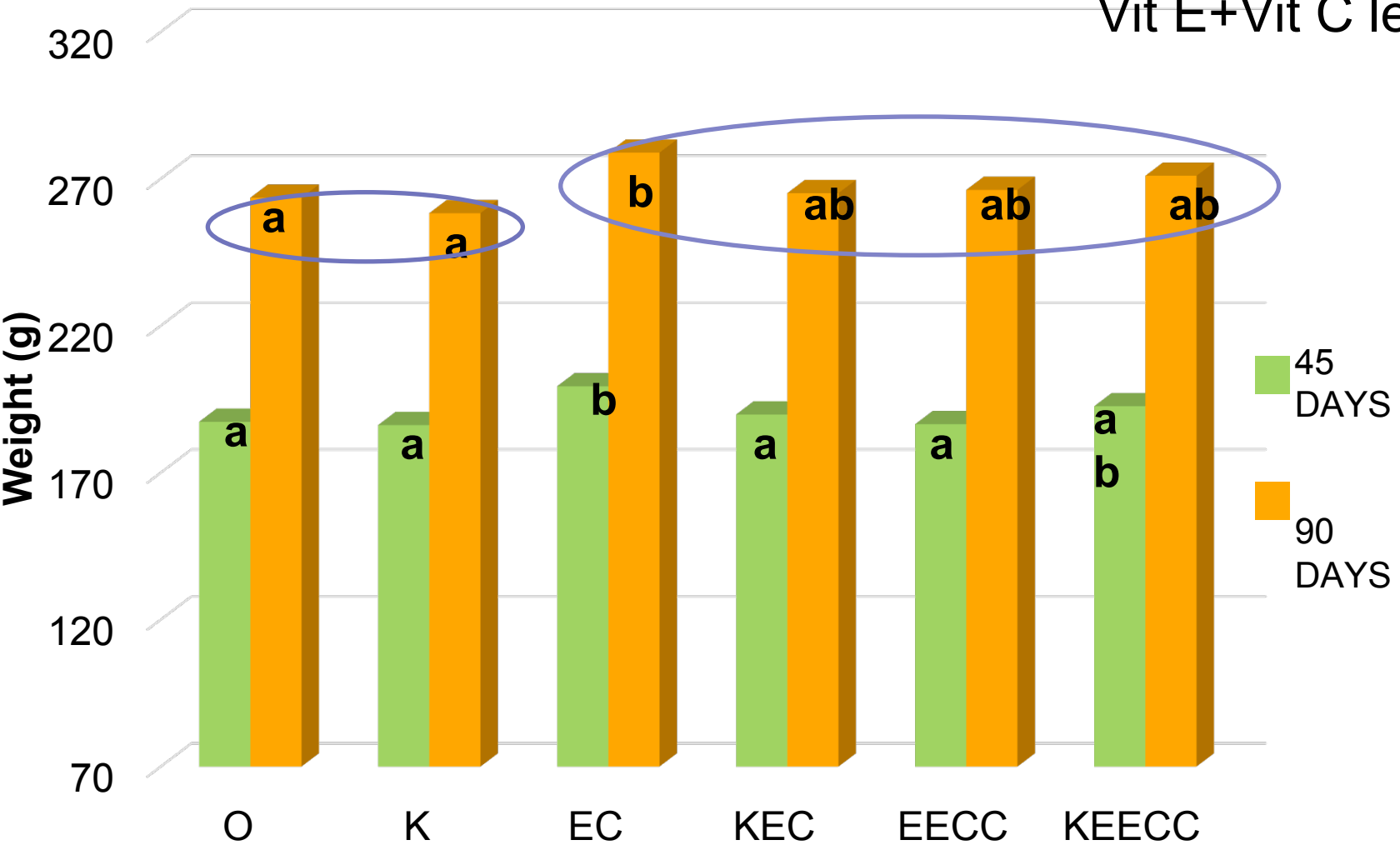
Survival was high (96-100 %) and no significantly affected by dietary vitamin levels

Survival

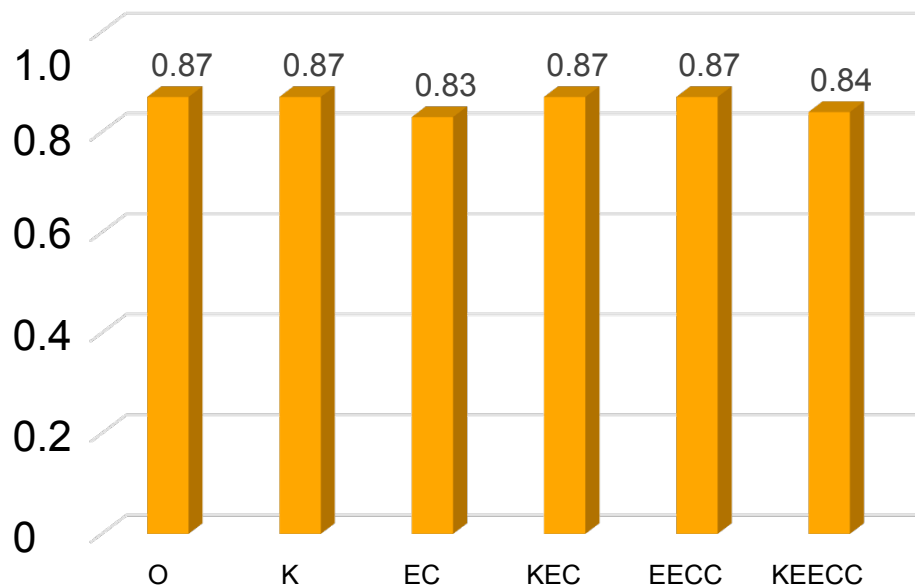


Growth was lower in meagre fed the lowest Vit E+Vit C levels

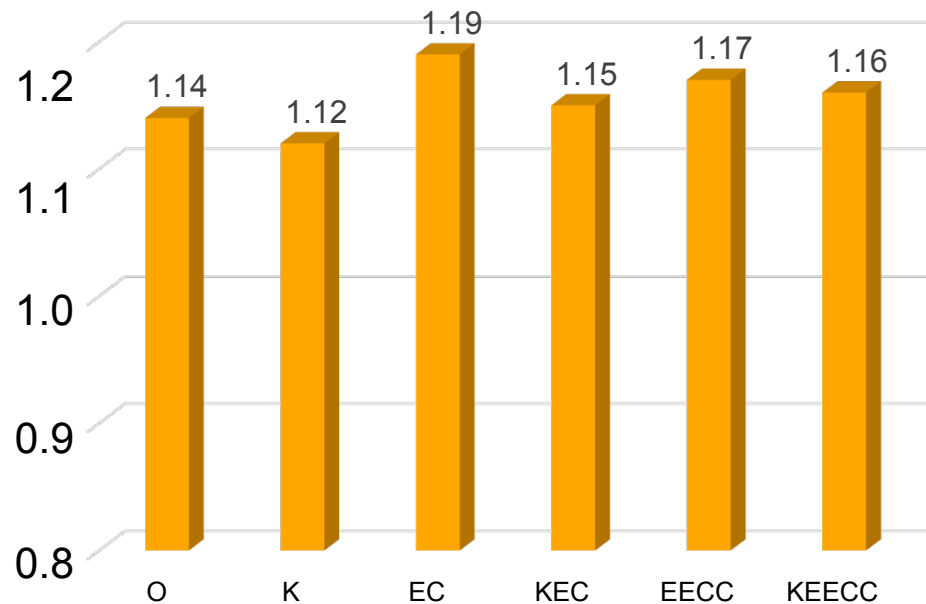
Final weight



FCR



SGR



FCR < 1
SGR 1.1-1.6



FCR ± 1.2-1.5
SGR 0.6-1.4



FCR ± 1.4-1.5
SGR 1.1-1.5



FCR ± 1
SGR 0.8-1.3

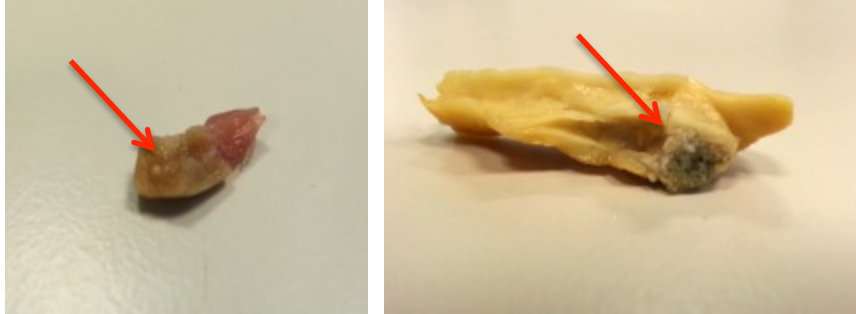
(Couto et al., 2016, Velazco, 2015, Chatzifotis et al., 2011)

(Bonaldo et al., 2009, Ortega, 2008, Gómez et al., 2003)

(Chebbaki et al., 2010, Hossu, et al., 2005, Robles, et al., 2006)

(Norman, 2014, Moreno, 2012, Handeland, et al., 2008)

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



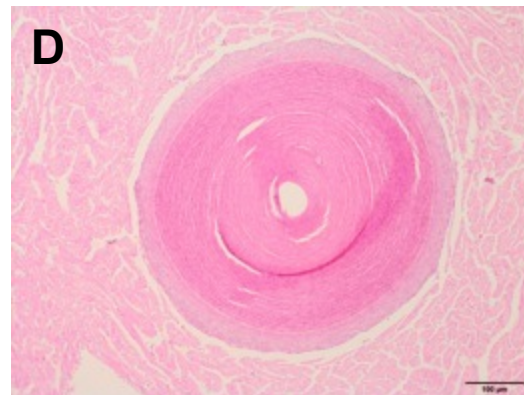
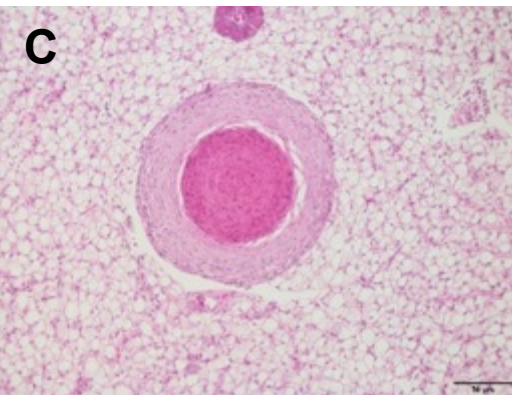
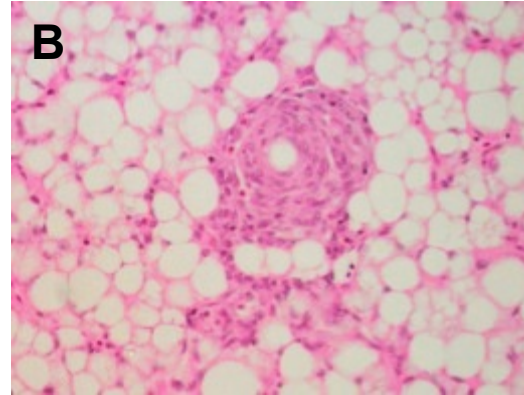
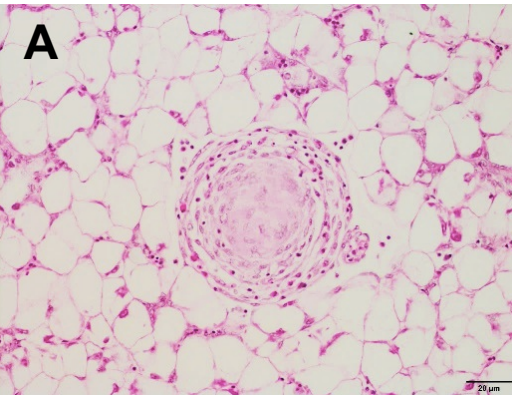
**Macroscopic and histological
description**

**(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**

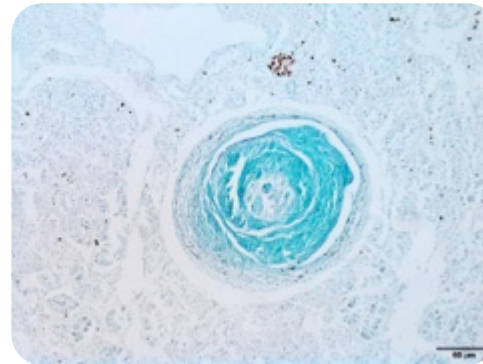
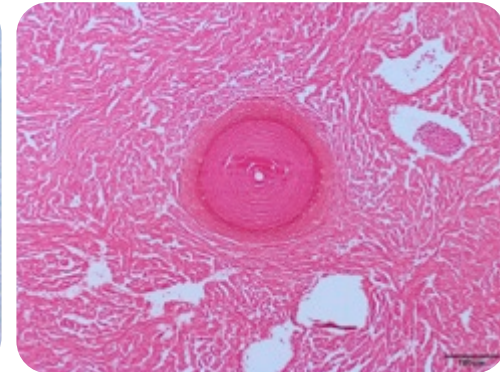
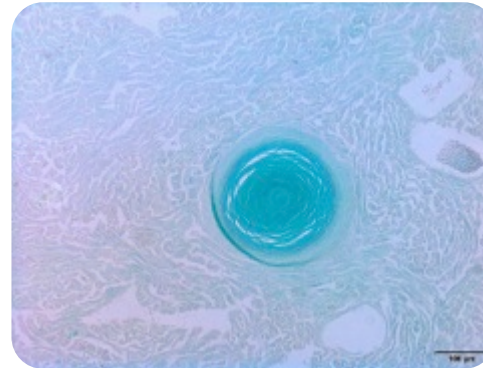


Ziehl-Neelsen / Fite-Faraco / Gram stain

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



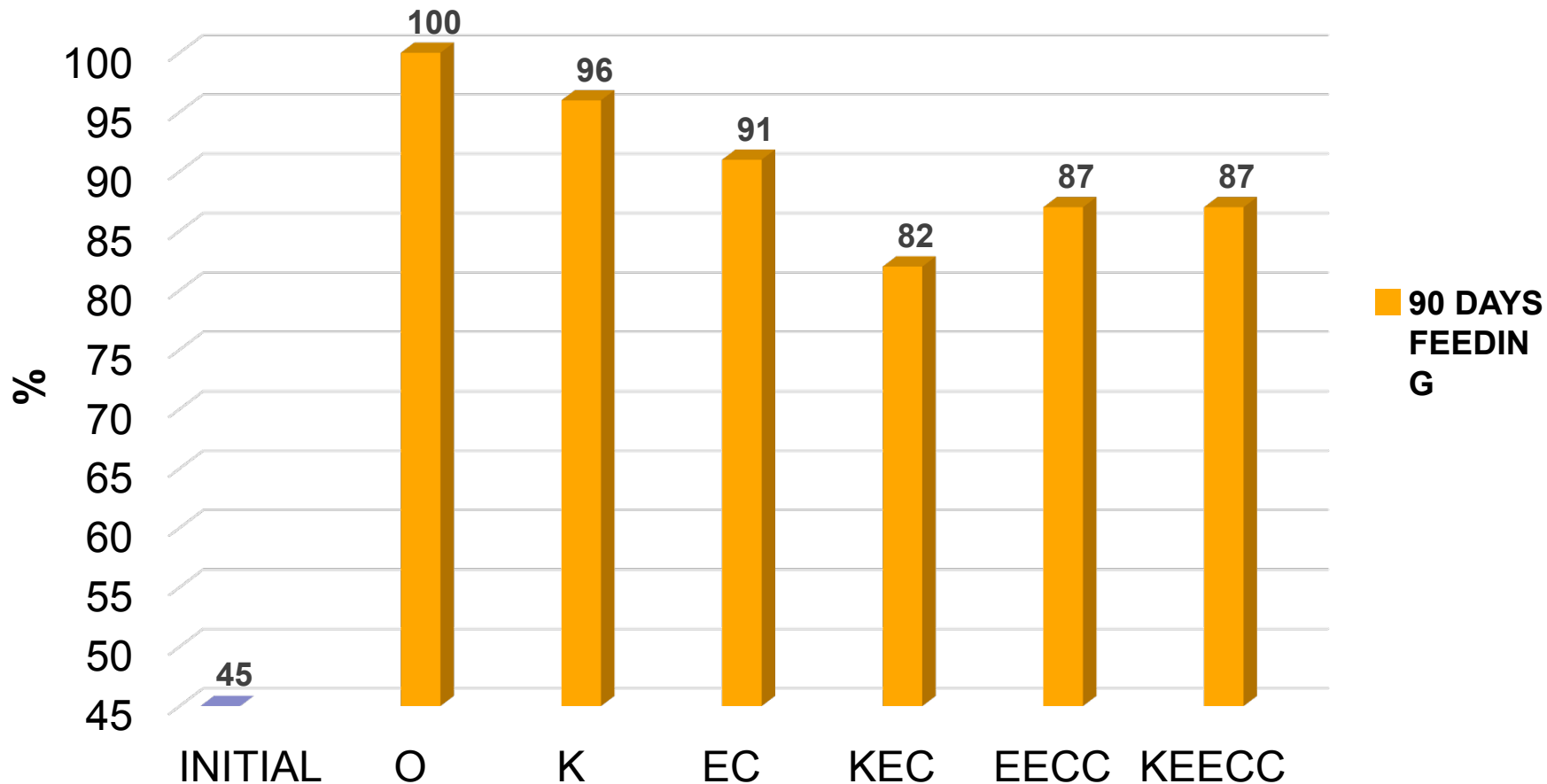
UNIVERSITY OF
STIRLING



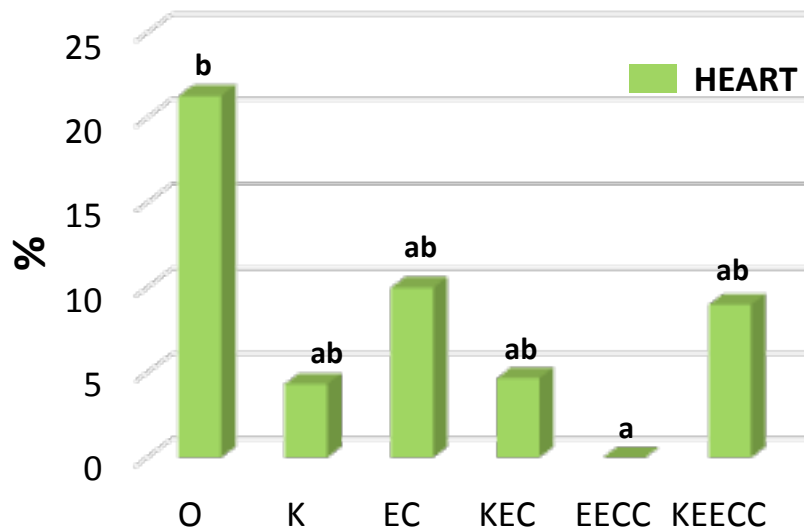
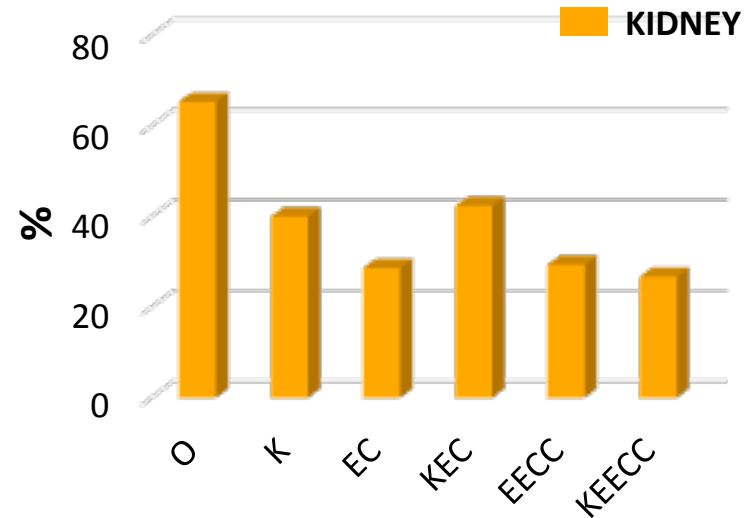
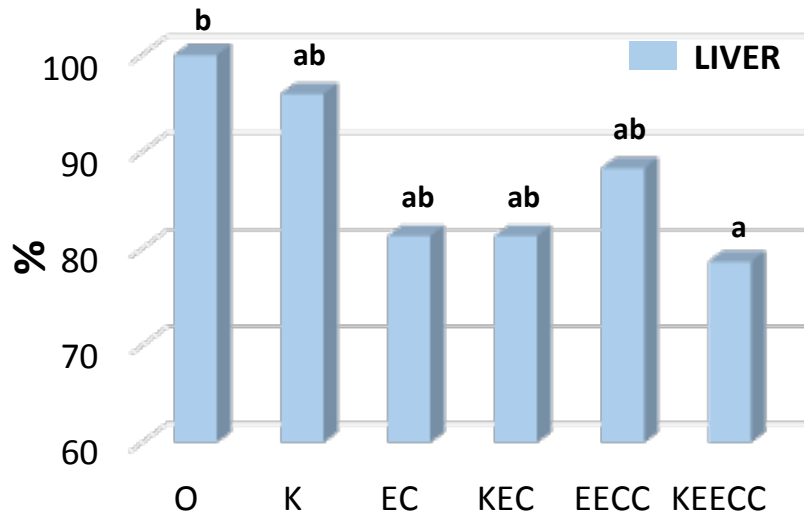
Negative results in Ziel-Neelsen, Fite-Faraco, Gram stain, bacterial cultures and PCR reinforce the non infectious character of the systemic granulomatosis in meagre (Deliverable 8.1).

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



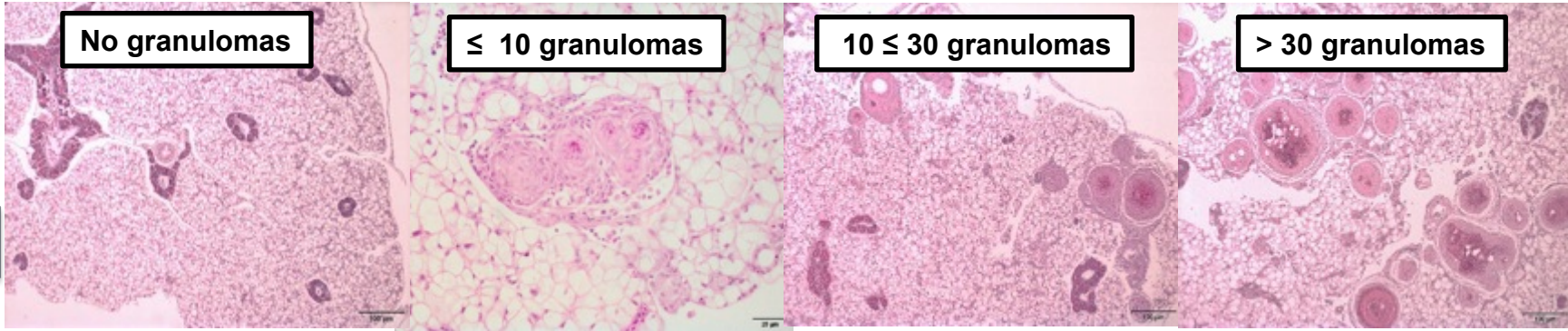
LIVER

No granulomas

≤ 10 granulomas

$10 \leq 30$ granulomas

> 30 granulomas



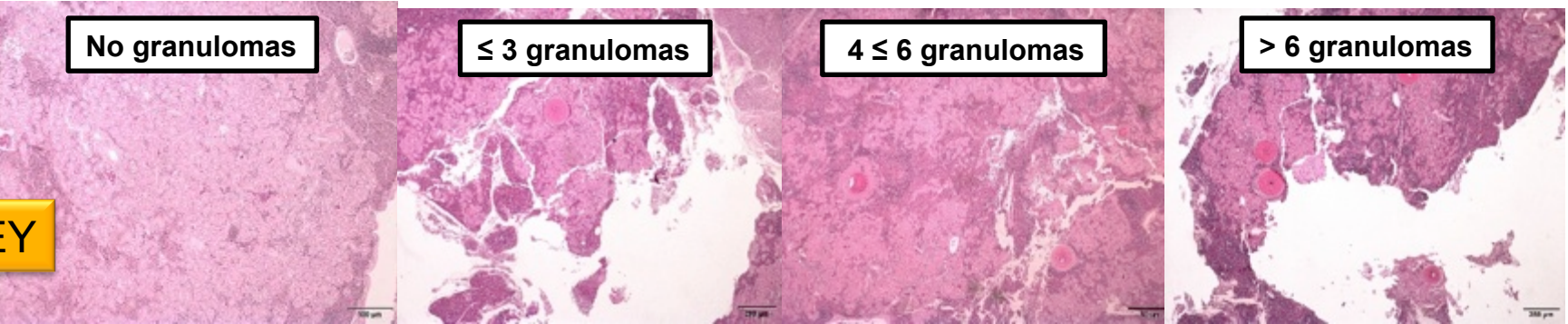
KIDNEY

No granulomas

≤ 3 granulomas

$4 \leq 6$ granulomas

> 6 granulomas



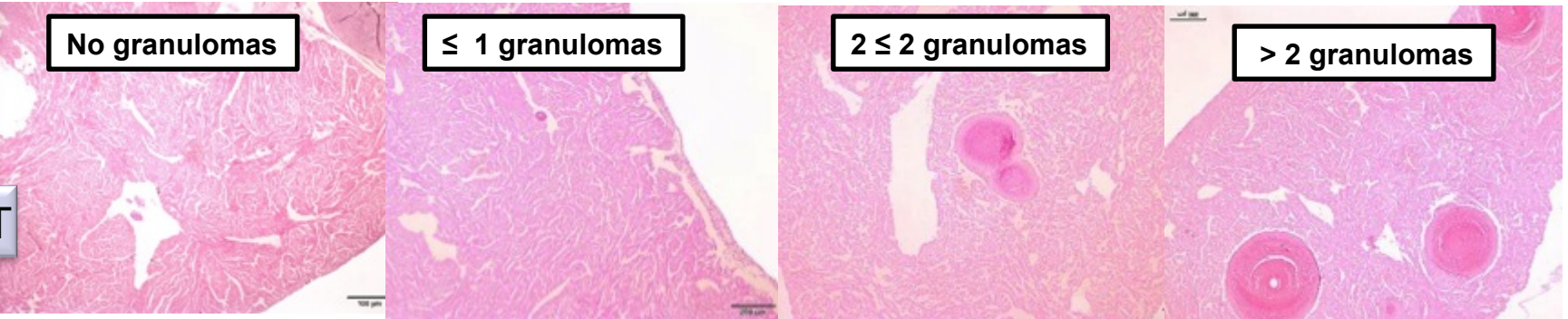
HEART

No granulomas

≤ 1 granulomas

$2 \leq 2$ granulomas

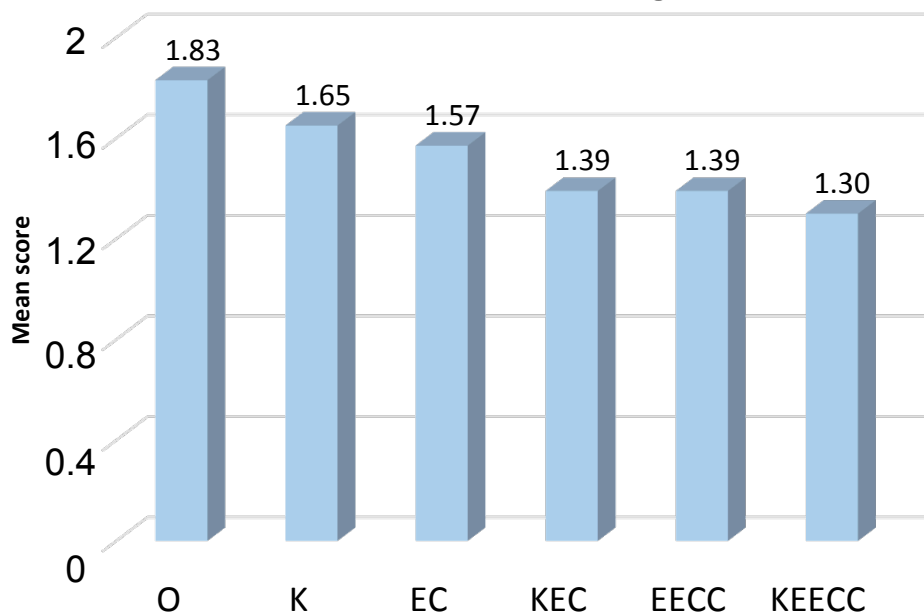
> 2 granulomas



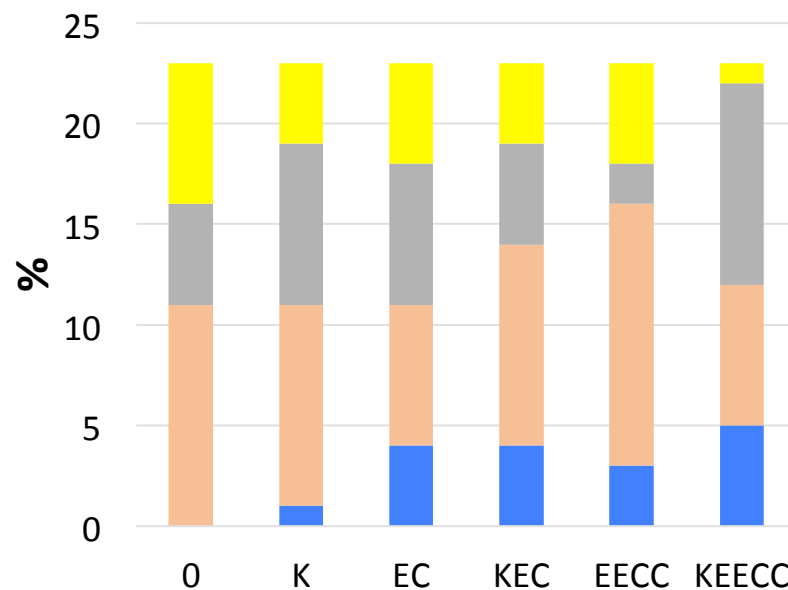
Effects of diets on granulomatosis severity



Granuloma severity in liver



% Fish with different severity of granuloma in liver



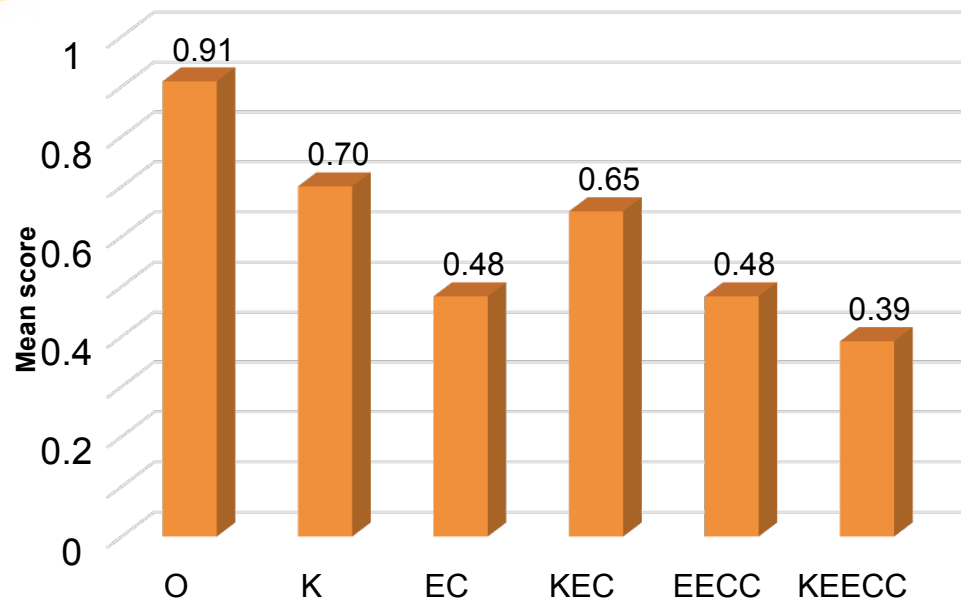
SCORING- SEVERITY



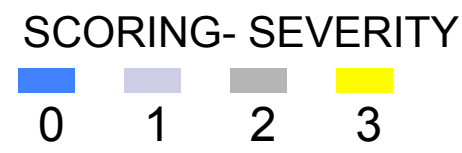
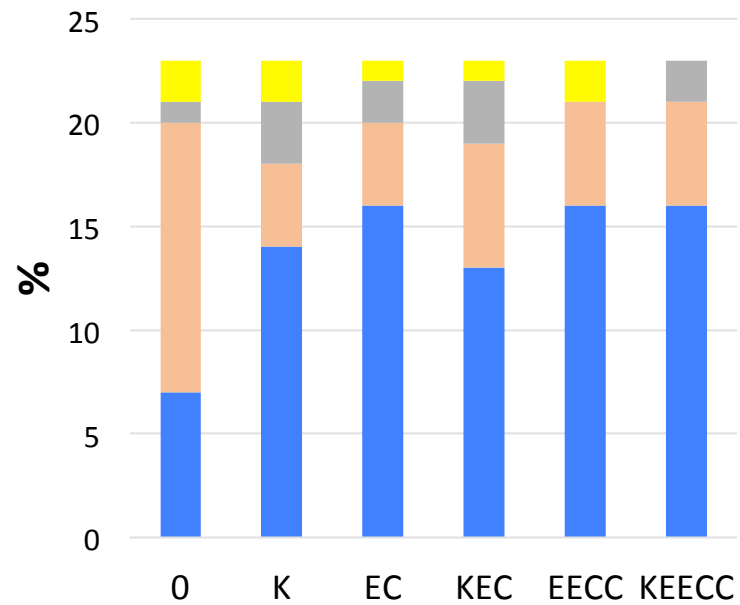
Effects of diets on granulomatosis severity



Granuloma severity in kidney



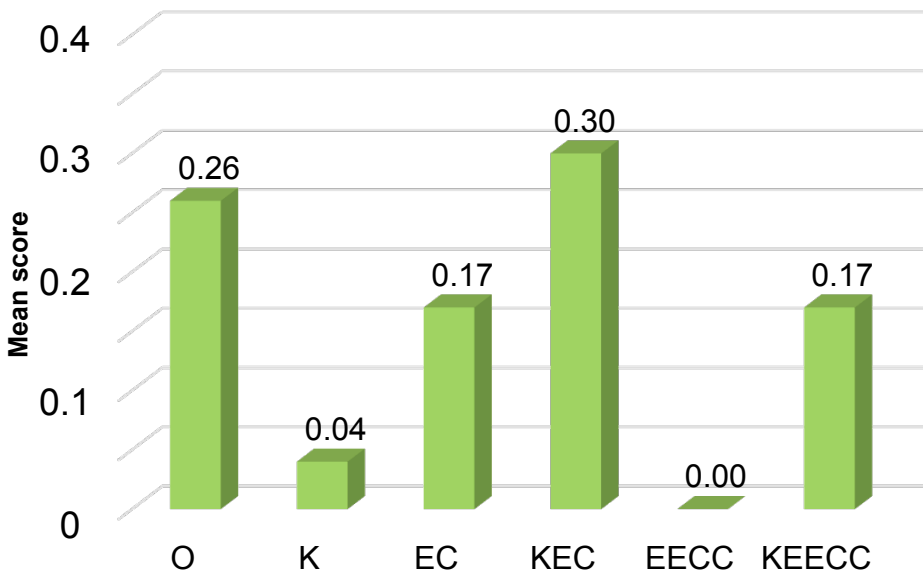
% Fish with different severity of granuloma in kidney



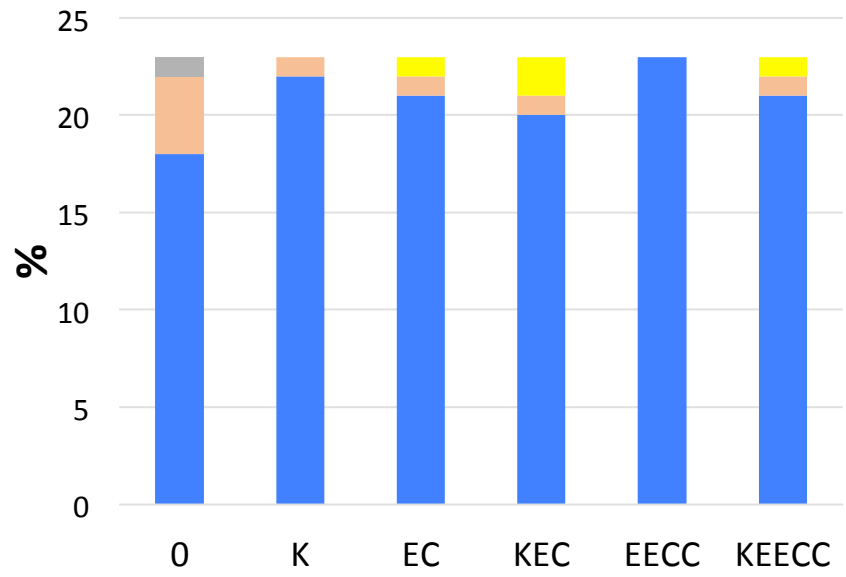
Effects of diets on granulomatosis



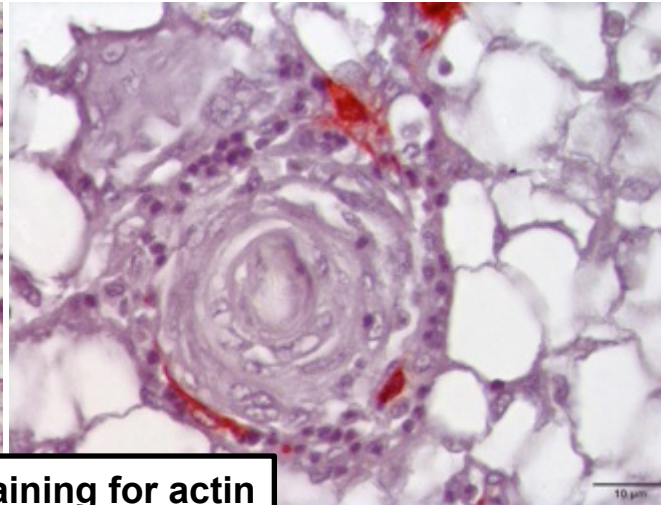
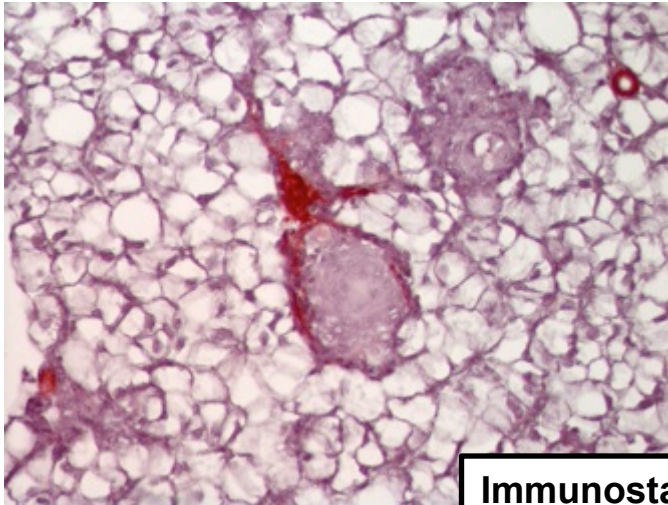
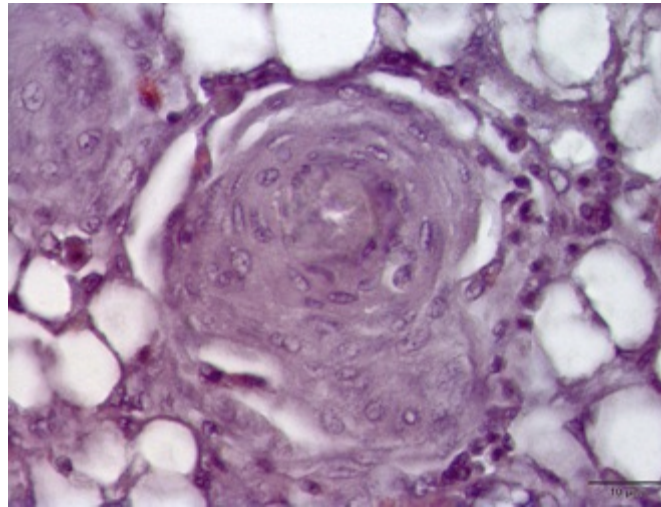
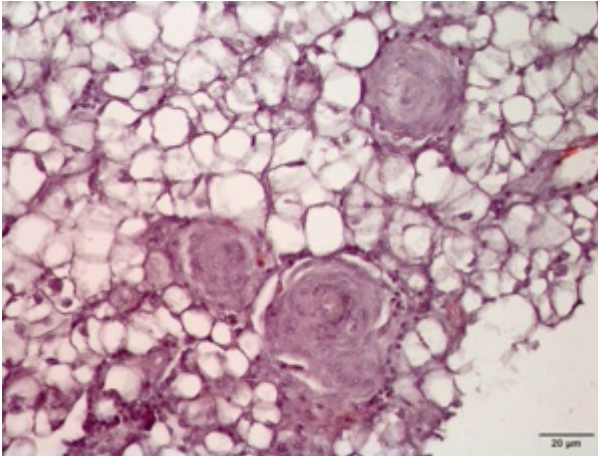
Granuloma severity in heart



% Fish with different severity of granuloma in heart



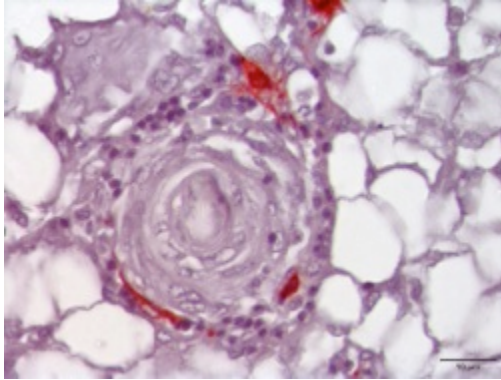
Granuloma formation



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

Summary

- Dietary addition of vitamins E and C improved meagre growth performance.
- Microscopic granuloma incidence did not affected feed efficiency or fish mortality.
- Granulomas were present in almost half of the initial meagre population and the incidence increased along the trial
- 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.
- No calcification was observed denoting that the origin of this granulomatosis is not directly related to mineral unbalances

THANK YOU FOR YOUR ATTENTION

