DIETARY USE OF PREBIOTICS IN GREATER AMBERJACK JUVENILES: EFFECTS ON GROWTH PERFORMANCE, IMMUNE GENE EXPRESSION AND DISEASE RESISTANCE AGAINST *Neobenedenia girellae*

Grupo de Investigación en Acuicultura

(GIA; www.giaqua.org)

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Research and Technology to enhance excellence in Aquaculture development under an Ecosystem approach

INTRODUCTION

Importance of the genus *Seriola* for aquaculture (150.000 Tn; FAO 2010)

25% of the total aquaculture production in Japan, (Ohara *et al.*, 2005)

Purposed as new aquaculture species for Europe

INTRODUCTION

Biosanitary problems

Main bottleneck for *S.dumerili* production

Ectoparasites
(Neobenedenia girellae)

Secondary bacterial infections

Introduction

Prebiotics: Mannan oligosaccharides

Dietary MOS (mannan oligosaccharides) has been demonstrated to promote fish performance and disease resistance by stimulating the systemic and GALT immune system. Torrecillas *et al.*, 2013

REVIEWS IN Aquaculture

Reviews in Aquaculture (2017) 0, 1–33 doi: 10.1111/raq.12201

Prebiotics as functional ingredients: focus on Mediterranean fish aquaculture

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ELSEVIER

Improved health and growth of fish fed mannan oligosaccharides: Potential mode of action

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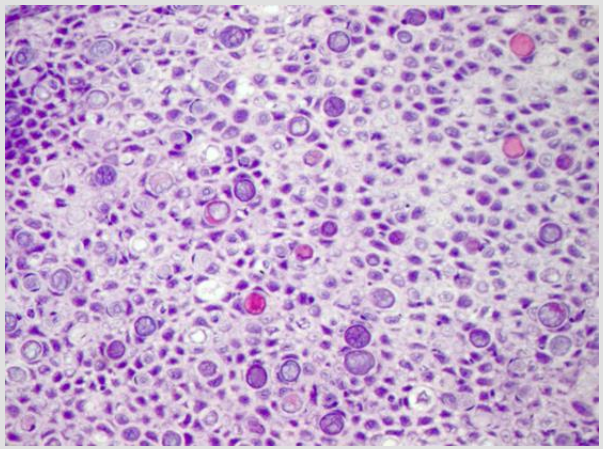
Introduction

MOS

- Not clear effects observed in skin immunology parameters in seabass (Torrecillas *et al*., 2011), but increase skin mucus production in rainbow trout (Rodriguez-Estrada *et al*., 2013)



Objective

Determine the effect of two commercial MOS on greater amberjack (*Seriola dumerili*) immune system and the impact on resistance to an experimental infection against the ectoparasite *Neobenedenia girellae*

Increase immune potential of greater amberjack (work package 25) for the start up of greater amberjack culture for the European aquaculture market in the 7th framework of the European project DIVERSIFY

Material & Methods

Experimental design

324 juveniles (331 ± 30g)

Randomly distributed into 18 1000l cylindroconical tanks

SKRETTING

Alltech®

4 diets: C (control-non-supplemented), (MOS), (cMOS) & (MOS + cMOS)

- Growth
- feed efficiency

Final sampling
- Gene expresión
- Skin mucus
- Serum

Parasite challenge

Parasite challenge final sampling
- Parasitation level
- Parasite samples of each tank

Start

30 days 60 days

90 days

100 days 115 days

Material & Methods

Final Sampling

- Extraction of gene expression samples
  - Posterior gut
  - Gill
  - Skin
  - Head kidney
  - Spleen

  } RNA later

- Skin mucus and serum extraction

- Bactericidal activity (Against *Photobacterium damselae)*
- Lysozyme activity

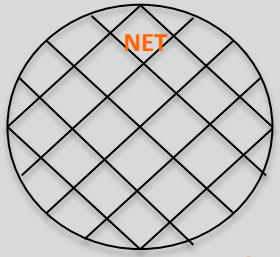
  (Guardiola et al. 2014; Quade and Roth 1997)

Experimentally egss culture

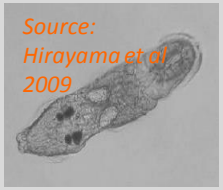
ADULT

Experimentally infestated fish

EGGS



ONCOMIRACIDIA



Controlled HOST ATTACHMENT- 10 days

Cohabitation in cages with experimental fish – 15 days

Parasitation level evaluation

Parasite measurements

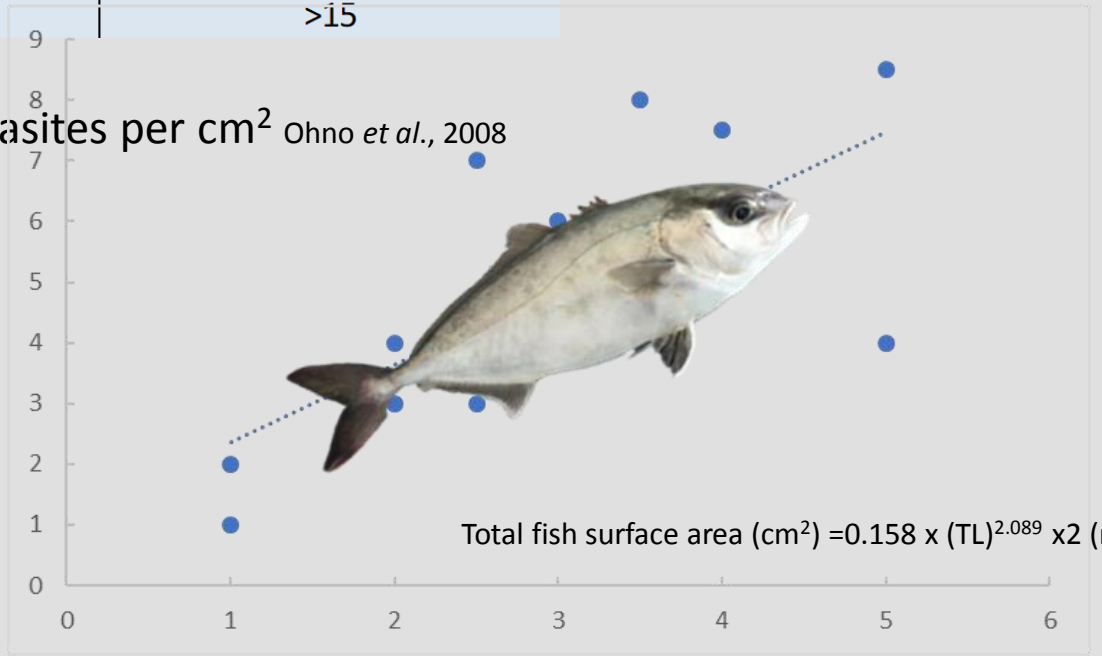
Material & Methods

Parasite challenge

- Parasitation level was made by direct observation by 3 different researchers following:

parasitation level	number of parasites per fish
0	0
1	1-5
2	5-15
3	>15

- Nº of parasites per cm^2 Ohno *et al.*, 2008

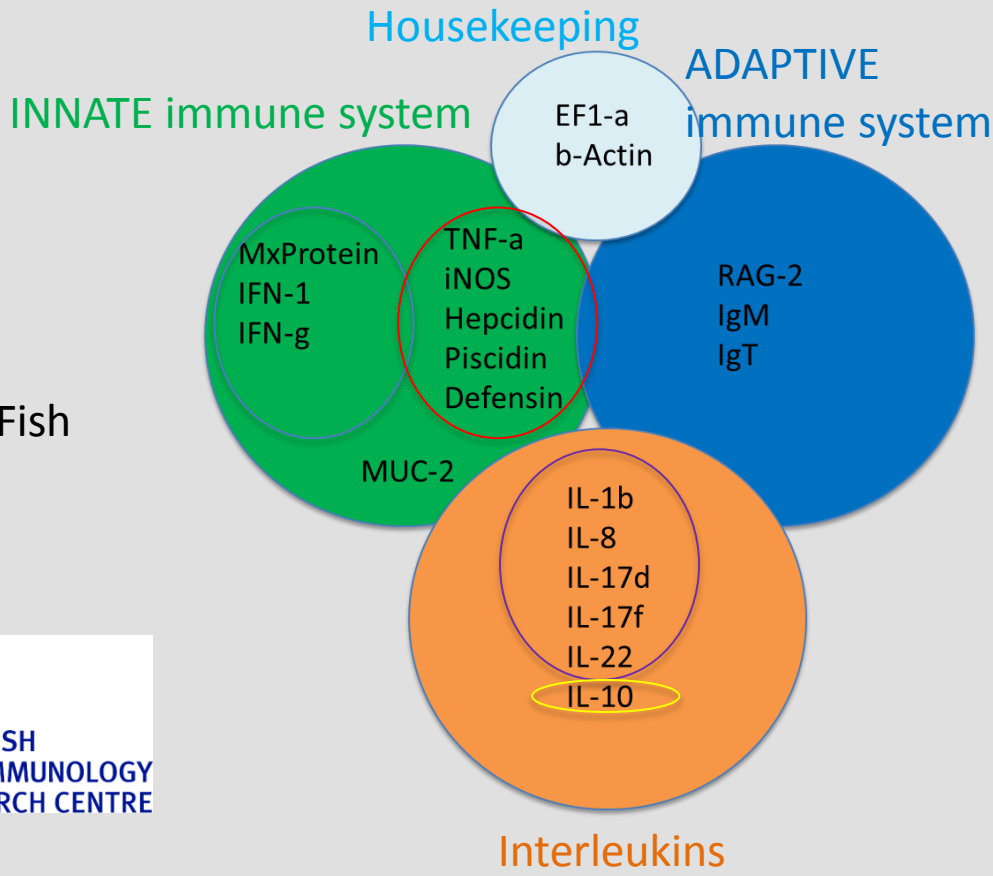


Material & Methods

Gene expression analyses

- Total RNA extraction by TRIZOL method
- CDNA synthesis
- RTPCR (sybergreen)

Primers designed by Scottish Fish Immunology Research Center



UNIVERSITY OF ABERDEEN

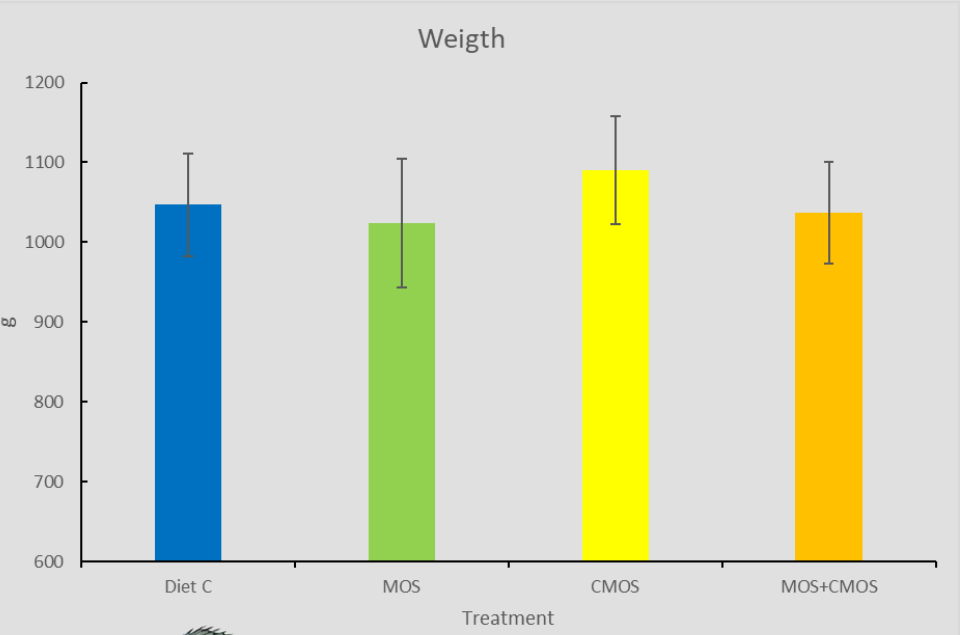
SCOTTISH FISH IMMUNOLOGY RESEARCH CENTRE

RESULTS

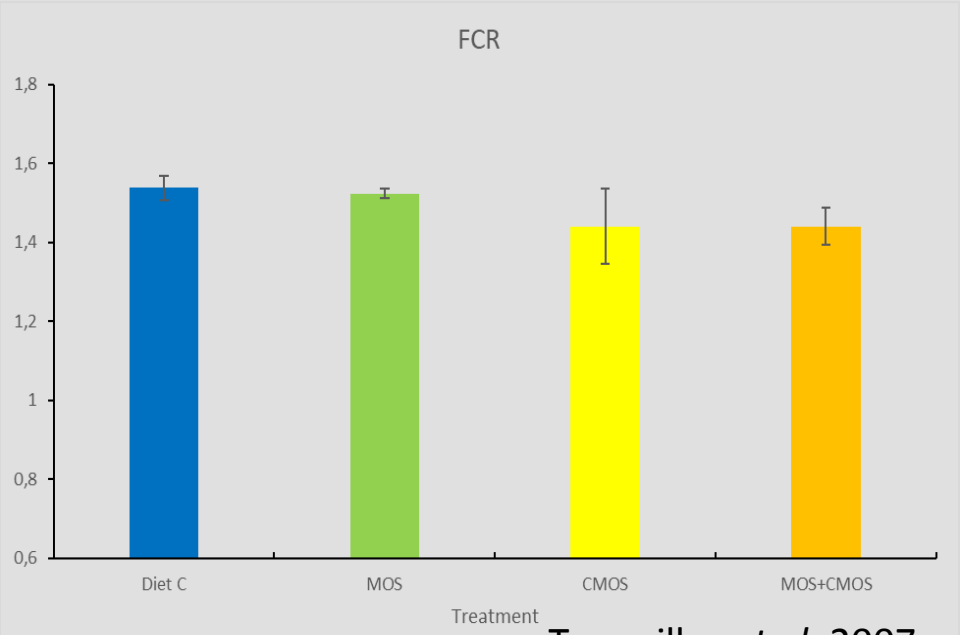
Results

Growth performance

(p>0.05)



(p>0.05)



Dimitroglou *et al.*,2010
Gültepe *et al.*,2011

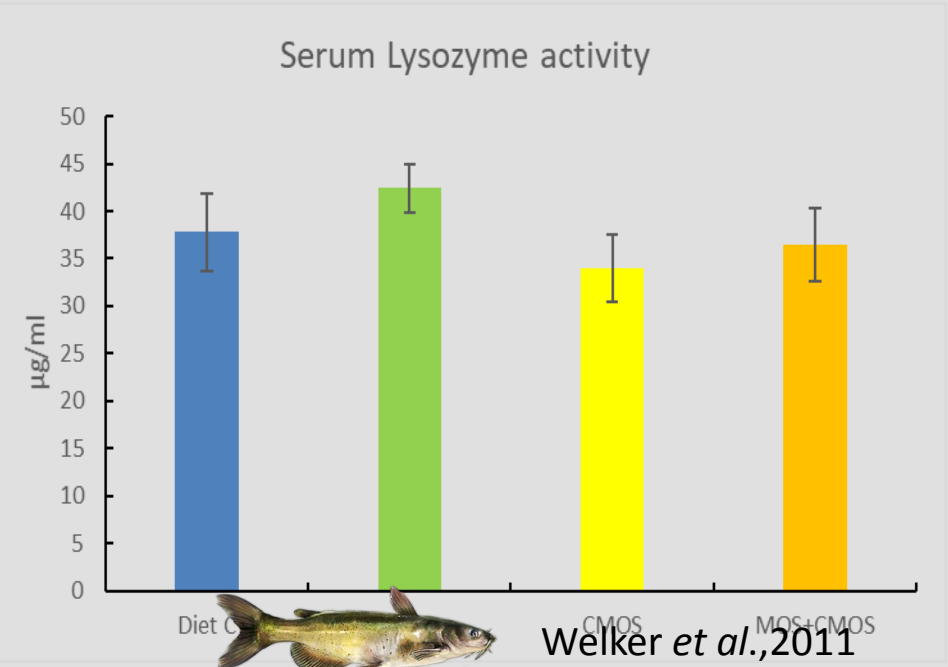
Rodriguez- estrada *et al.*,2008

Torrecillas *et al.*,2007, 2011, 2013, 2015, 2016
Salem *et al.*,2016

Samrongpang *et al.*,2008

Mucus and serum immunological parameters

(p>0.05)

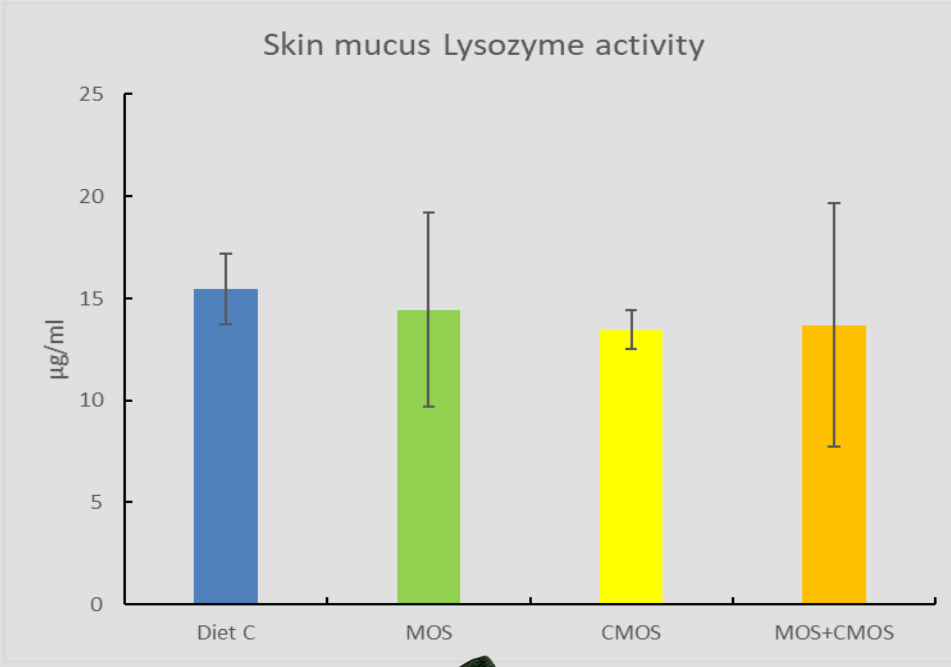


Welker *et al.*,2011

Ye *et al.*,2011

Dimitroglou *et al.*,2011

(p>0.05)



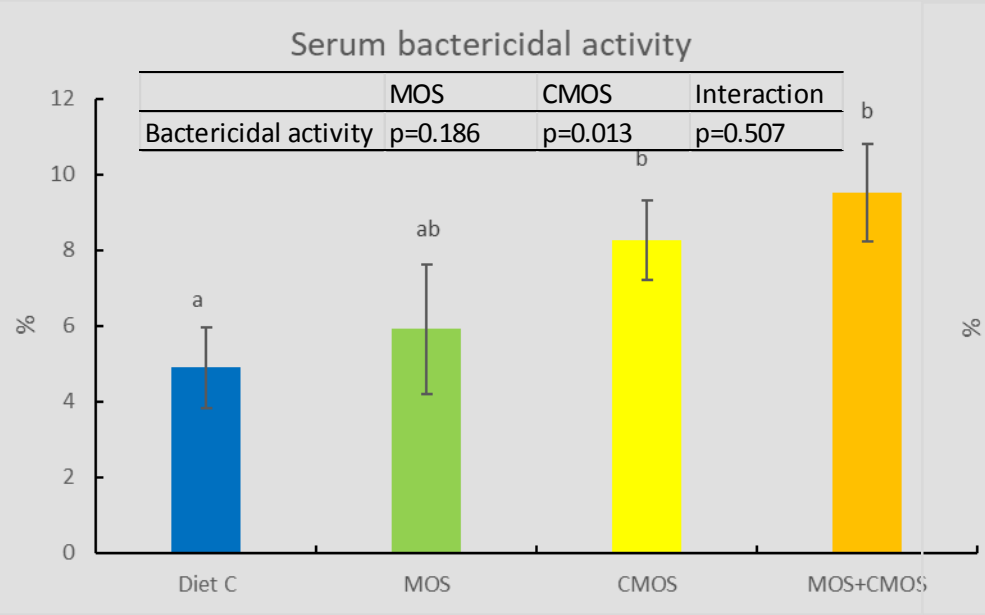
Rodriguez-estrada *et al.*,2008

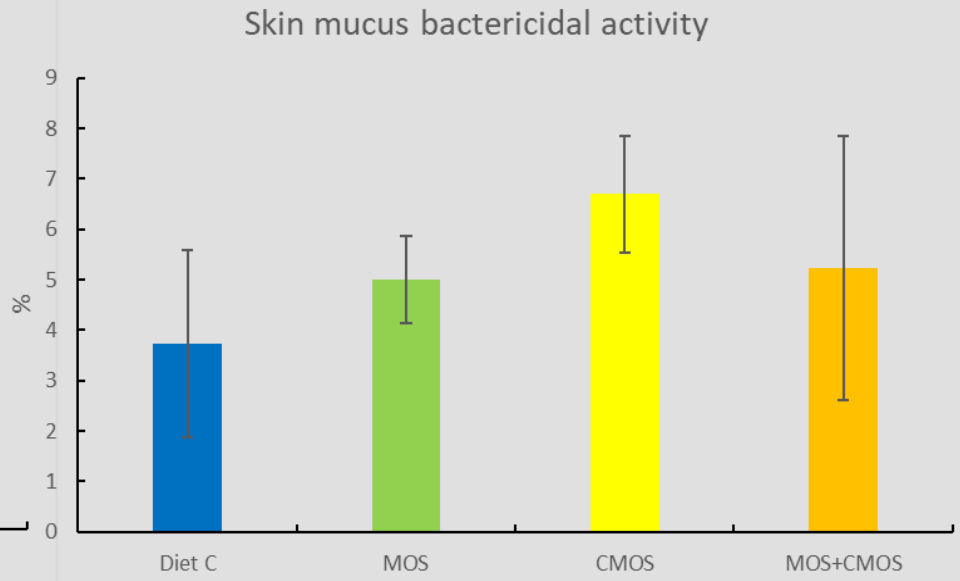
Torrecillas *et al.*,2011

Mucus and serum immunological parameters

(p<0.05)

(p>0.05)





Torrecillas *et al.*,2011

Welker *et al.*,2011

Torrecillas *et al.*,2011

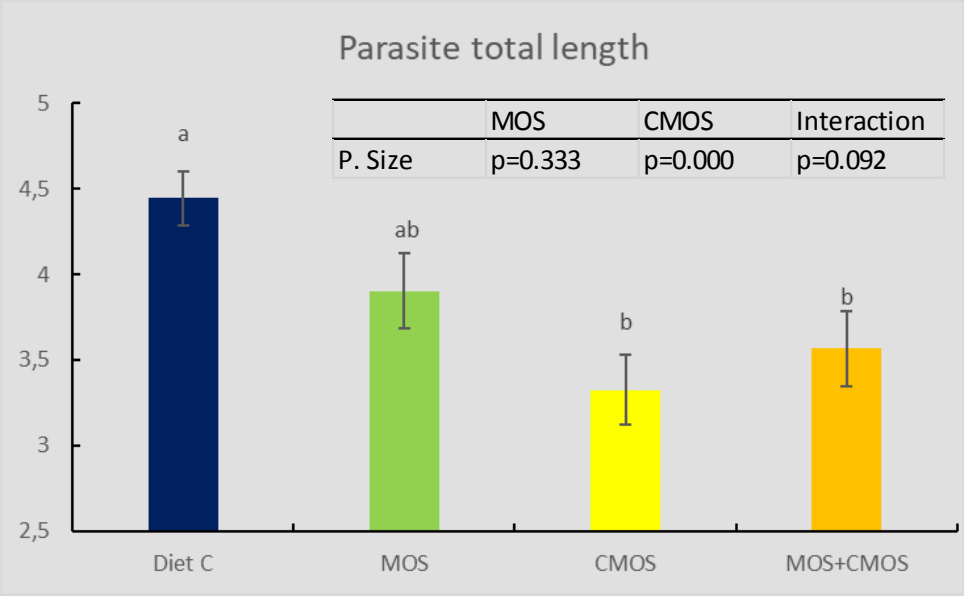
Rodriguez- estrada *et al.*,2008

Parasite Challenge

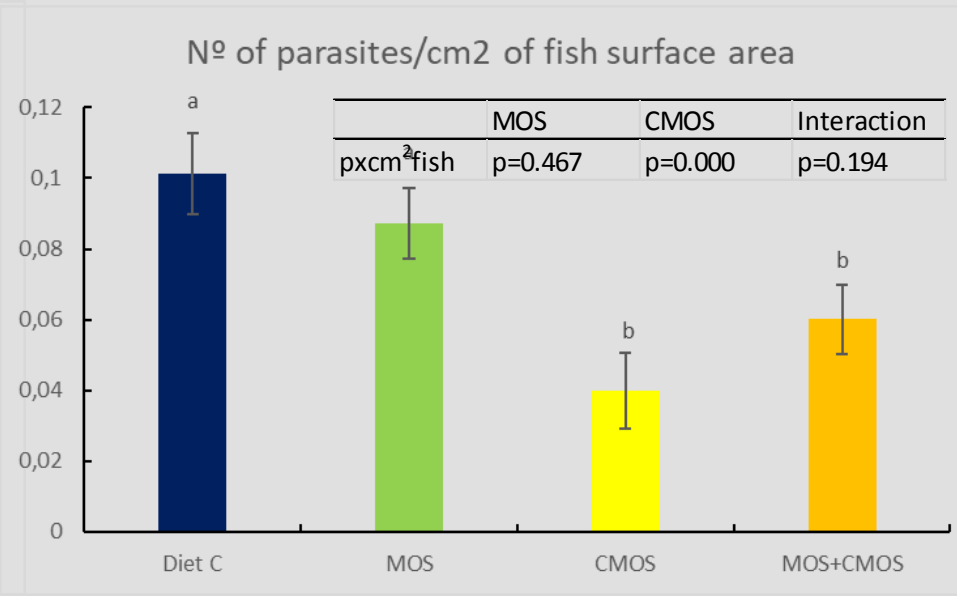
KW ($p<0.05$)

	DIET C	MOS	CMOS	MOS+CMOS
Parasitation level (range)	2-3[a]	2[a]	1-2[b]	1-2[b]

($p<0.05$)



($p<0.05$)

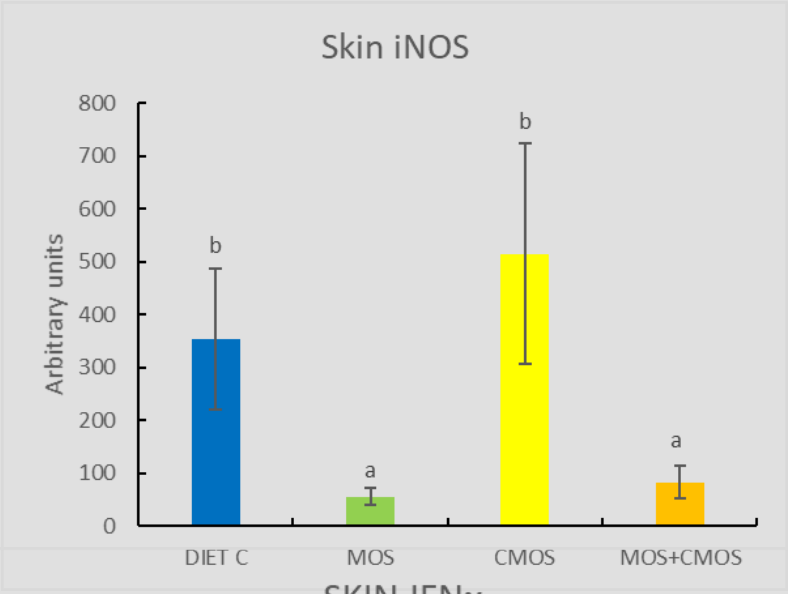


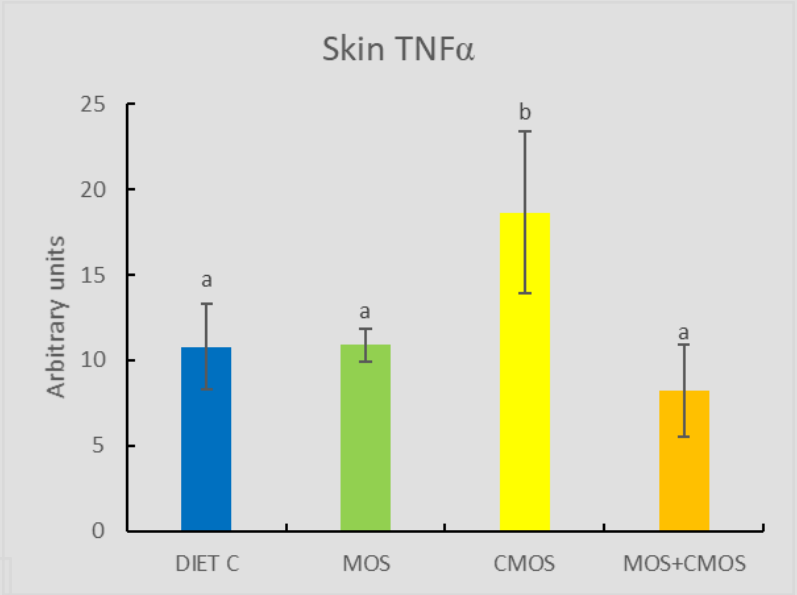
Gene expression analyses

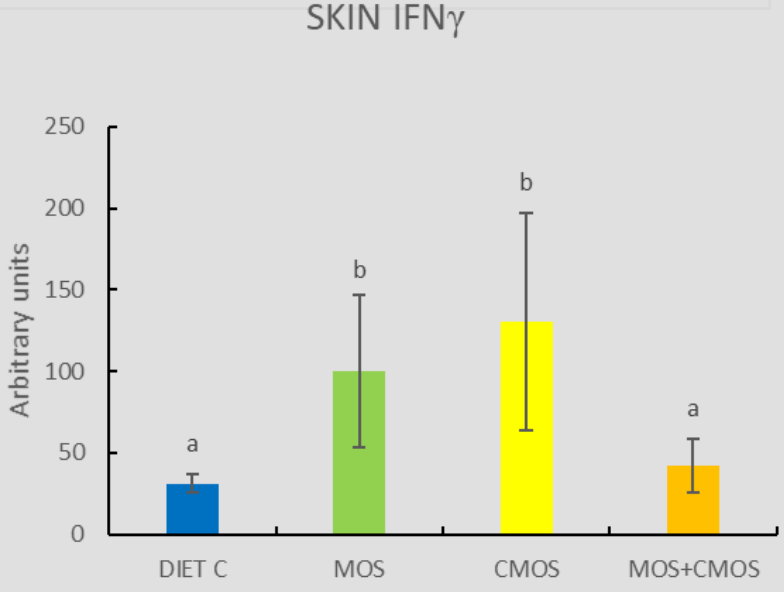
Results

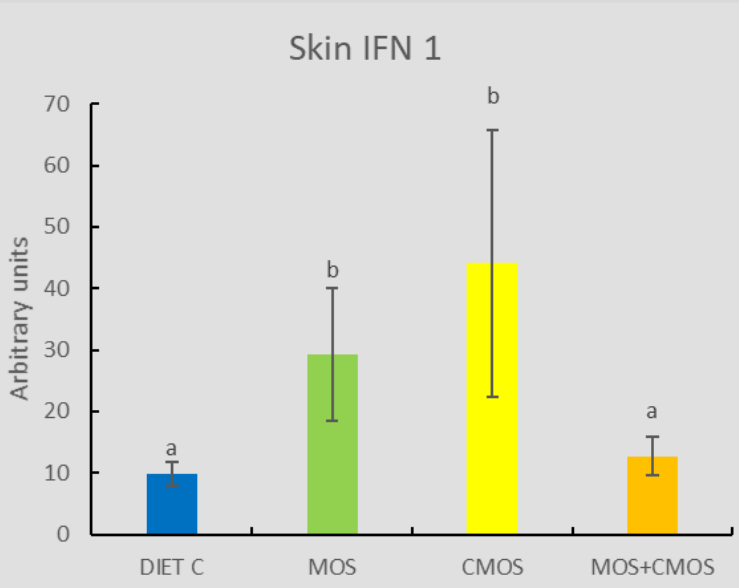
SKIN (INNATE IMMUNE PARAMETERS)

P<0.05

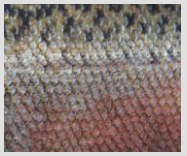






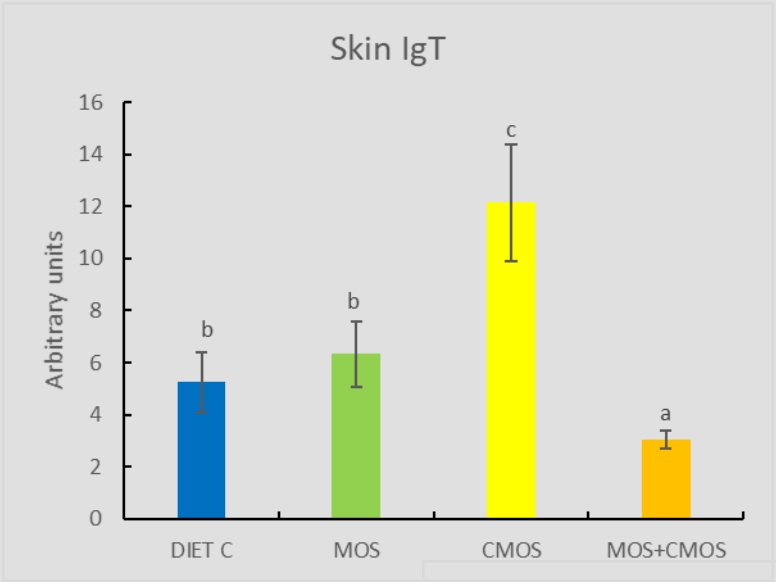


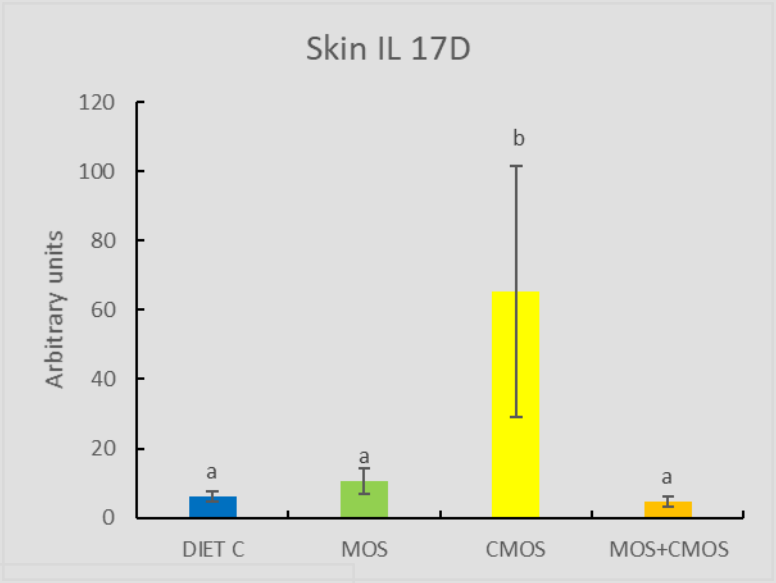
Gene expression analyses

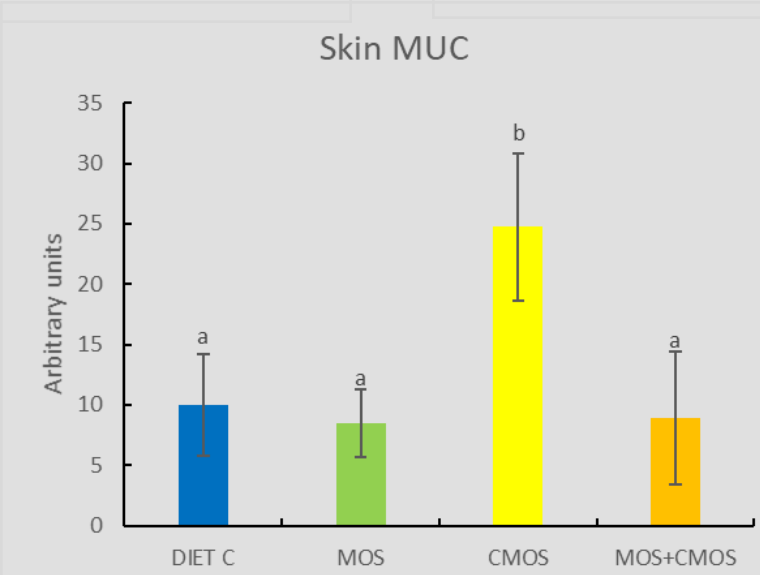


SKIN(ADAPTIVE IMMUNE PARAMETERS, INFLAMATORY RESPONSE AND MUCINE PRODUCTION)

$p<0.05$

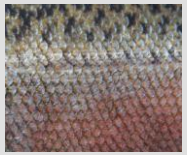


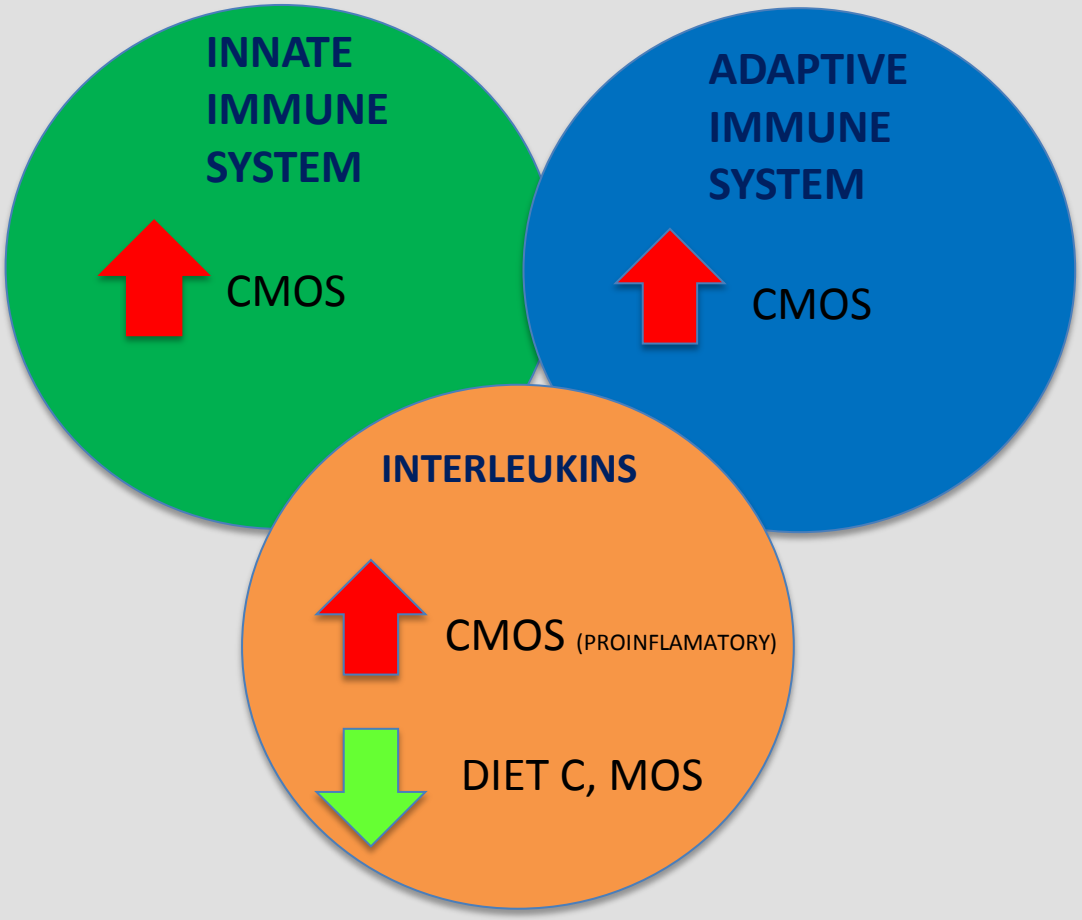




Gene expression analyses

SKIN



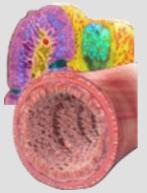


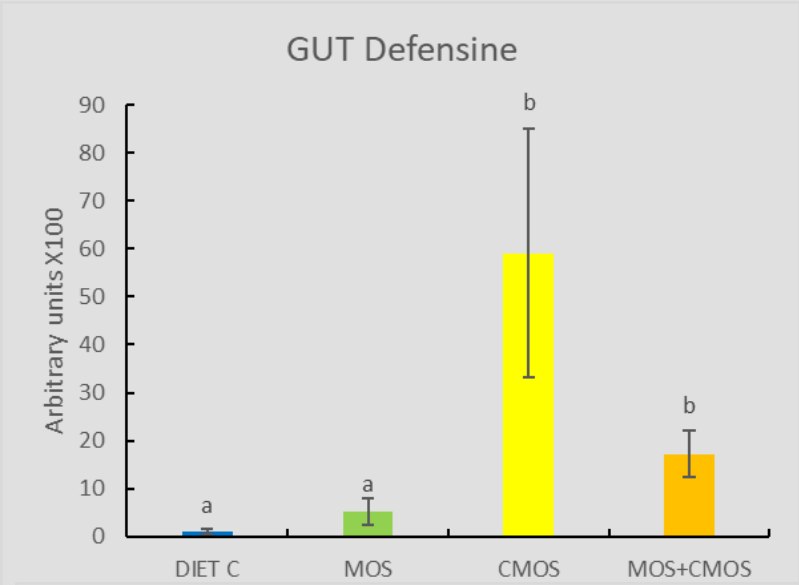
Gene expression analyses

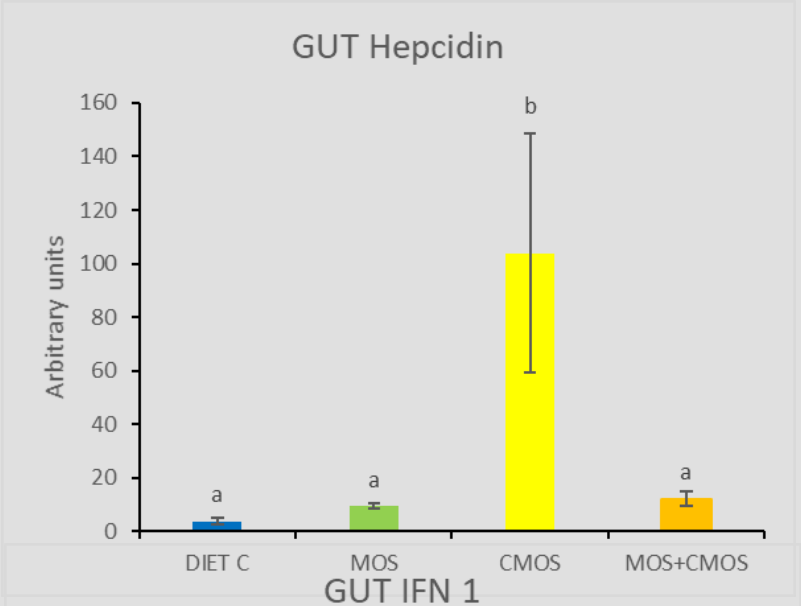
Results

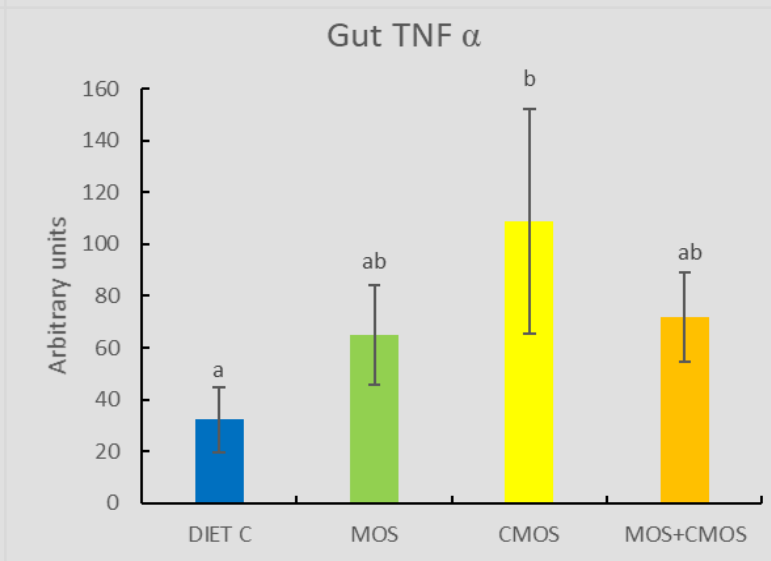
POSTERIOR GUT (INNATE IMMUNE PARAMETERS)

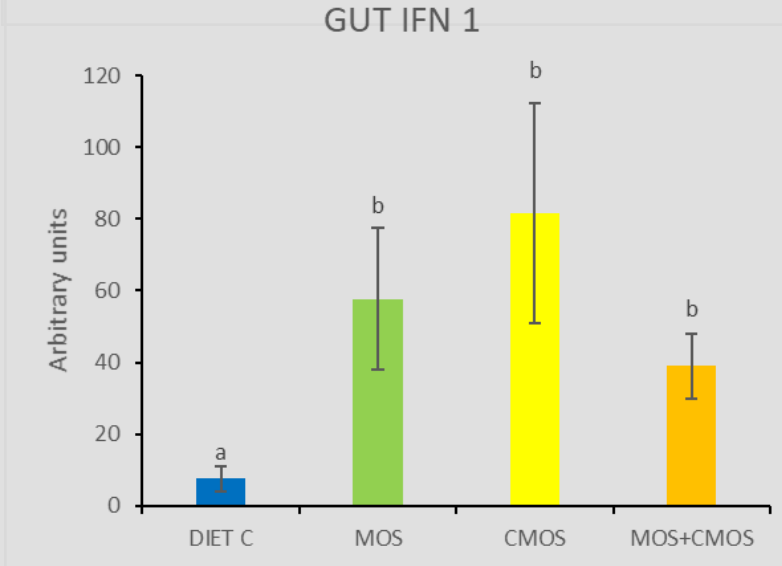
P<0.05





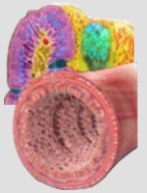






Gene expression analyses

POSTERIOR GUT (INTERLEUKINS)



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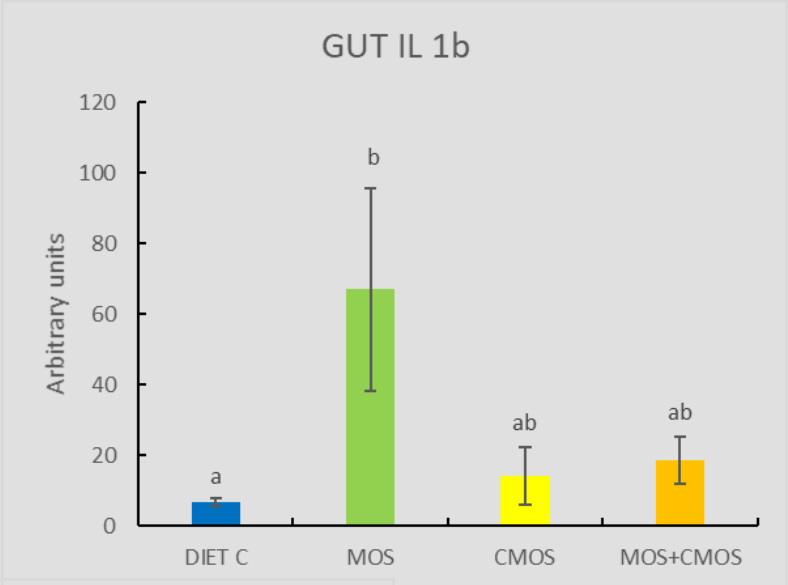
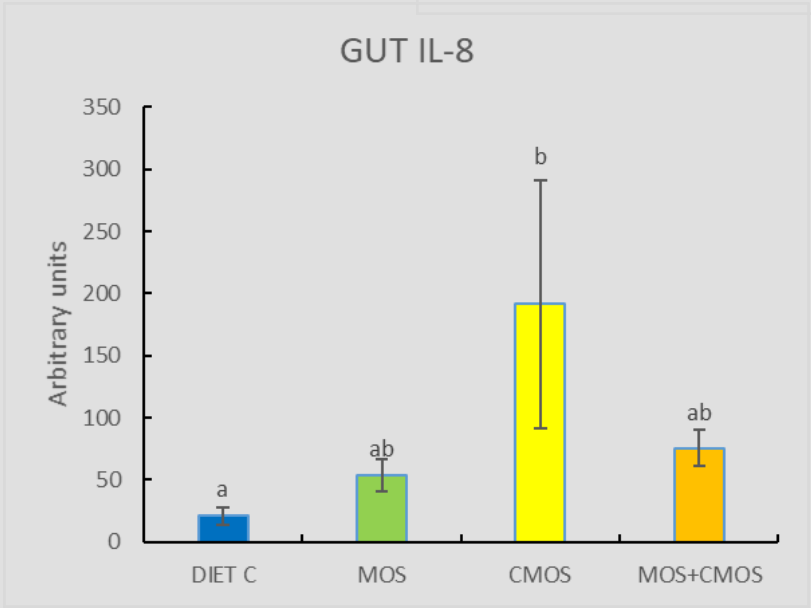
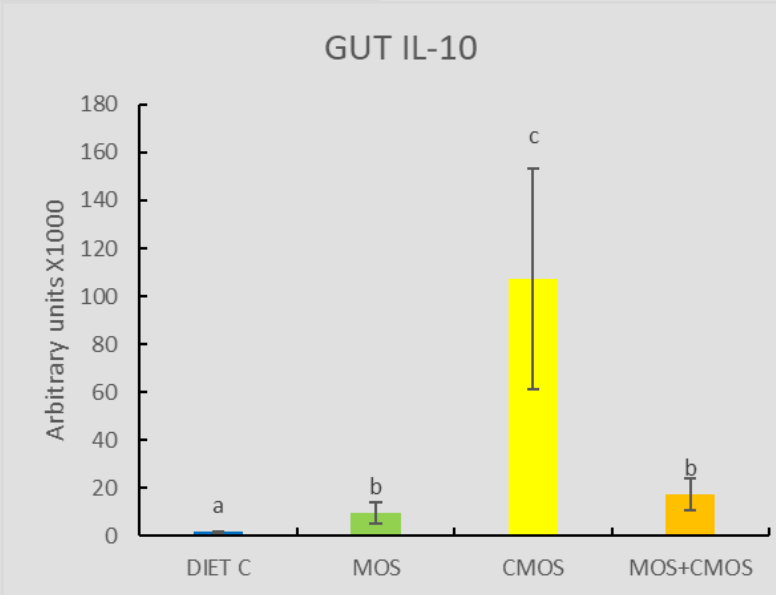
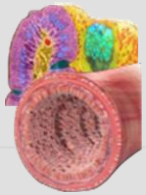




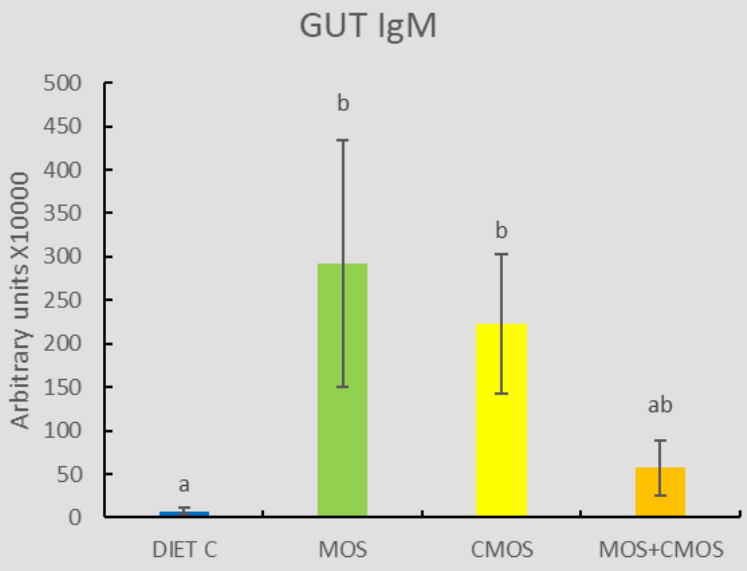


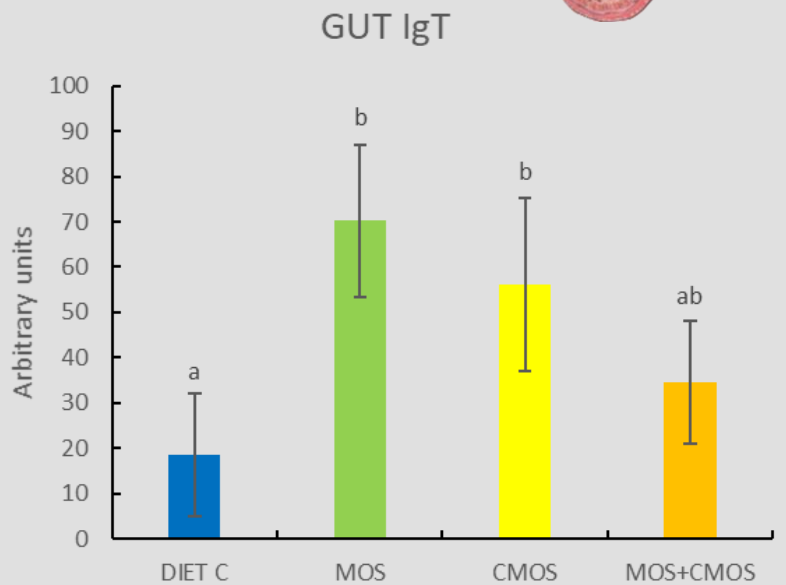
Gene expression analyses

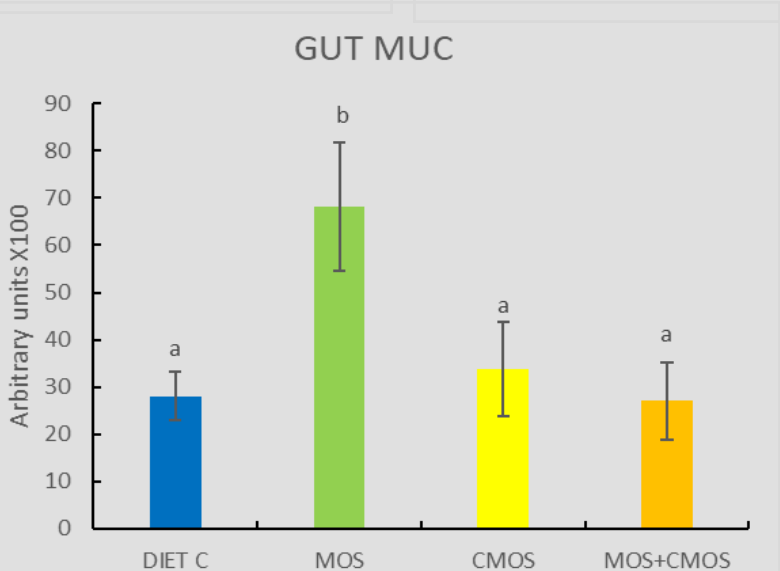


POSTERIOR GUT (ADAPTIVE IMMUNE PARAMETERS AND MUCINE PRODUCTION)

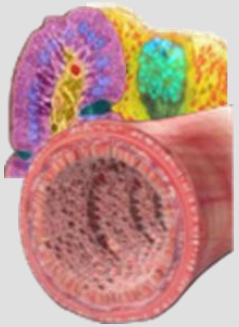
$p<0.05$



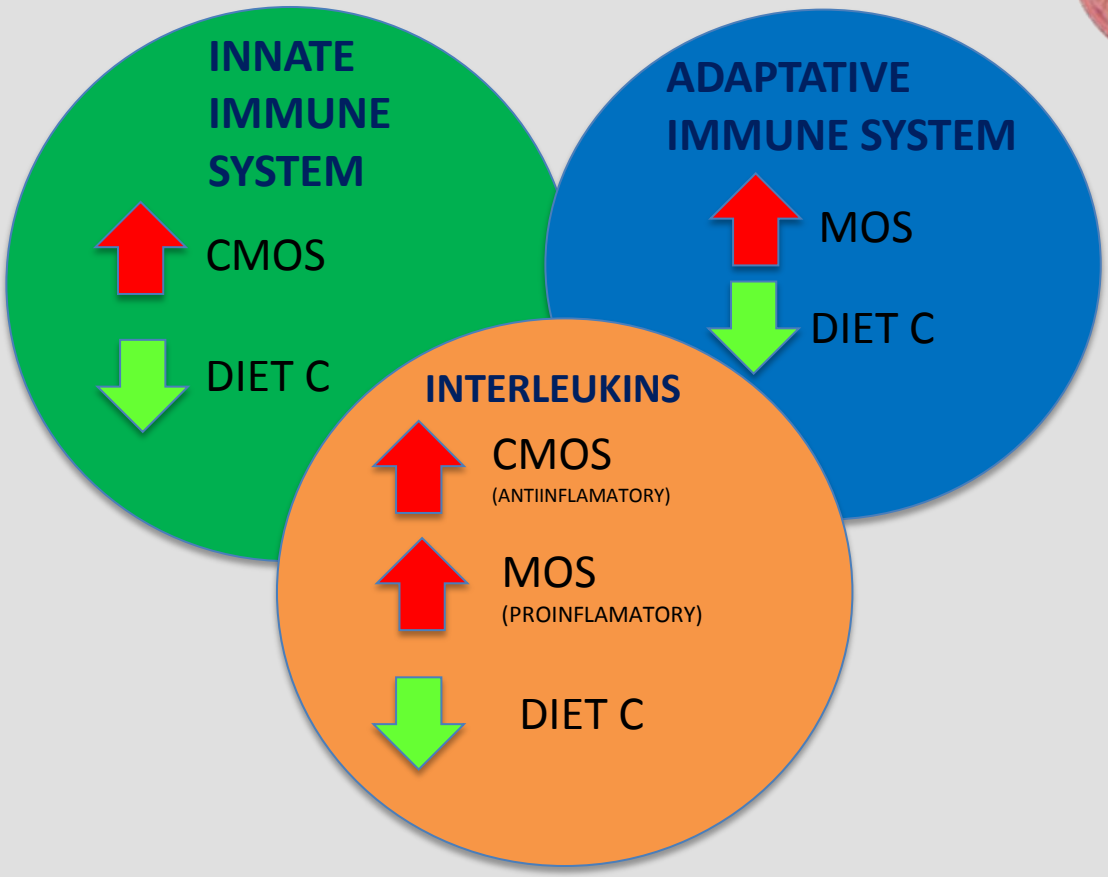




Gene expression analyses

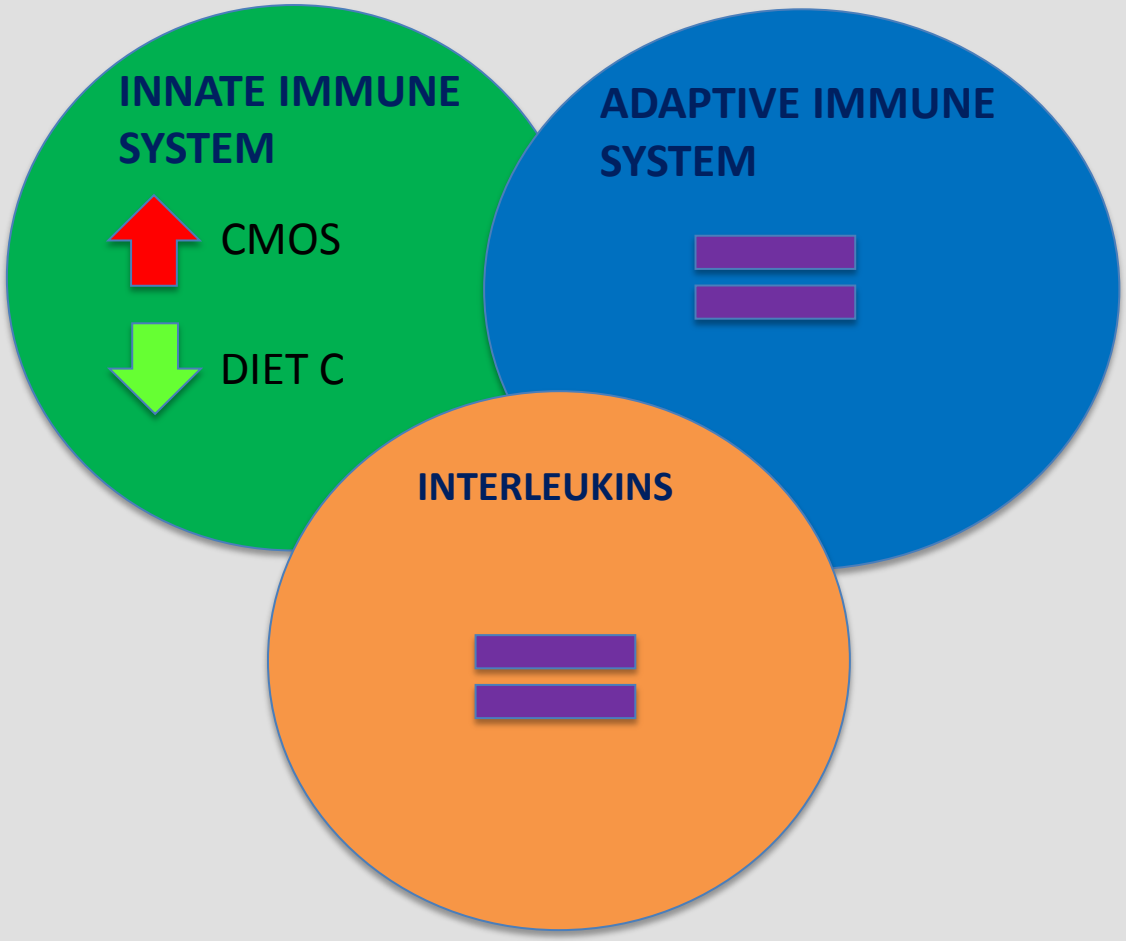


POSTERIOR GUT



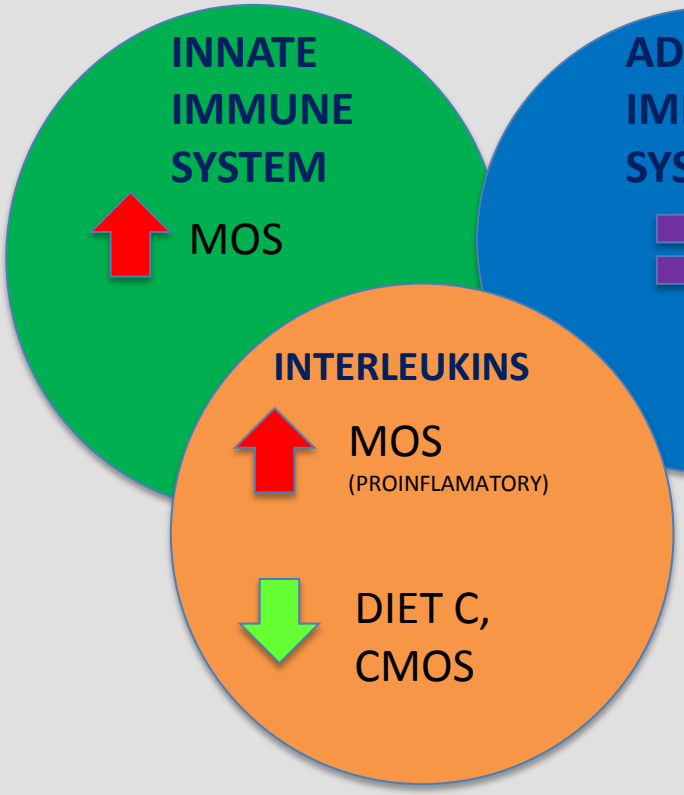
Gene expression analyses

GILL



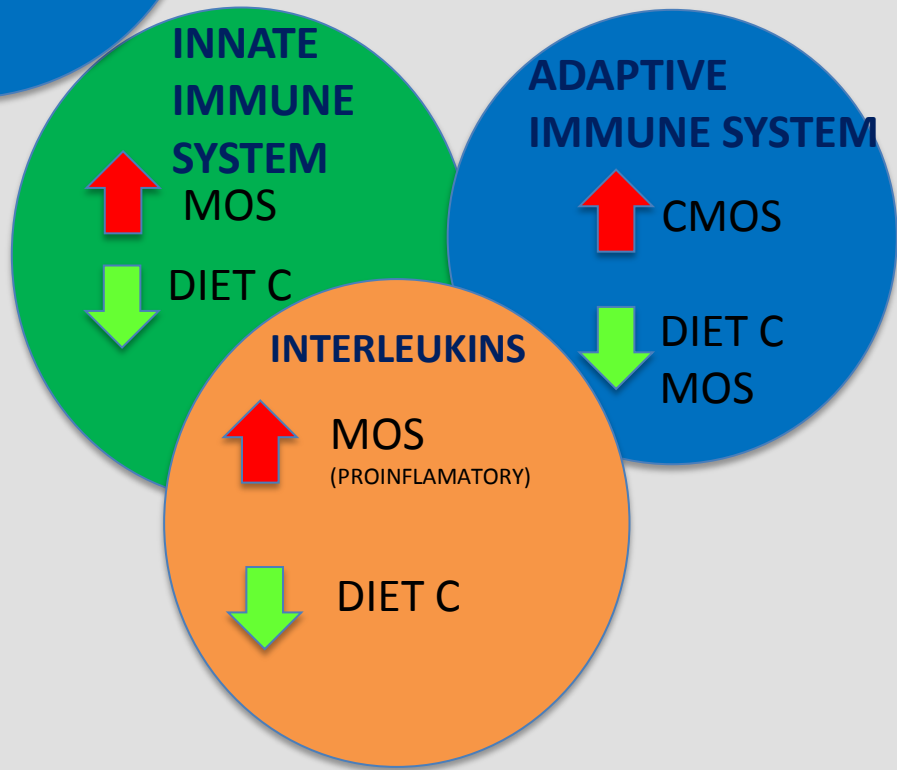
Gene expression analyses

Spleen and Head kidney



Spleen

Head kidney



SUMMARY

1. Growth performance and feed conversion ratio were not altered by dietary MOS supplementation in *S.dumerili* at the concentration used.

2. Dietary cMOS supplementation stimulated serum bactericidal activity, however no effect was detected on lysozyme activity.

3. Dietary use of cMOS reduce *S.dumerilli* infestation by *N.girellae* after 3 months of supplementation.

4. cMOS presented a response more focus on ectomucoses (skin and gill), meanwhile MOS focused more the response in gut and systemic immunity (head kidney and spleen).

Acknowledgements

- EU & DIVERSIFY

- Univ. of Aberdeen

- Univ. of Las Palmas de Gran Canaria

- Skretting and ALLTECH

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