

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



WP 25.3

Grupo de Investigación en Acuicultura
(GIA; www.giaqua.org)



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



INTRODUCTION

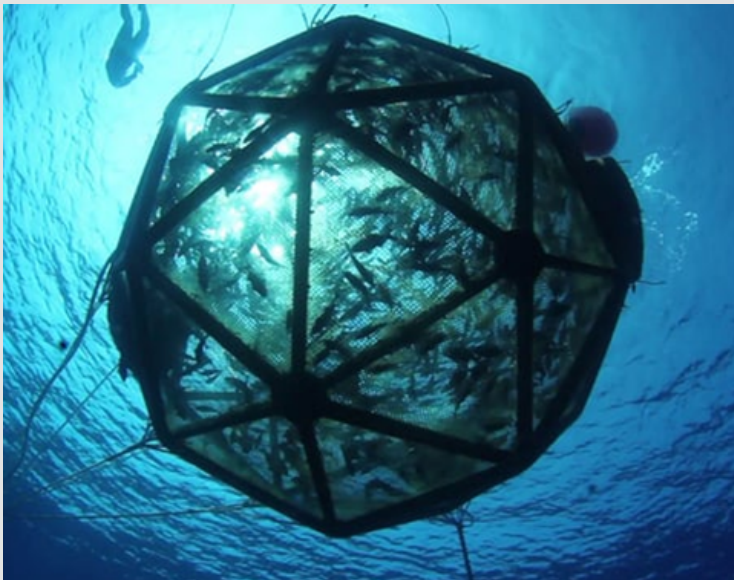
Importance of the genus *Seriola* for aquaculture (150.000 Tn; FAO 2010)



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



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
 Inês Guerreiro^{1,2} , Aires Oliva-Teles^{1,2} and Paula Enes¹

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Fish & Shellfish Immunology 36 (2014) 525–544

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 journal homepage: www.elsevier.com/locate/fsi



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



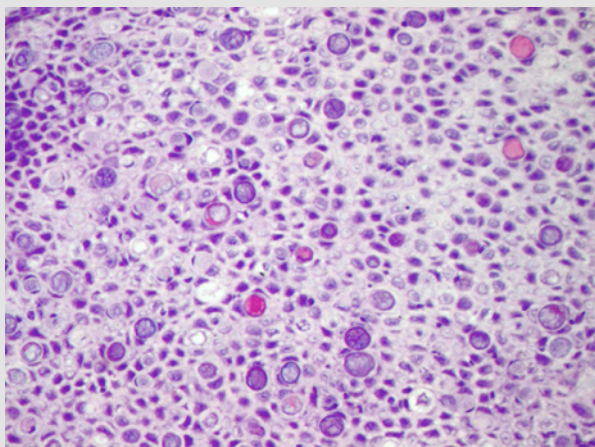
Silvia Torrecillas*, Daniel Montero, Marisol Izquierdo

Grupo de Investigación en Acuicultura (GIA), Universidad de Las Palmas de Gran Canaria, Transmontaña s/n, Arucas, 35416 Las Palmas de Gran Canaria, Canary Islands, Spain

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

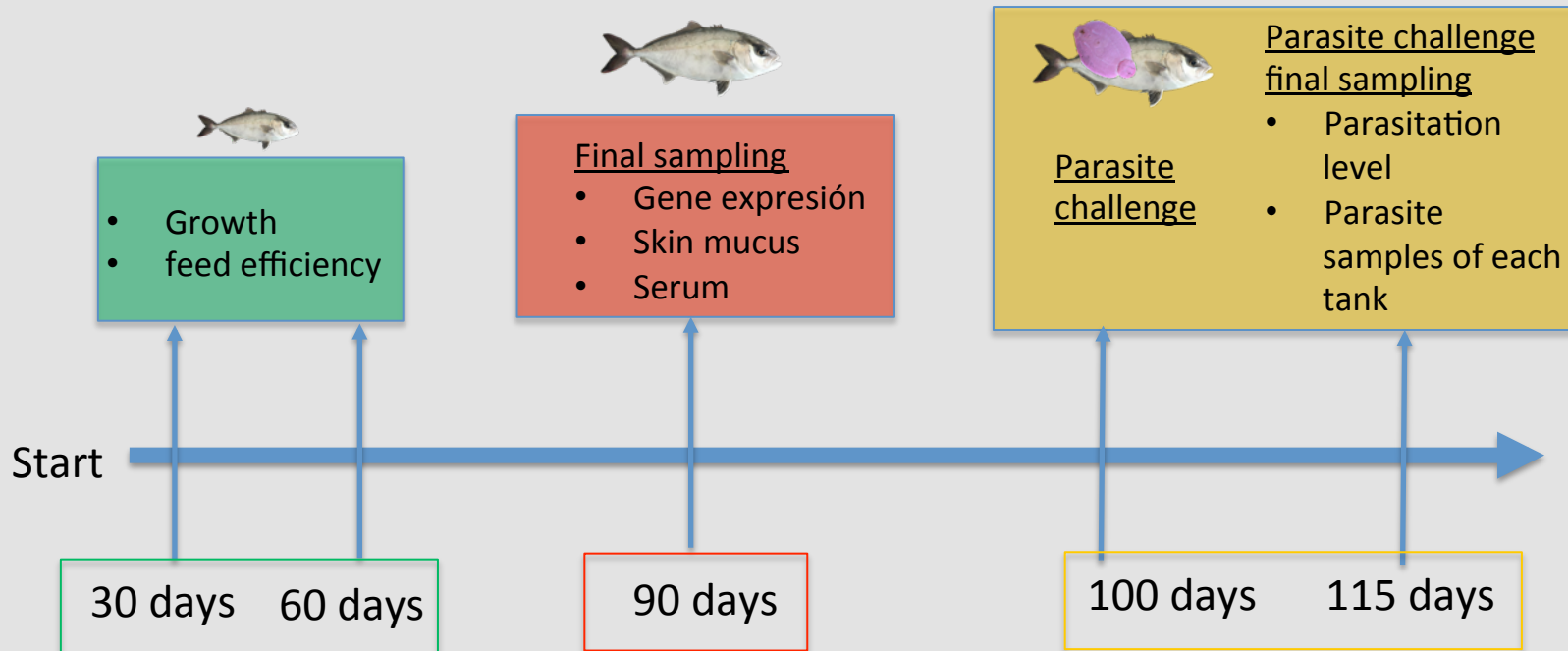
Experimental design

324 juveniles (331 ± 30g)

Randomly distributed into 18 1000l cylindroconical tanks



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



Material & Methods

Final Sampling

- Extraction of gene expression samples
 - Posterior gut
 - Gill
 - Skin
 - Head kidney
 - Spleen
- Skin mucus and serum extraction

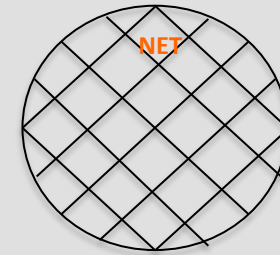
} RNA later



- Bactericidal activity
(Against *Photobacterium damsela*)
- Lysozyme activity
(Guardiola et al. 2014;
Quade and Roth 1997)



Experimentally eggs culture



ONCOMIRACIDIA

Source: Hirayama et al 2009



Controlled HOST ATTACHMENT- 10 days



Highly parasited animals



EGGS

ADULT



Experimentally infestated fish

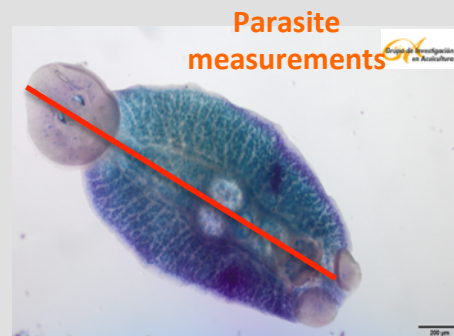


Cohabitation in cages with experimental fish - 15 days

Parasitation level evaluation



Freshwater bath



Parasite measurements

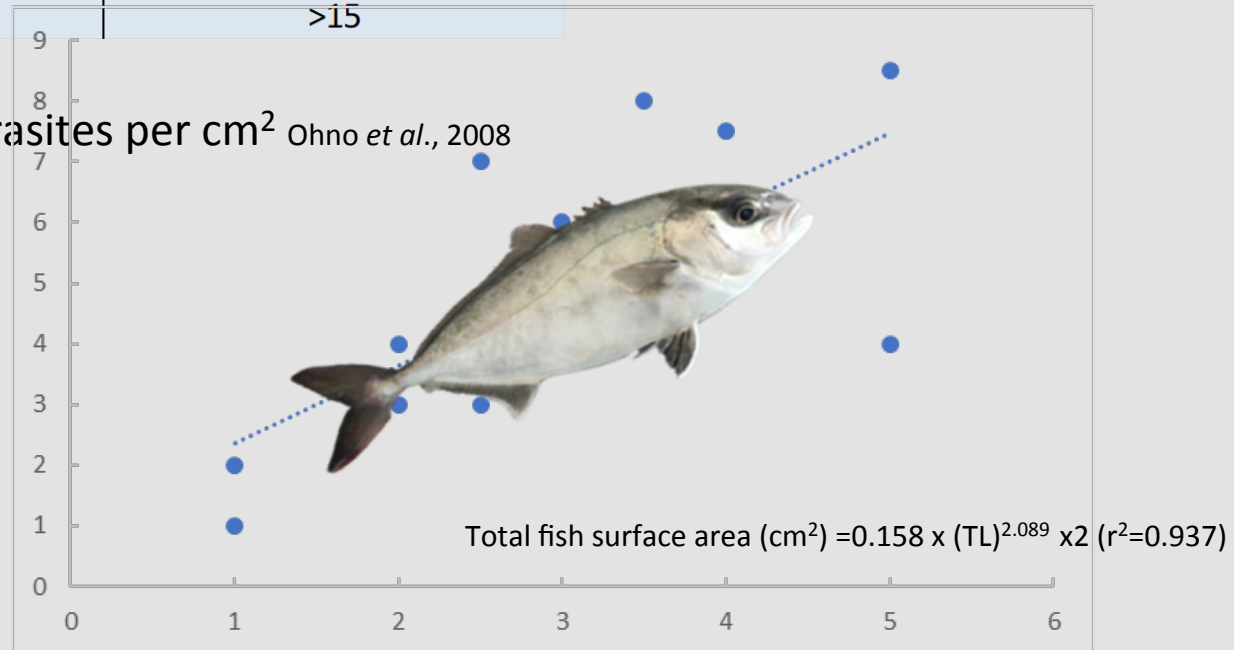
Material & Methods

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 cm² Ohno *et al.*, 2008

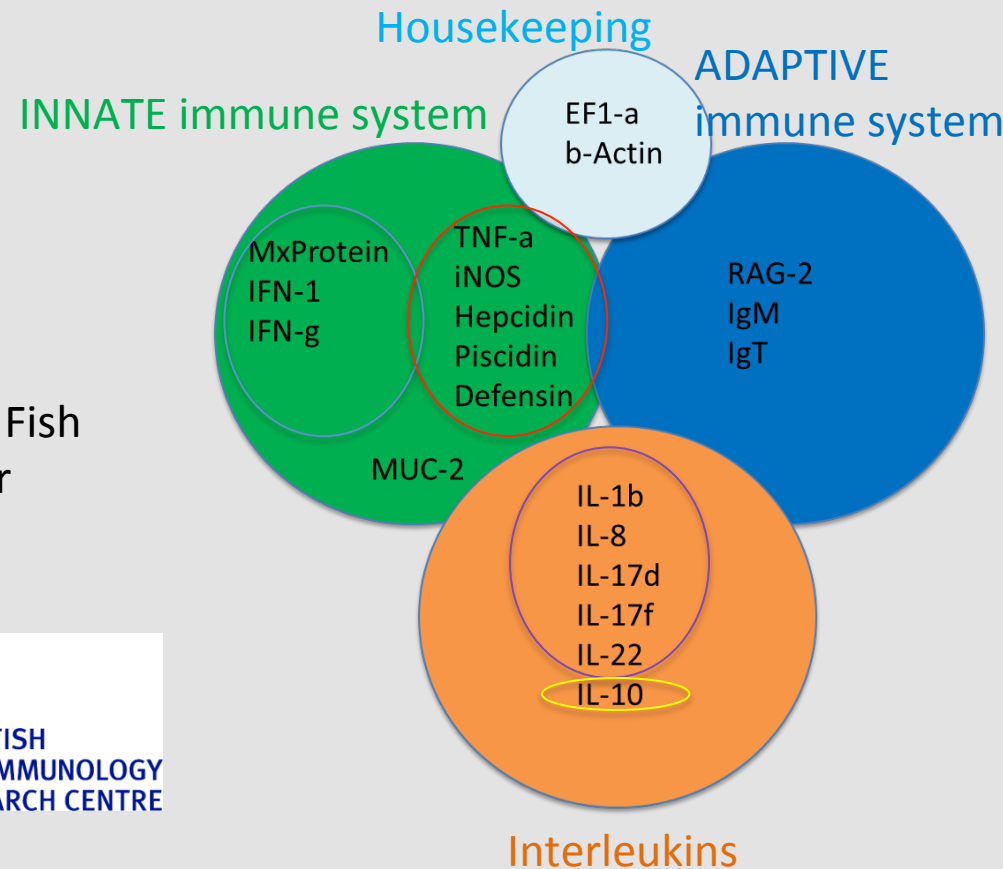


Material & Methods

Gene expression analyses

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

Primers designed by Scottish Fish Immunology Research Center





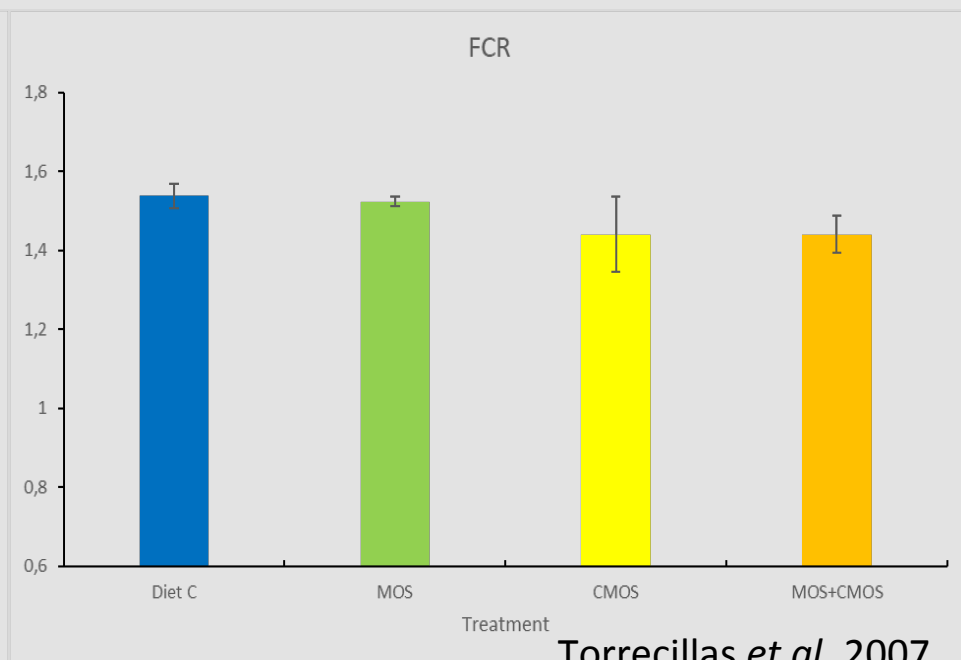
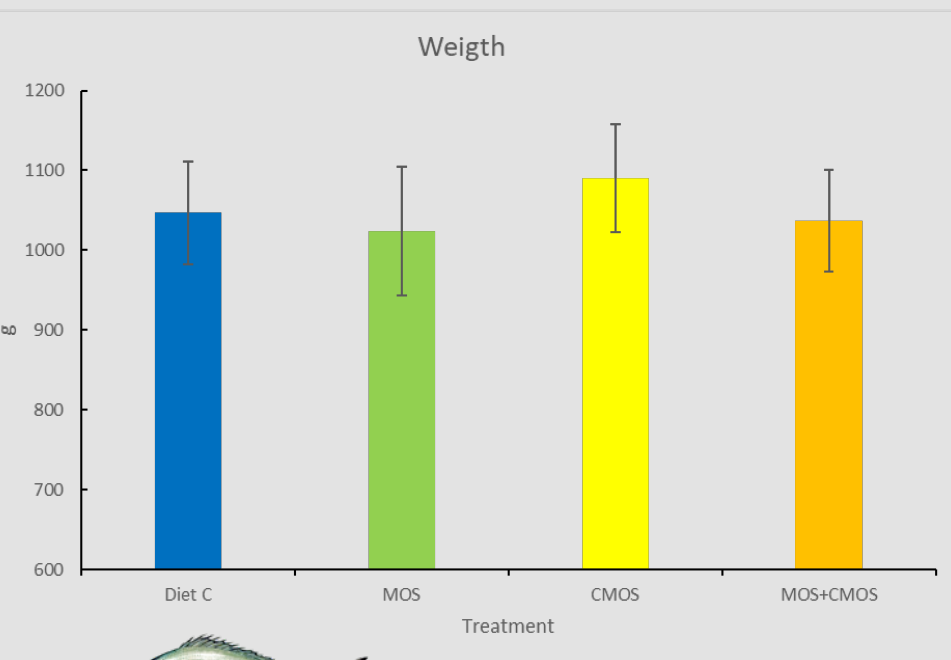
RESULTS



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



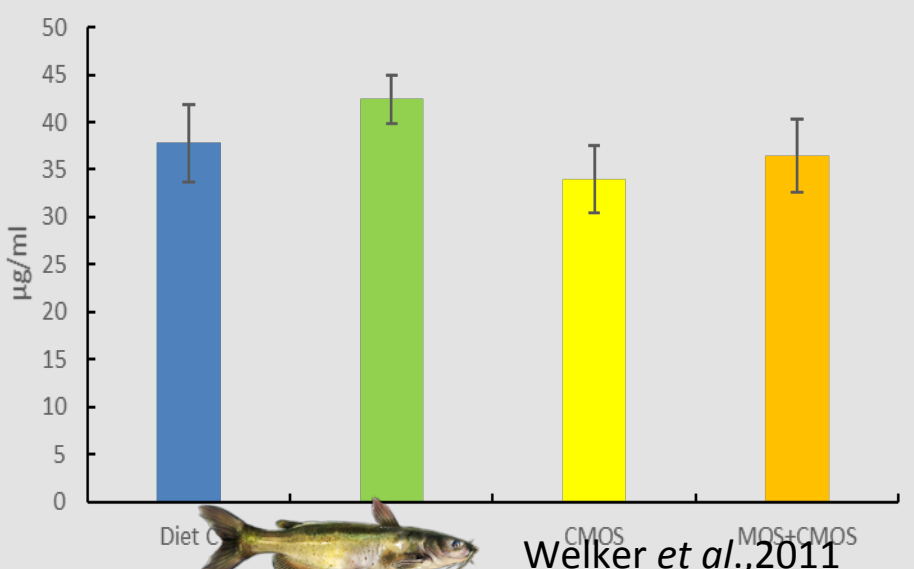
Results

Mucus and serum immunological parameters

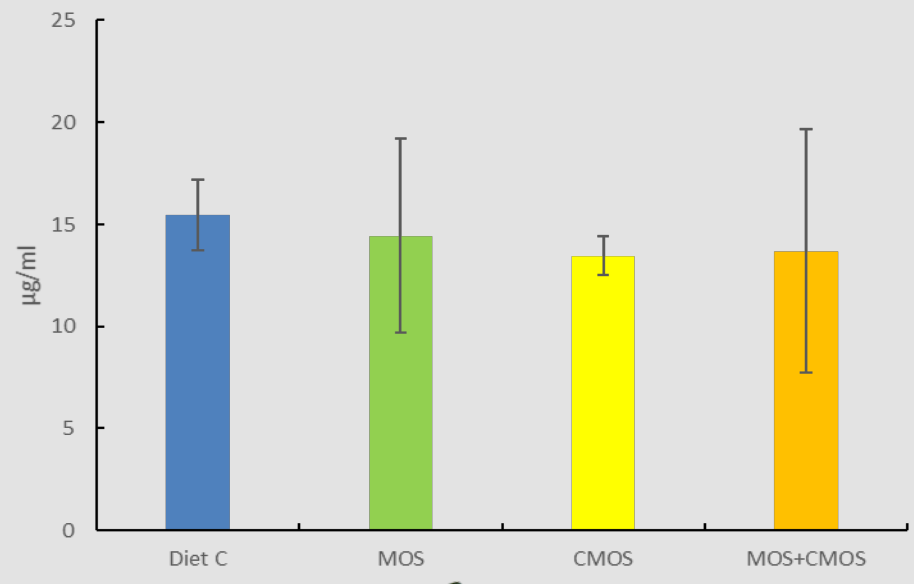
($p > 0.05$)

($p > 0.05$)

Serum Lysozyme activity



Skin mucus Lysozyme activity



Ye et al., 2011



Dimitroglou et al., 2011



Rodriguez-estrada et al., 2008



Torrecillas et al., 2011



Results

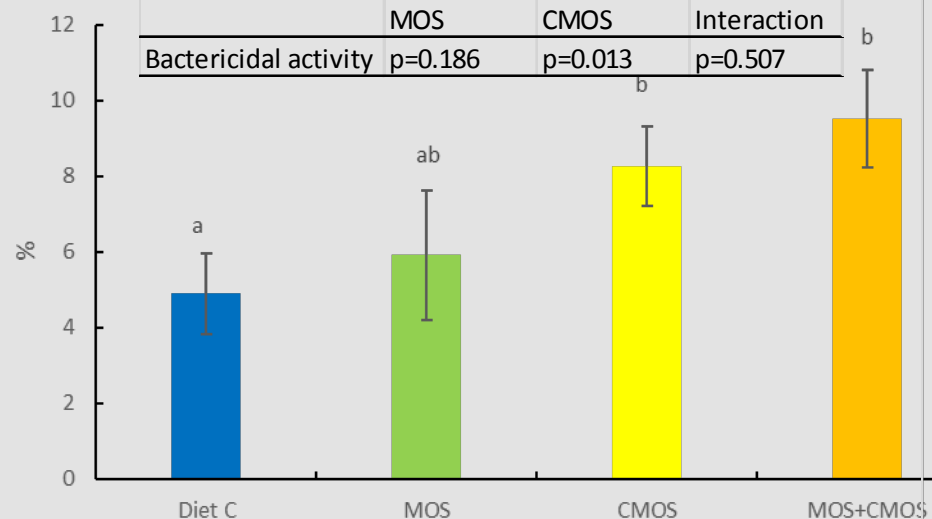
Mucus and serum immunological parameters

($p < 0.05$)

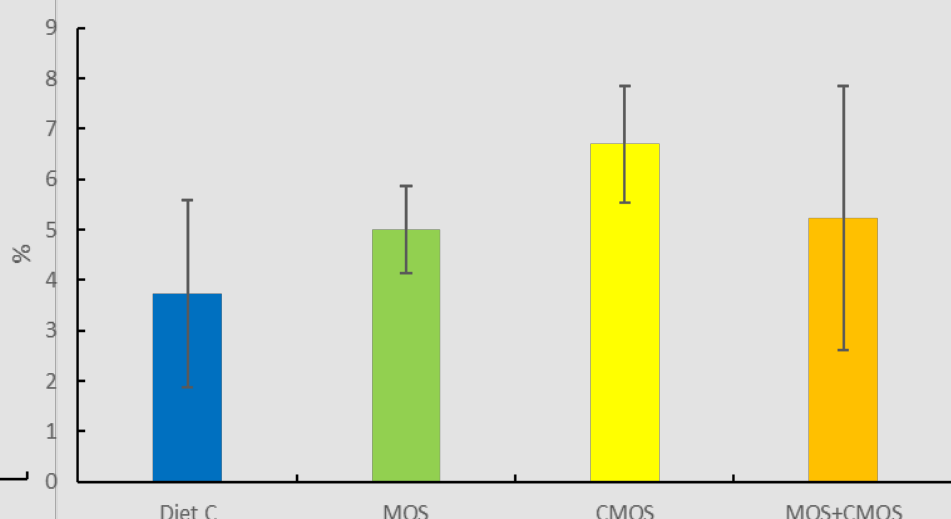
($p > 0.05$)

Serum bactericidal activity

| | MOS | CMOS | Interaction |
|-----------------------|-----------|-----------|-------------|
| Bactericidal activity | $p=0.186$ | $p=0.013$ | $p=0.507$ |



Skin mucus bactericidal activity





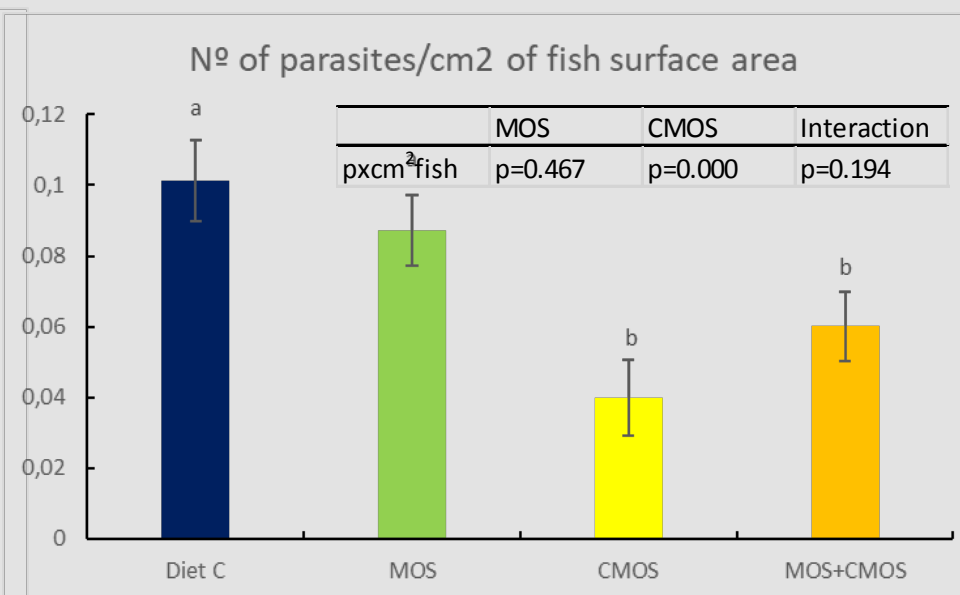
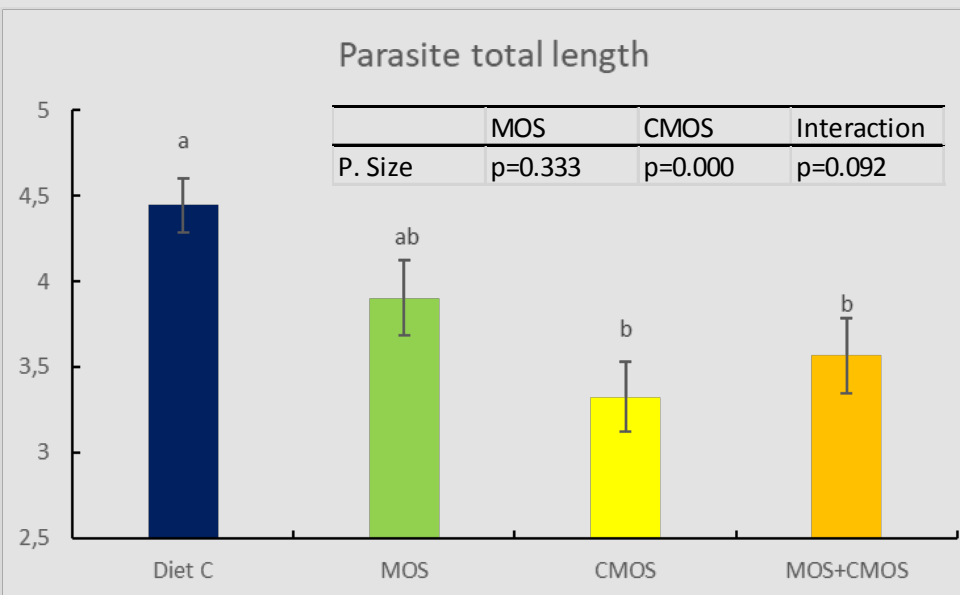
Parasite Challenge

KW ($p < 0.05$)

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

($p < 0.05$)

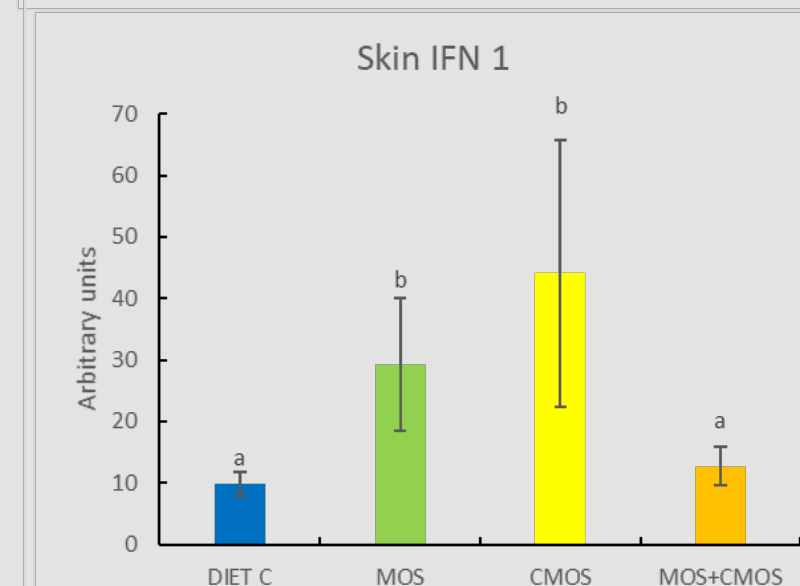
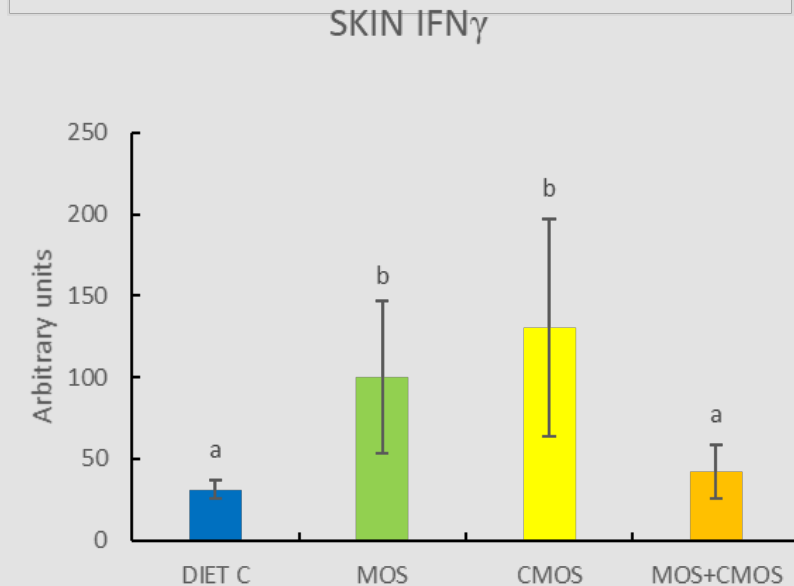
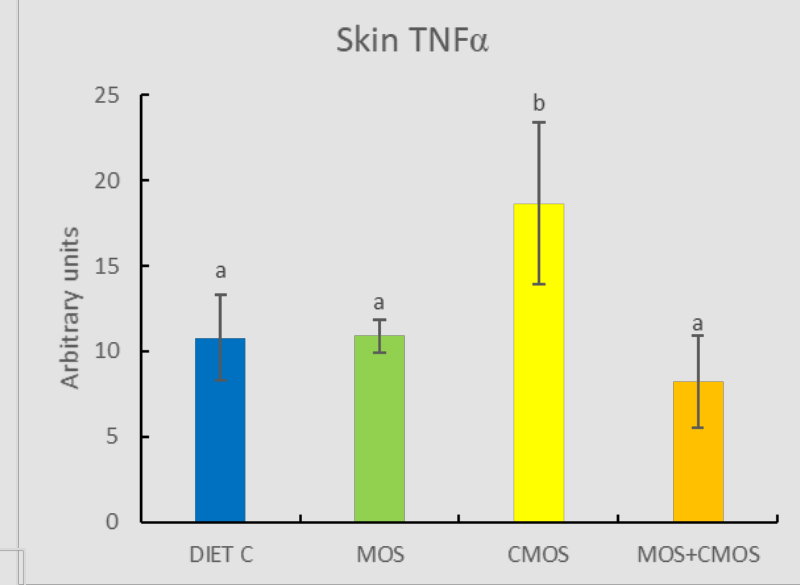
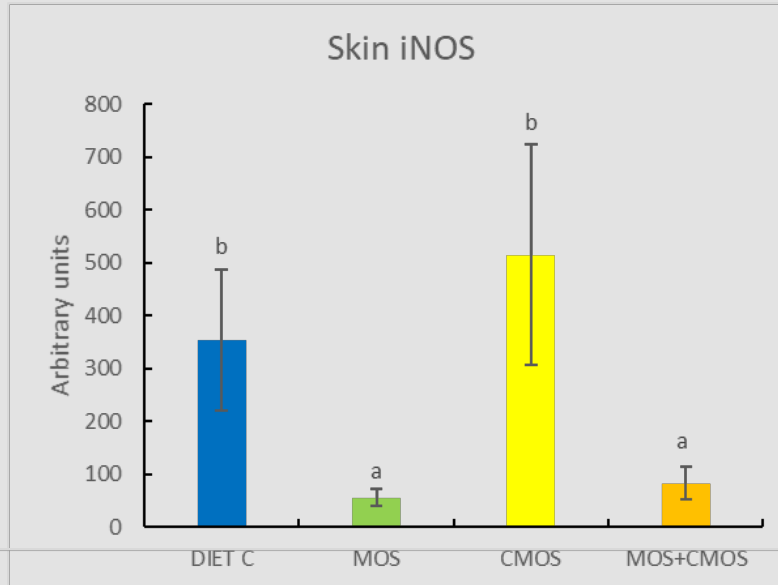
($p < 0.05$)



Gene expression analyses

SKIN (INNATE IMMUNE PARAMETERS)

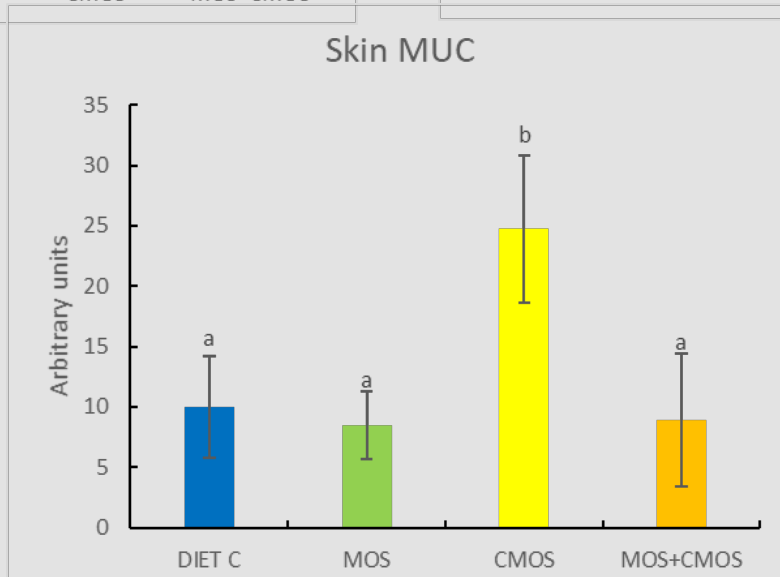
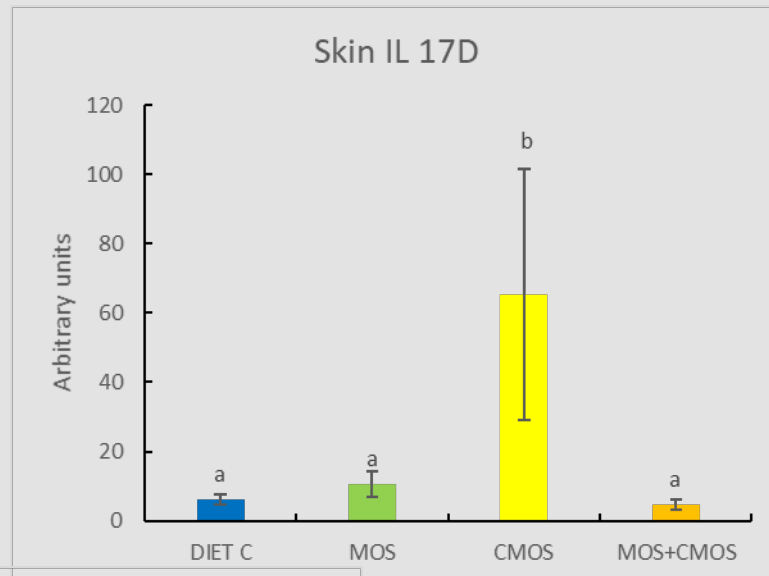
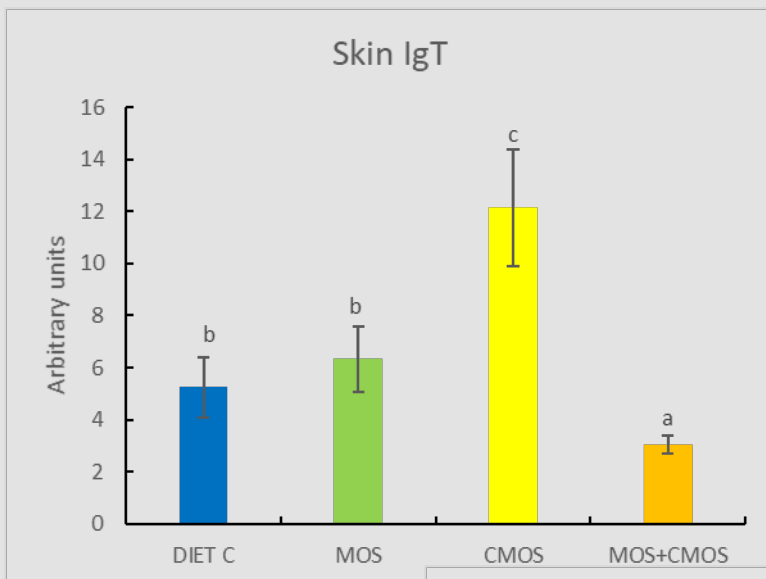
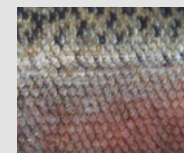
P<0.05



Gene expression analyses

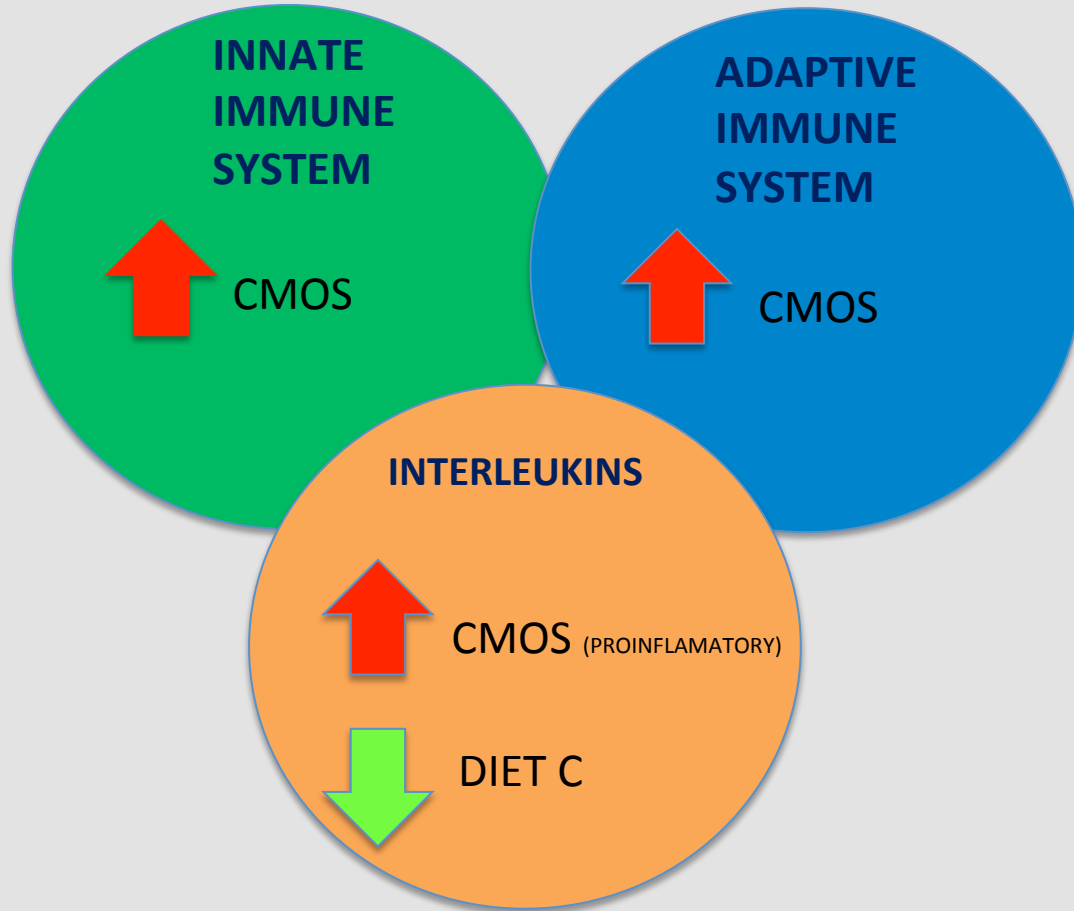
SKIN(ADAPTIVE IMMUNE PARAMETERS, INFLAMMATORY RESPONSE AND MUCINE PRODUCTION)

p<0.05



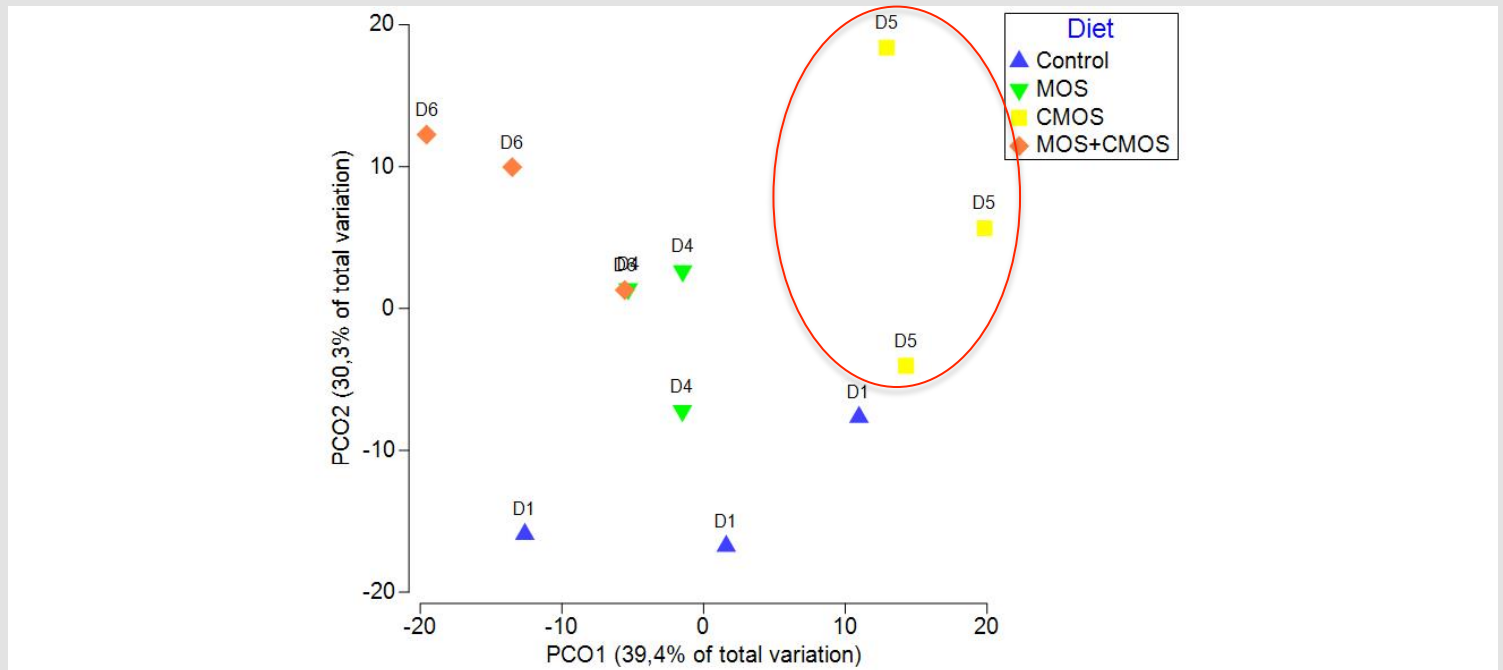
Gene expression analyses

SKIN



Gene expression analyses

SKIN PCO



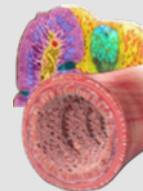
PERMANOVA

| MOS | cMOS | INTER. |
|--------------|--------------|--------------|
| P-PERM.<0.05 | P-PERM.<0.05 | P-PERM.<0.05 |

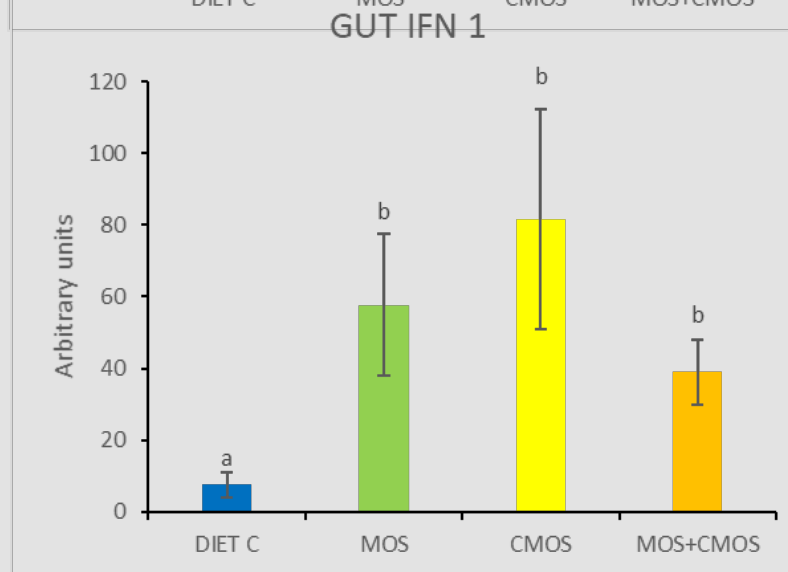
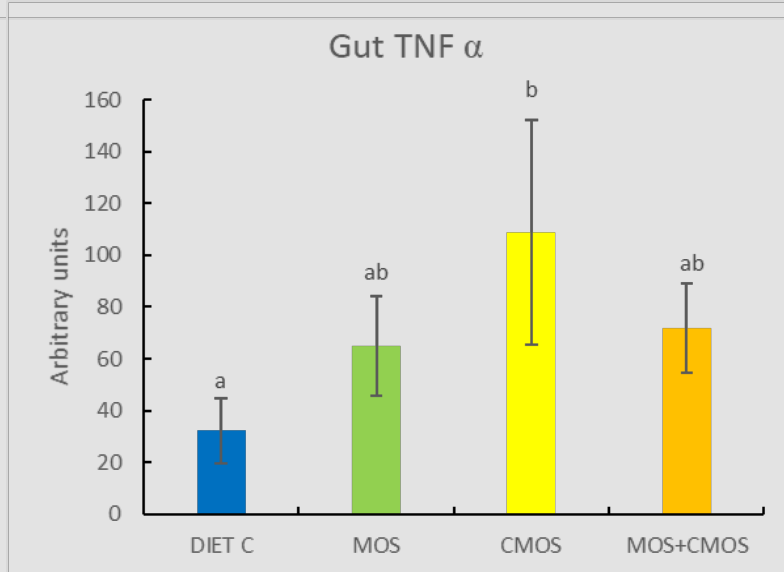
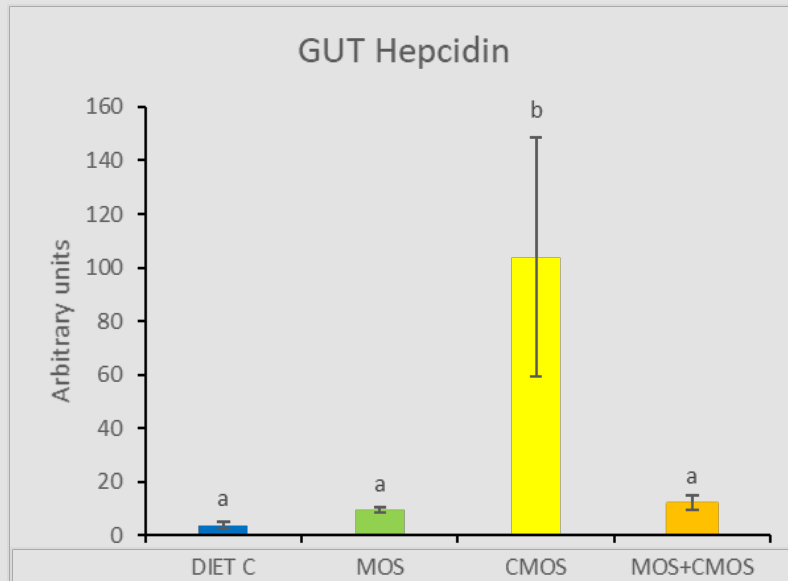
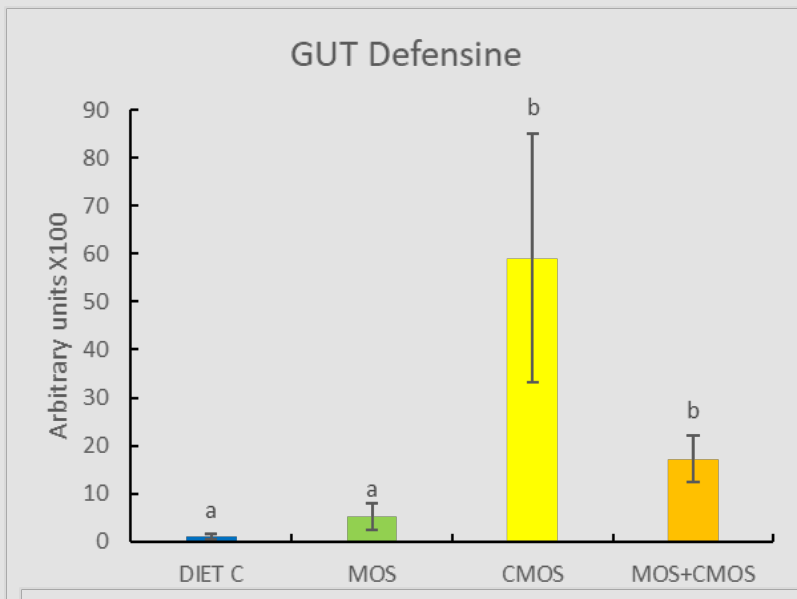


Gene expression analyses

POSTERIOR GUT (INNATE IMMUNE PARAMETERS)

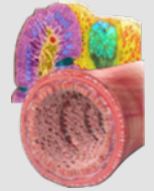


P<0.05

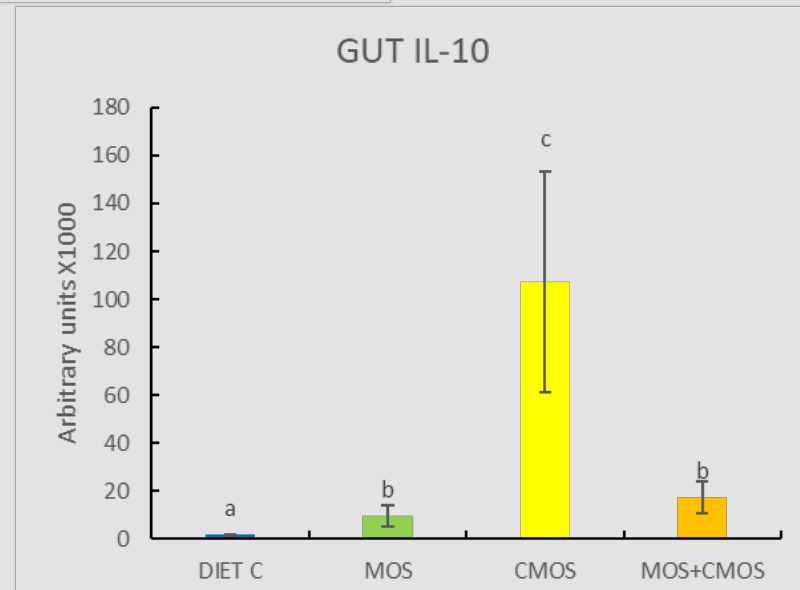
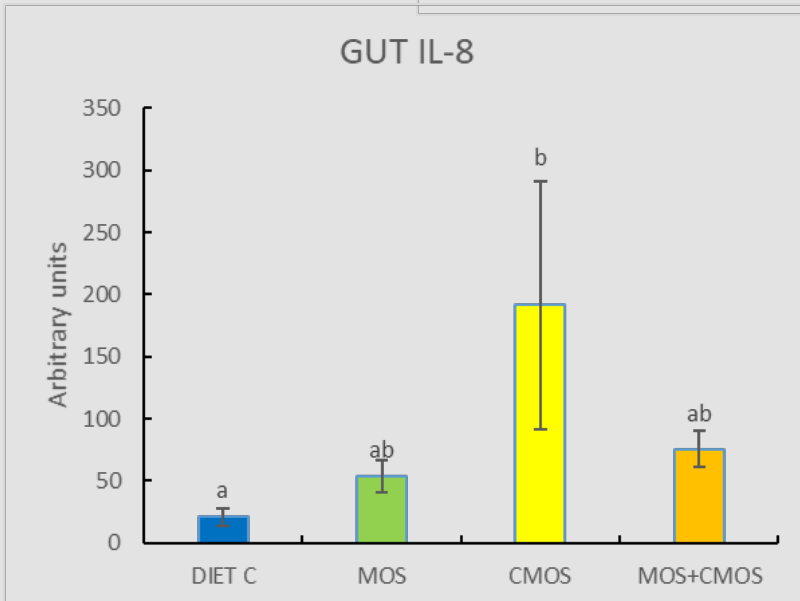
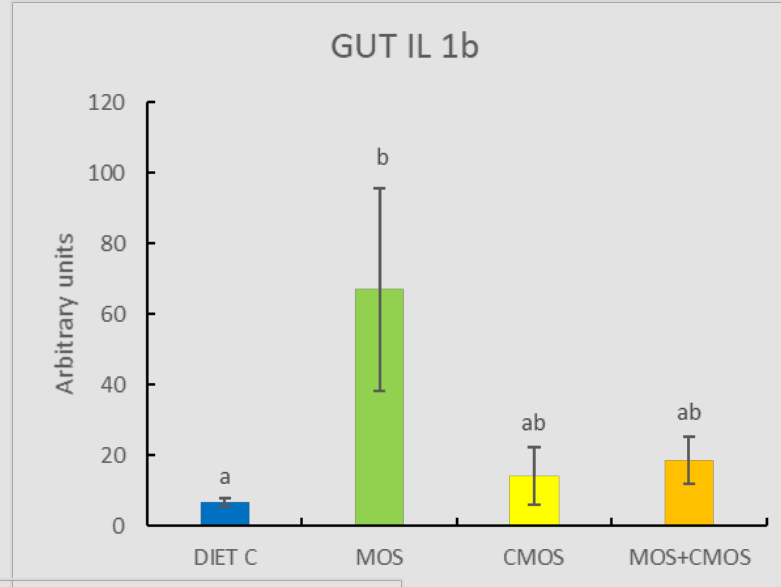


Gene expression analyses

POSTERIOR GUT (INTERLEUKINS)



P<0.05



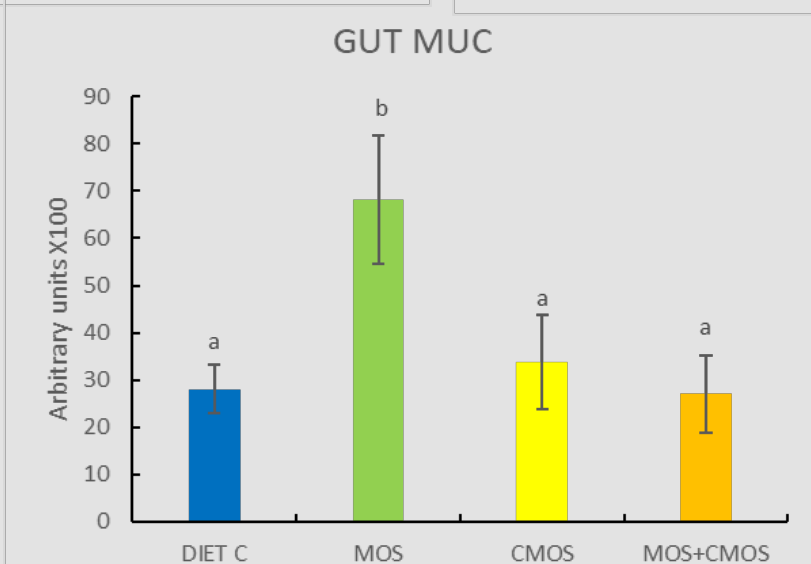
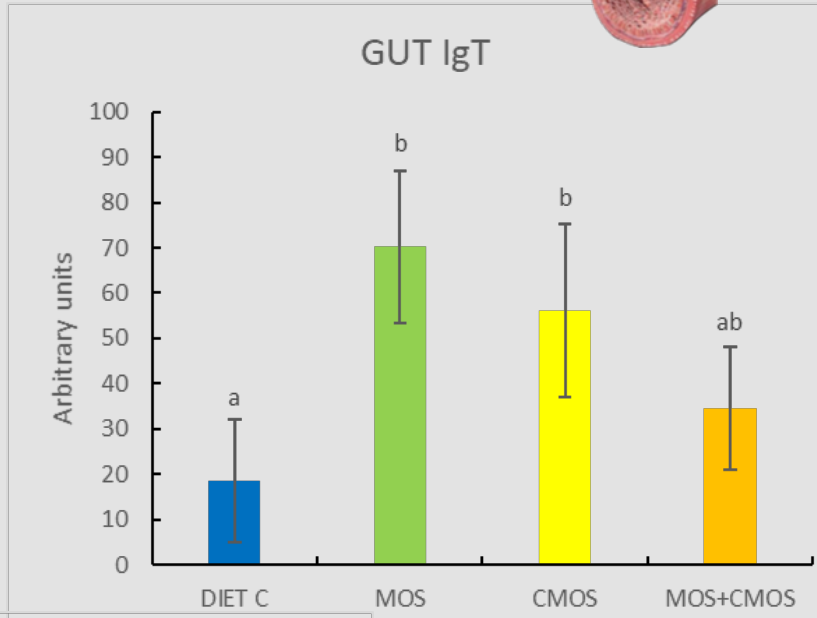
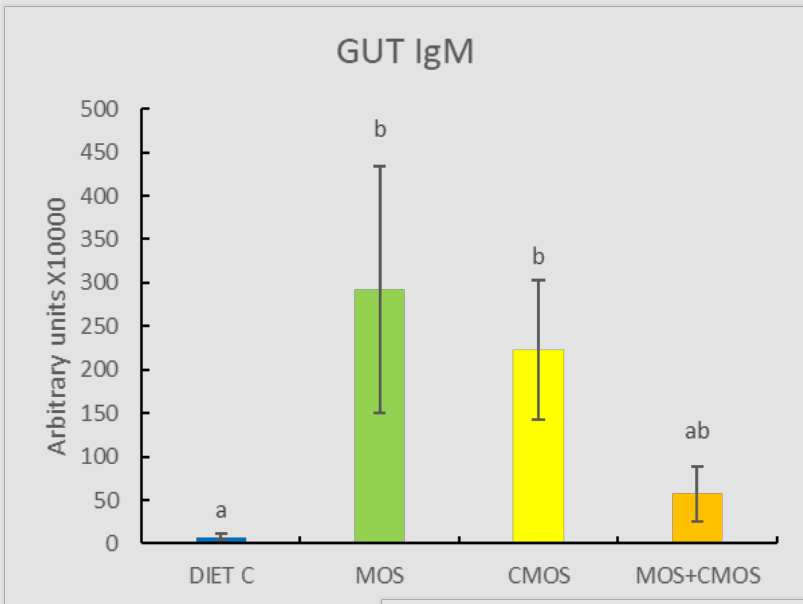


Gene expression analyses

POSTERIOR GUT (ADAPTIVE IMMUNE PARAMETERS AND MUCINE PRODUCTION)

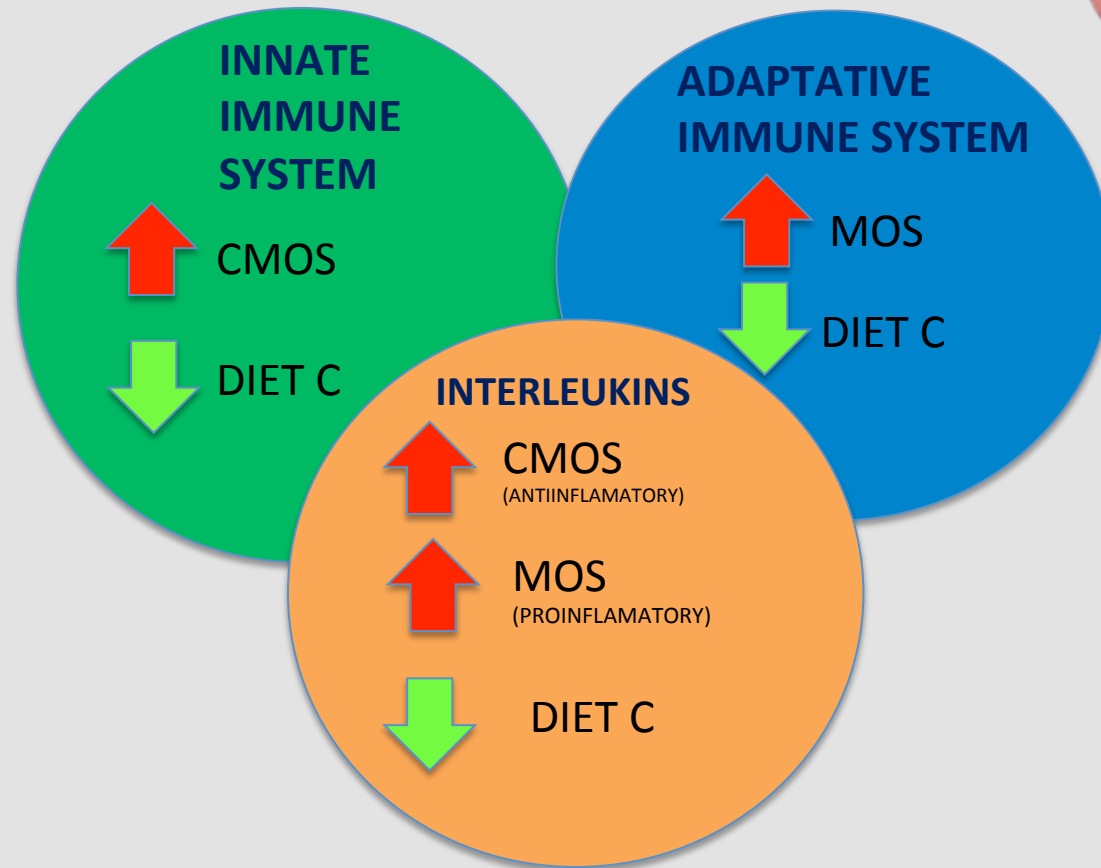
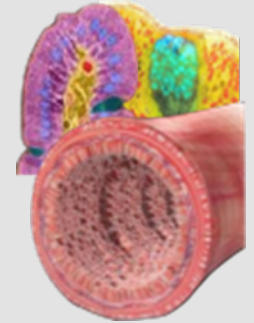


p<0.05



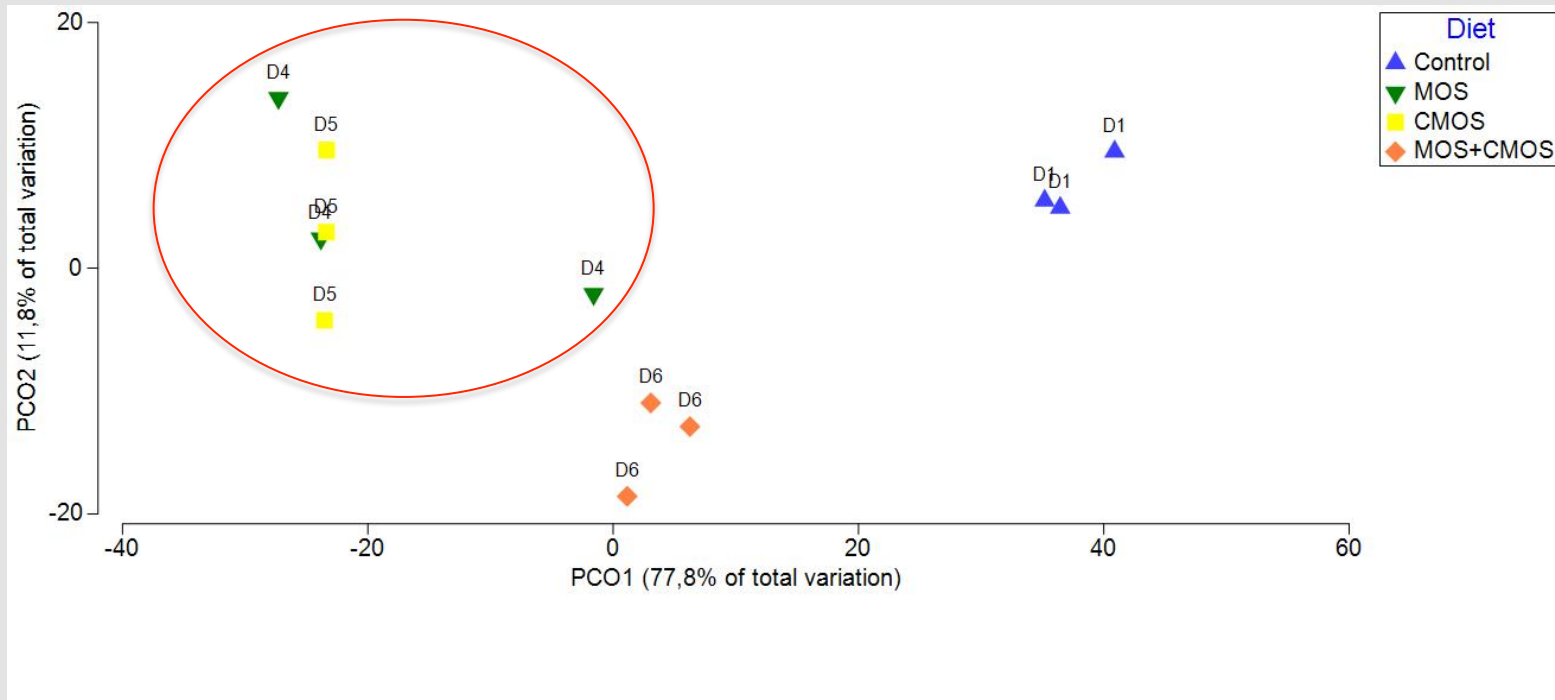
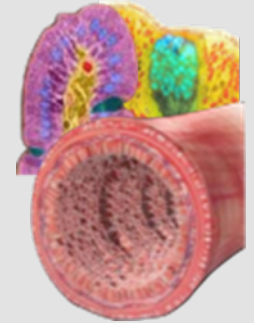
Gene expression analyses

POSTERIOR GUT



Gene expression analyses

POSTERIOR GUT PCO



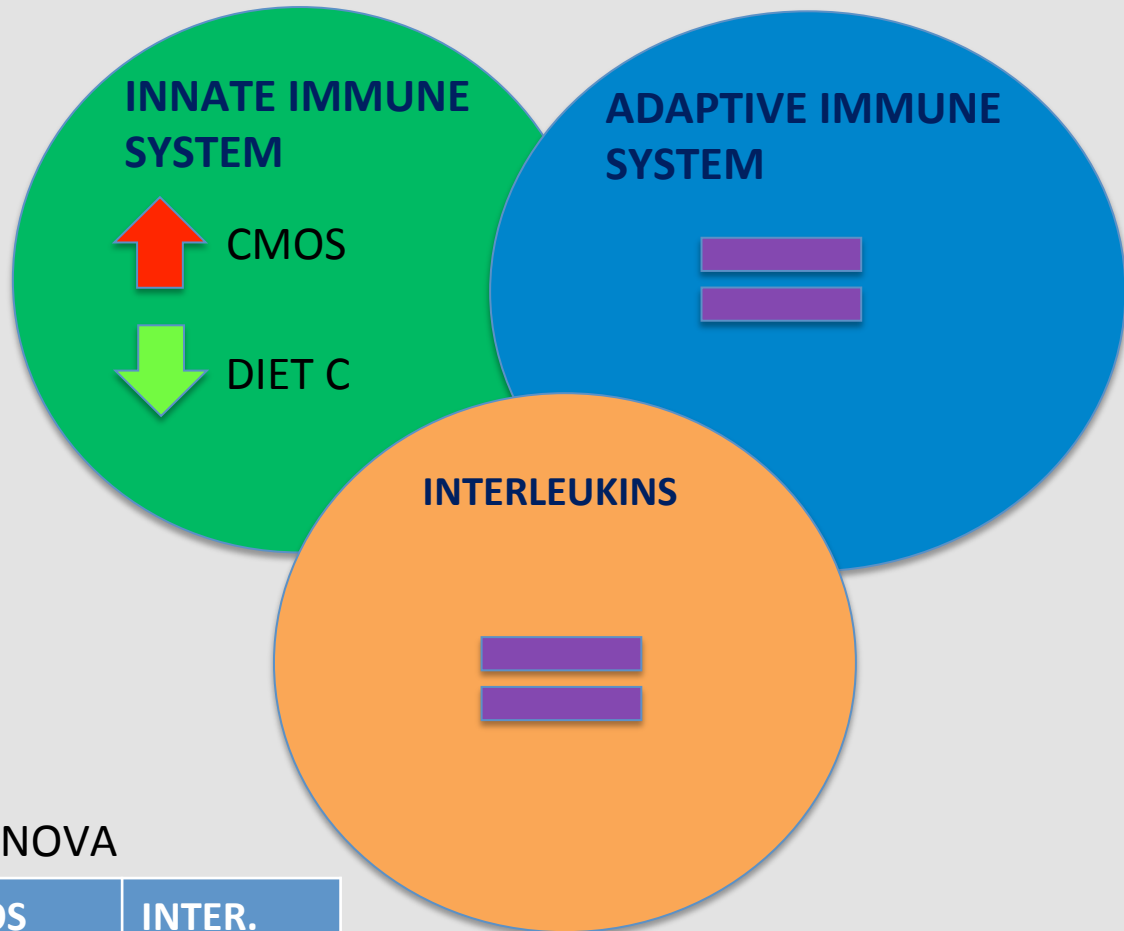
PERMANOVA

| MOS | cMOS | INTER. |
|--------------|--------------|--------------|
| P-PERM.<0.05 | P-PERM.<0.05 | P-PERM.<0.05 |



Gene expression analyses

GILL



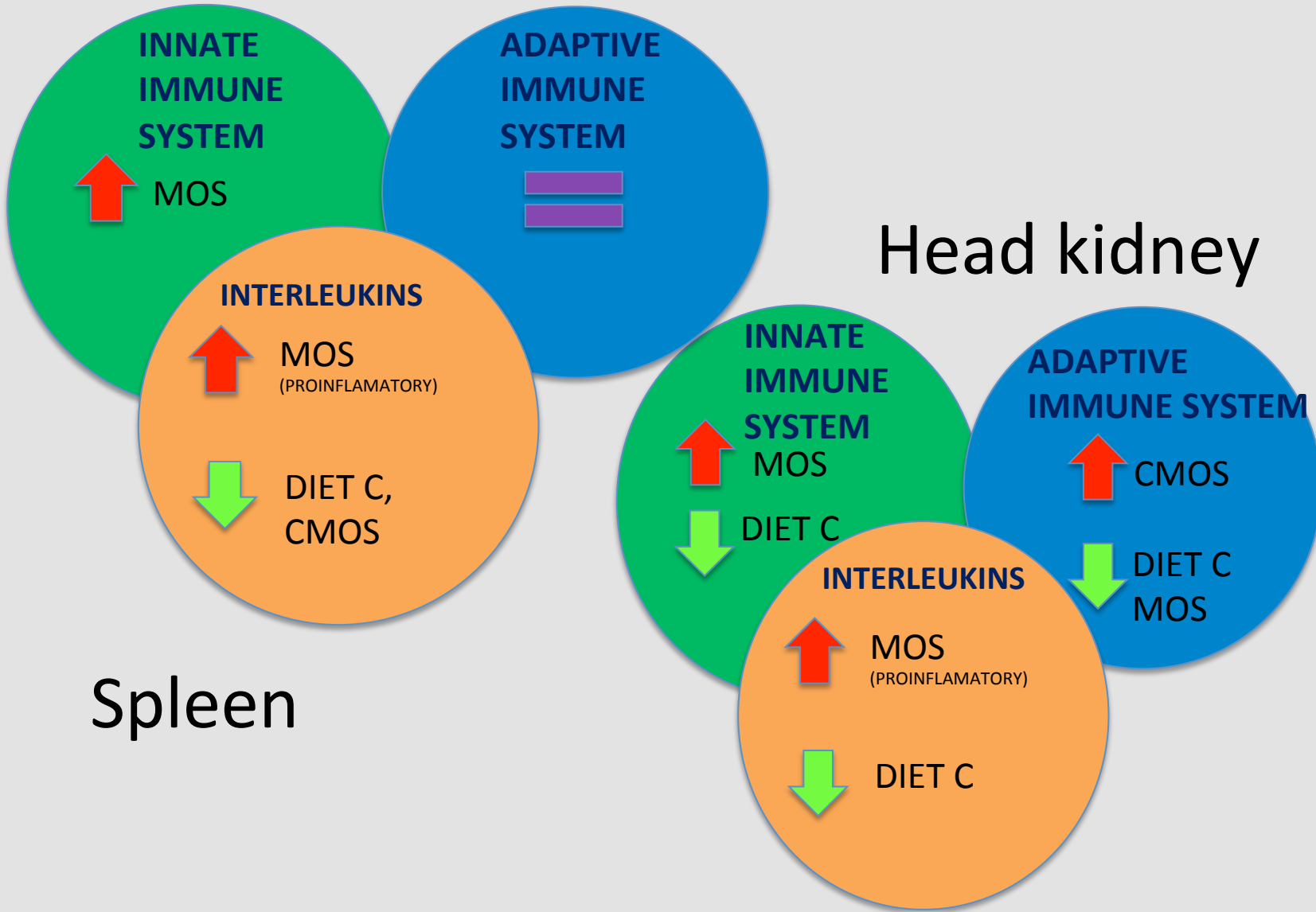
PERMANOVA

| MOS | cMOS | INTER. |
|--------------|--------------|--------------|
| P-PERM.>0.05 | P-PERM.>0.05 | P-PERM.>0.05 |



Gene expression analyses

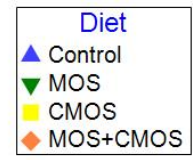
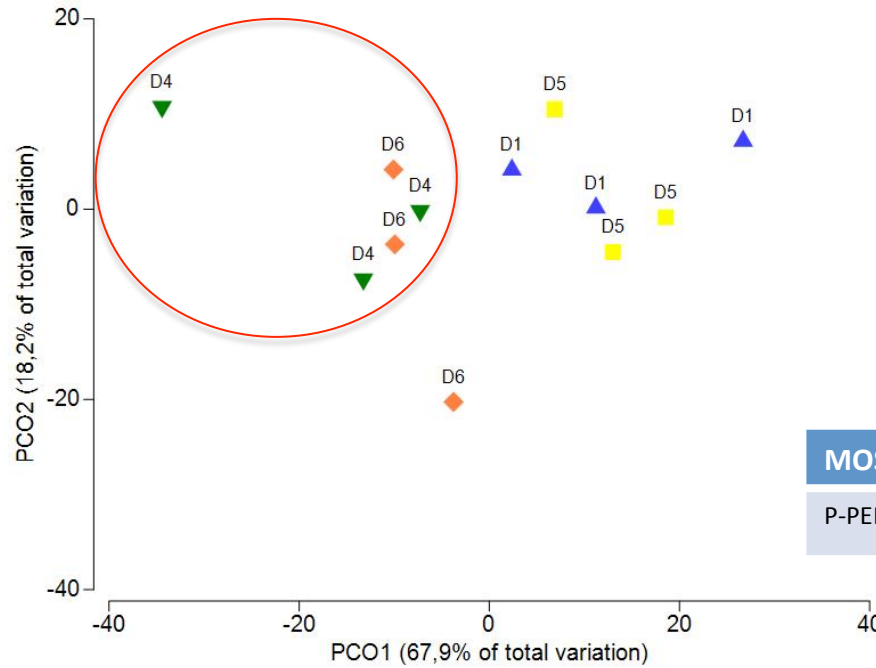
Spleen and Head kidney



Spleen

Head kidney





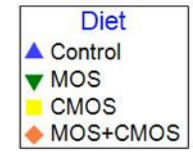
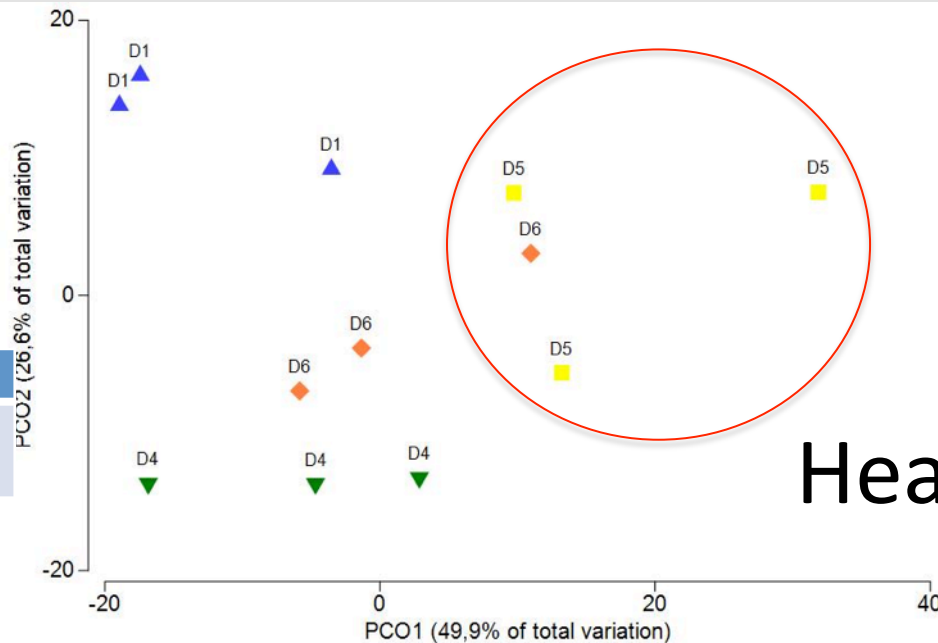
Spleen

PERMANOVA

| MOS | cMOS | INTER. |
|--------------|--------------|--------------|
| P-PERM.<0.05 | P-PERM.>0.05 | P-PERM.<0.05 |

PERMANOVA

| MOS | cMOS | INTER. |
|--------------|--------------|--------------|
| P-PERM.<0.05 | P-PERM.<0.05 | P-PERM.<0.05 |



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



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