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Increased parasite resistance of greater amberjack (*Seriola dumerili* Risso 1810) juveniles fed a cMOS supplemented diet is associated with upregulation of a discrete set of immune genes in mucosal tissues

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2 **a cMOS supplemented diet is associated with upregulation of a discrete set of immune genes**
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4

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15

16

17 Abstract

18 The main objective of this study was to determine the effect of two forms of mannan
19 oligosaccharides (MOS: Bio-Mos® and cMOS: Actigen®, Alltech Inc, USA) and their combination
20 on greater amberjack (*Seriola dumerili*) growth performance and feed efficiency, immune
21 parameters and resistance against ectoparasite (*Neobenedenia girellae*) infection. Fish were
22 fed for 90 days with 5 g kg^{-1} MOS, 2 g kg^{-1} cMOS or a combination of both prebiotics, in a
23 *Seriola* commercial base diet (Skretting, Norway). At the end of the feeding period, no
24 differences were found in growth performance or feed efficiency. Inclusion of MOS also had no
25 effect on lysozyme activity in skin mucus and serum, but the supplementation of diets with
26 cMOS induced a significant increase of serum bactericidal activity. Dietary cMOS also reduced
27 significantly greater amberjack skin parasite levels, parasite total length and the number of
28 parasites detected per unit of fish surface following a cohabitation challenge with *N. girellae*,
29 whereas no effect of MOS was detected on these parameters. Of 17 immune genes studied
30 cMOS dietary inclusion up-regulated hepcidin, defensin, Mx protein, interferon-γ (IFNγ),
31 mucin-2 (MUC-2), interleukin-1β (IL-1B), IL-10 and immunoglobulin-T (IgT) gene expression in
32 gills and/or skin. MOS supplementation had a larger impact on spleen and head kidney gene
33 expression, where piscidin, defensin, iNOS, Mx protein, interferons, IL-1β, IL-10, IL-17 and IL-22
34 were all upregulated. In posterior gut dietary MOS and cMOS both induced IL-10, IgM and IgT,
35 but with MOS also increasing piscidin, MUC-2, and IL-1β whilst cMOS induced hepcidin,
36 defensin and IFNγ. In general, the combination of MOS and cMOS resulted in fewer or lower
37 increases in all tissues, possibly due to an overstimulation effect. The utilization of cMOS at the
38 dose used here has clear benefits on parasite resistance in greater amberjack, linked to
39 upregulation of a discrete set of immune genes in mucosal tissues.

40

41 Keywords

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42 MOS; prebiotics, MALT; amberjack; ectoparasites; cytokines

43

44

45

46 1.Introduction

47 *Seriola* aquaculture has traditionally been focused on yellowtail kingfish (*S. lalandi*) and 48 Japanese amberjack (*S. quinqueradiata*) [1]. In Europe, greater amberjack (*Seriola dumerili*, 49 Risso 1810) is considered an emerging aquaculture species due to its high commercial value 50 and fast-growth [2], where under appropriate culture conditions they can reach 6 kg in 2.5 51 years [3]. Nevertheless, greater amberjack production in sea cages is limited by several 52 bottlenecks, with monogenean ectoparasite outbreaks a key concern [4, 5, 6].

53 *Neobenedenia girellae* is a monogenean ectoparasite that has become one of the main causes 54 of greater amberjack parasitic infections. It is characterised by a broad host range and wide 55 distribution in warm waters, with an important prevalence in aquaculture farms [4, 7]. Its 56 lifecycle is highly dependent of seasonal temperature [8, 9, 10, 11] which promotes the 57 parasite attachment to the host. Furthermore, parasite attachment to fish skin produces 58 important alterations [5, 12] such as wounds and ulcers, promoting secondary infections [13], 59 thereby increasing mortality. To fight secondary infections, especially those caused by fungi 60 and bacteria, several different strategies have been adopted, mainly based on the use of 61 antibiotics and topical treatments that have some risks [14]. Nowadays, one of the most 62 common strategies to avoid the use of antibiotics is to boost the immune system to enable fish 63 to overcome pathogen infections [15, 16]. These strategies include dietary inclusion of 64 prebiotics and use of functional feeds, some of which have been shown to affect ectoparasite 65 prevalence [17, 18].

66 Prebiotics are commonly used in the animal production industry due to their effects on the 67 immune system leading to pathogen protection [19]. It has been well established that the by-68 products produced when beneficial commensal bacteria ferment prebiotics play a key role in 69 improving host health [20]. New prebiotics have been showing successful results [21], 70 including mannan oligosaccharide (MOS) by-products [22, 23, 24, 25]. Studies of MOS 71 beneficial effects have focused on growth performance and health, especially the modulation 72 of intestinal microbiota and promotion of gut integrity in adult and juvenile fish [23, 26]. 73 However, MOS effects are known to be highly dependent upon the biotic parameters of the 74 cultured fish, including the species, culture conditions, duration of the supplementation, age 75 and size [27], [21].

76 Previous studies have shown that an inclusion level of 4g MOS kg^{-1} in diets increases growth 77 performance, feed efficiency and feed intake in salmonids and seabass after 67 days of 78 supplementation [28, 22]. In contrast, in gilthead seabream and channel catfish no effect was 79 observed on these parameters using this inclusion level during 63 and 42 days respectively [29, 80 30], but changes of the immune system were found. Similarly, in rainbow trout [31] fed a 81 functional diet with 2 g MOS kg^{-1} during 42 and 90 days improved antibody production and 82 lysozyme activity were found, and in Japanese flounder, after 56 days of dietary inclusion of 5 83 g MOS kg^{-1} gave higher lysozyme activity, although no differences were observed in the 84 numbers of cells undergoing phagocytosis or the phagocytic index [32]. However, in Atlantic

85 salmon (200g) fed a diet supplemented with 10 g MOS kg^{-1} for 4 months no effects on the
86 innate immune system were seen [33]. Such studies suggest that the effects are not consistent
87 between species or that there is a limited duration of the MOS effect on the host immune
88 response. Recently the study of key regulatory cytokines as markers has also become a useful
89 indicator of the immune system status in fish. For instance, previous studies with Atlantic cod
90 showed that MOS dietary inclusion produces changes in gut cytokine expression levels after 35
91 days of supplementation [34]. Clearly future studies on cytokines are warranted to shed light
92 on MOS effects.

93

94 Little information is available about the immune system of greater amberjack [10, 35] and few
95 studies have investigated the use of immunostimulants with this species [36, 37, 38], with
96 none using MOS or concentrated MOS (cMOS) inclusion. For this reason, the objective of the
97 present work was to determine the effect of MOS and cMOS (Bio-Mos® and Actigen®) and
98 their combination on greater amberjack juveniles, focusing on immune parameters, protective
99 effects against a *N. girellae* and any impact on growth/feed efficiency.

100

101 2. Materials and methods

102 The present study was conducted at the Scientific and Technologic Park of the University of Las
103 Palmas de Gran Canaria (Las Palmas, Canary Islands, Spain). The animal experiments described
104 comply with the guidelines of the European Union Council (2010/63/EU) for the use of
105 experimental animals and were approved by the Bioethical Committee of the University of Las
106 Palmas de Gran Canaria. For the whole trial, a tank is considered as an experimental unit.

107 2.1. Experimental fish and conditions

108 Two hundred and sixteen fish (mean weight 331.4 ± 30 g) were distributed in twelve
109 cylindroconical 1,000 L tanks with an open circulation (18 fish/tank). Water conditions were
110 monitored daily, maintaining salinity at 37 mg L^{-1}, oxygen values at 6.0 ± 1 ppm O_2 and
111 temperature at 23°C ± 0.3 during July, August and September. Fish were fed by hand 3 times
112 per day to apparent satiety. Uneaten pellets were recovered, dried and weighed.

113 2.2. Diets

114 The diets used combined a *Seriola* base diet designed by Skretting (Stavanger, Norway) and
115 containing 55% protein, 55% fish meal and 10% fish oil, with two different prebiotics, namely
116 MOS and cMOS (Bio-Mos® and Actigen® developed by Alltech, Inc.). Diet C (control) was
117 composed exclusively of the *Seriola* base diet, the MOS diet included 5 g Bio-Mos® kg^{-1}, the
118 cMOS diet 2 g Actigen ® kg^{-1}, and the MOS + cMOS diet had 5 g Bio-Mos ® kg^{-1} and 2 g Actigen
119 ® kg^{-1}. Each diet was randomly assigned to triplicate groups of fish (n=3x3).

120

121 2.3. Sampling procedures

122 Sampling was conducted after 0, 30 days, 60 days and 90 days of feeding, where growth and
123 feed utilization parameters were evaluated. Additionally, at the end of the feeding trial head
124 kidney, spleen, gills, posterior gut and skin of 3 fish per tank were sampled for immune gene

125 expression analysis. Skin mucus and blood (serum) were also collected from 3 fish per tank.
126 Finally, a parasite challenge against *N. girellae* was performed (as outlined below).

127

128 2.4. Fish performance parameters

129 Specific growth rate (SGR) and feed efficiency were calculated as follows:

130 SGR = (Ln (final weight) – Ln (initial weight))*100/feeding time (days)

131 Feed efficiency= (feed intake/ weight gain)

132

133 2.5. Gene expression analyses

134 Samples for gene expression analyses were collected in RNAlater and stored for 48 h at 6°C.
135 Total RNA was subsequently extracted using the Trizol reagent method (Invitrogen) according
136 to the manufacturer's instructions. RNA concentration and purity were determined by
137 spectrophotometry measuring the absorbance at 260 and 280 nm (NanoDrop2000, Thermo
138 Fisher Scientific, Madrid, Spain). Electrophoresis in agarose gels was conducted to check
139 extracted RNA quality by visualization of RNA bands. DNase treatment was applied to the
140 extracted RNA, according to the manufacturer's instructions, to remove possible
141 contaminating genomic DNA (AMPD1–1KT, Sigma–Aldrich, Broendby, Denmark). Total RNA
142 was reverse transcribed in a 20 µL reaction volume containing 2 µg total RNA, using a
143 ThermoScript ™ Reverse Transcriptase (Invitrogen) kit, until cDNA was obtained in a
144 thermocycler (Mastercycle ® nexus GSX1, Eppendorf AG, Hamburg, Germany) run according to
145 the manufacturer's instructions. The samples were then diluted 1:20 in miliQ water and stored
146 at -20°C.

147 Specific primers were designed to target genes found in genbank from species phylogenetically
148 related with *S. dumerili* (Table 1), following the methodology described in [39]. The primers
149 were used to amplify products using amberjack cDNA obtained from a pool of gill, posterior-
150 gut, head kidney and spleen tissue, and the products cloned and sequenced. At least a partial
151 sequence was obtained for all the target genes and these partials were sufficient in length to
152 determine gene identity and develop qPCR primers. qPCR was conducted with SYBRgreen and
153 truestar taq following a programme of: 1 cycle of 6 min denaturalization at 95°C, 45 cycles of
154 amplification (25 s at 95°C, 30 s at the annealing temperature, 25 s at 70°C for the extension,
155 and 5 s at 82°C), 1 cycle for the melting curve of 5 s at 95°C and 1 min at 75°C, ending with 1
156 cycle of cooling for 1 min at 40°C. MUC-2 was only analysed in the mucosal tissues and not
157 head kidney and spleen.

158

159 2.6. Blood and mucus immunological parameters

160 Serum was obtained by centrifuging the collected blood after clotting overnight at 4°C. Skin
161 mucus was obtained following the methodology described by Guardiola et al [40] with some
162 modifications. Skin mucus was collected by gently scrapping the surface of the fish skin with
163 autoclaved microscopy slides and diluted 1:1 with filtered and autoclaved salt water. Lysozyme
164 activity was determined as described by Ellis [41]. Lysozyme activity was expressed in units ml^{-}
165 ^{1}, were one unit of lysozyme was considered as the quantity of enzyme needed for reducing

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166 absorbance by 0.001 per millilitre of serum and mucus per minute. Bactericidal activity was
167 measured with a modification of the method described by Sunyer and Tort [42], using
168 *Photobacterium damselae*.

169 2.7. Parasite infection

170 The parasite source was a tank (10,000 L) of *S. dumerili* naturally infested with *Neobenedenia*
171 *girellae* at high parasite density. Nets (0.14 mm pore diameter) were placed into the tank to
172 entangle the eggs and collect them. After 24 h eggs were introduced into a 1,000 L tank with
173 200 uninfected *S. dumerili* juveniles. After 10 days, all the fish were infected to the same
174 degree. Then, 96 infected animals from the source tank were placed into twelve $0.03m^3$ cages
175 (8 infected fish per cage and one cage per experimental tank) for 15 days, to enable a
176 cohabitation challenge after 100 days of prebiotic inclusion. After 15 days of cohabitation, the
177 remaining one hundred eighty experimental fish were sampled, and a visual evaluation of
178 infection level for each fish was carried out by 3 different trained researchers. The levels were
179 scored between 0 (no parasites observed), 1 (between 1 and 5 parasites), 2 (between 6 and
180 15) and 3 (more than 15). After that, the fish were introduced into freshwater to release all of
181 the attached parasites, and the parasites counted and measured. The number of parasites per
182 fish was converted into the number of parasites per square centimetre of fish surface area,
183 calculated following the method described in Ohno et al [43]. Total length of 50 adult parasites
184 per tank was recorded using a profile projector (Mitutoyo, PJ-A3000).

185 2.8. Statistical analyses

186 The statistical analyses followed the methods outlined by Sokal and Rolf [44], with means and
187 standard deviations (SD) calculated for each parameter measured. All data were tested for
188 normality and homogeneity of variance. Data were subjected to one-way ANOVA and
189 differences were considered significant when $P < 0.05$. Two-way ANOVA was conducted for
190 MOS, cMOS and the interaction among treatments. If the variances were not normally
191 distributed, data were transformed (log_{10}) and the Kruskall-Wallis non-parametric test applied.
192 Kruskall-Wallis analysis was also used for range-comparison statistical analyses. Analyses were
193 performed using SPSS software (SPSS for windows 10).

194 Multivariant analyses and their plots were performed using PRIMER 7 and PERMANOVA. The
195 number of permutations was established at 999. PERMANOVA analysis considered differences
196 significant when the permutation p-value (p perm.) was below 0.05.

197

198 3. Results

199

200 3.1. Growth performance

201 No effect of MOS, cMOS or their combination was observed in final weight, SGR or feed
202 efficiency among fish fed the different dietary treatments ($p>0.05$), although fish fed the cMOS
203 diet tended to perform better (+4% SGR) (Table 2).

204

205 3.2. Serum and skin mucus immunological parameters

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206 After 90 days of feeding, two way-ANOVA analysis revealed a significant increase in serum
207 bactericidal activity in fish fed MOS (F=6.68, P=0.04) and cMOS (F=17.56, P=0.02), whereas no
208 effect was detected when measured in mucus (Table 2). Lysozyme activity in mucus and serum
209 was not affected by MOS or cMOS dietary supplementation. No interaction between MOS and
210 cMOS was detected for the mucus and serum immune parameters evaluated (Table 2).

211

212 3.3. Parasite challenge

213 Greater amberjack given dietary supplementation of cMOS for 90 days had significantly
214 reduced skin parasite levels (F=6.17, P=0.01), parasite total length (F=15.47, P=0.01) and the
215 number of parasites by unit of fish surface (F=52.36, P=0.01) following challenge with *N.*
216 *girellae*. No specific effect of MOS was found on these parameters (Table 2) and no interaction
217 between MOS and cMOS was detected.

218

219 3.4. Gene expression

220 At the end of the feeding trial (90 days), two way-ANOVA analyses showed that dietary cMOS
221 up-regulated skin hepcidin, MUC-2, IL-1ß, IL-10 and IgT (Table 3). On the other hand, a down-
222 regulation of skin iNOS gene expression was detected after dietary MOS supplementation, and
223 supplementation with both products resulted in a down-regulation of skin IL-10, IL-17D and IgT
224 and a reduced impact on IFN expression vs the single supplements (Table 3).

225 In gills, dietary cMOS up-regulated hepcidin, defensin, Mx protein and IFNγ transcript levels
226 (Table 4). No effects of dietary MOS were found. However, supplementation with both
227 products resulted in down-regulation of gill IgT and reduced the cMOS effect on defensin and
228 Mx protein gene expression in gills (Table 4).

229 Regarding fish posterior gut, two way-ANOVA analysis showed that dietary cMOS up-regulated
230 expression of hepcidin, defensin, IFNγ, IL-10, IgM and IgT. Additionally, dietary MOS up-
231 regulated piscidin, MUC-2, IL-1β, IL-10, IgM and IgT gene expression. However,
232 supplementation with both products down-regulated IFNγ (F= 1.09, P= 0.02) and IgM (F=2.41,
233 P= 0.02) gene expression and lost the effects on IL-10 and IgT (Table 5).

234 Head kidney gene expression analyses showed that dietary cMOS up-regulated hepcidin, IFNd,
235 IL-10 and IL-22, while MOS up-regulated iNOS, Mx protein, IFNd, IL-10, IL-17D and IL-22.
236 Supplementation with both products resulted in up-regulation of defensin and Mx protein but
237 down-regulated IL-10 transcript levels relative to single supplementation (Table 6). In addition,
238 the effects on IFNd and IL-22 were lost.

239 Lastly, cMOS down-regulated spleen hepcidin gene expression whilst dietary MOS induced
240 expression of piscidin, defensin, IFNγ, IL-1β and IL-17D in this tissue. Supplementation with
241 both products further increased defensin expression (Table 7).

242 Multivariant analyses comparing gene expression data presented different responses for each
243 tissue and are presented in Annex 1 (supplementary files). Principal coordinates analysis (PCO)
244 of skin clearly separated responses in fish fed the cMOS diet from fish fed the other dietary
245 treatments, with the main sources of variation due to anti-microbial peptides (AMPs) (piscidin
246 and defensin), MUC-2, iNOS, TNFα, Mx Protein, IL-8, IL-10, IL-17 and IFN genes. PERMANOVA

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247 analysis indicated differences in gene expression between MOS and cMOS, with an interaction
248 effect more related to PC1 (p-perm. <0.05).

249 PCO analysis in gill partially separated the MOS and cMOS effects due to AMPs and IFNs.
250 Nonetheless, PERMANOVA analysis showed no difference between MOS and cMOS in this
251 tissue (p-perm. >0.05).

252 PCO analysis of posterior gut clearly separated dietary treatments into three different groups:
253 control, MOS and cMOS, and MOS+cMOS. This variation was due to the effect on AMPs, IL-10,
254 IFNs and iNOS gene expression. Hence, the posterior gut PCO PERMANOVA analysis found
255 differences between MOS, cMOS and an interaction effect more related to PC2 (p-perm.
256 <0.05).

257 PCO analysis of head kidney discriminated cMOS from the other treatments due to the effect
258 of this prebiotic on Igs and AMP gene expression. MOS treatment was also differentiated from
259 the other treatments in the spatial distribution by PCO analysis due to effects on IFNs, ILs,
260 defensin and TNFα gene expression. PERMANOVA comparisons showed differences in the
261 MOS and cMOS dietary effects and also on interaction (p-perm. <0.05).

262 In spleen PCO analysis discriminated MOS from the other treatments mainly due to its effect
263 on piscidin and IgM gene expression. PERMANOVA analysis only showed a difference for the
264 MOS treatment (p-perm. <0.05).

265 Fish fed cMOS were differentiated from other groups in skin and posterior gut, together with
266 MOS in this last tissue, with differences found using PERMANOVA (p-perm. <0.05) in terms of
267 increasing immune parameters compared with control fish. Fish fed dietary MOS showed an
268 up-regulation in immune parameters in spleen and head kidney (p-perm. <0.05), with cMOS
269 responsible for increased Ig levels.

270 4. Discussion

271 The present study examined the effects of dietary supplementation with MOS and cMOS on
272 greater amberjack growth, immunity and disease resistance. No effects on growth
273 performance were found, in agreement with previous studies on hybrid tilapia (*Oreochromis*
274 *niloticus x O. aureus)* or channel catfish (*Ictalurus punctatus*) [45, 30]. In contrast, in studies
275 conducted with European sea bass (*Dicentrarchus labrax*), MOS and cMOS enhanced fish
276 growth performance and improved FCR [22, 23]. Similarly, in fresh water species such as
277 rainbow trout (*Oncorhynchus mykiss*), MOS dietary inclusion increases growth performance
278 and reduces FCR [31]. These effects are likely related with the enhanced nutrient availability
279 due to changes in digestive enzyme activity or in gut morphology, that subsequently increase
280 absorption efficiency [46]. However, such differences in the impact of MOS on growth
281 parameters among species suggest that these effects are highly dependent on the
282 supplementation level, fish species and age, rearing conditions and diet composition [27].

283 An increase in mucus production has been shown to be a key factor for reducing ectoparasite
284 adhesion in fish species such as Atlantic salmon (*Salmo salar*)[47]. MOS promotes both the
285 enhancement of the innate immune system and mucus production (for reviews see [27, 46,
286 16]), reducing bacterial and parasite adherence to the host. In the present study, cMOS
287 induced an up-regulation of skin MUC-2 compared with fish fed the other dietary treatments,
288 suggesting it promotes mucus production. Dietary MOS showed a similar effect on the gut, in
289 agreement with previous results in European sea bass [23]. Whilst the impact of prebiotics on

290 ectoparasite resistance is poorly studied [18], cMOS showed a clear effect on parasite
291 adhesion in the present work. cMOS not only prevented parasite attachment but also reduced
292 the growth and development of the parasites concomitant with increased immune responses
293 (see below). A mobilization of fish defences to the skin mucus has been described as an effect
294 of prebiotics [48], and could prevent the correct development of parasites as they attempt to
295 overcome the first physical and chemical barriers of the host. In line with this, red drum
296 (*Sciaenops ocellatus*) show a reduced mortality and parasite level after challenge with
297 *Amyloodinium ocellatum*, when receiving a diet supplemented with MOS at 10 g kg^{-1} for 30
298 days [17]. Similarly, Atlantic salmon fed for 98 days with 4 g MOS kg^{-1} had a significantly
299 reduced parasite load [18].

300 MOS has shown a more consistent effect on the immune system, improving parameters such
301 as lysozyme activity in fish species including channel catfish, Japanese flounder (P*aralichthys*
302 *olivaceus*), rainbow trout or European sea bass when supplemented at similar doses [46].
303 Whilst skin mucus and serum lysozyme activity were unaffected by dietary MOS in the present
304 study, serum bactericidal activity was increased in fish fed the supplemented diets. This
305 indicates that other molecules within the innate immune system that effect antimicrobial
306 responses are affected by these prebiotics [49]. Indeed, the results of the present study show
307 there is upregulation of antimicrobial peptide (AMP) gene expression in all of the tissue
308 studied, and these molecules are an important part of the innate immune system in fish. AMPs
309 are stored in cells so that they are readily available after an infection [50, 51]. That MOS mainly
310 increased piscidin whilst cMOS mainly increased hepcidin and defensin is curious. It is known
311 that different cytokines can have unique specificity regarding AMP gene induction [53, 54, 55]
312 and may be a factor here. The kinetics of AMP induction can also vary, as seen in rainbow trout
313 after dietary inclusion of peptidoglycans [56].

314 Adaptive immunity also plays a key role in the host response against ectoparasites [52, 47]. IgT
315 is considered a mucosal associated immunoglobulin in fish [57, 58, 59]. The increase of IgT
316 transcript levels in skin after feeding cMOS in the present study supports the key role of this
317 immunoglobulin at mucosal surfaces, and could be related with the reduction of the parasite
318 load induced by cMOS. The mode of action of this immunoglobulin is not completely
319 understood, although an up-regulation in IgT expression in skin has been observed as a
320 response to sea lice infection in Atlantic salmon [60], as well as to parasites in the gills and gut
321 [57], [61].

322 Key genes of the immune system have traditionally been selected as markers of immune
323 system activation by prebiotics, including TNFα, IL-1β, IL-8, IL-10, iNOS, IFNs, IgM, TLRs and
324 MHC [62]. As discussed above, there is a direct linkage between MOS administration and
325 innate immune system modulation [26, 15], with the skin a key point of entry of potential
326 pathogens in fish [63]. In humans an increase of TNFα expression with no IL-10 response is
327 associated with an increase of mucosal IL-17 [64, 65, 46], similar to the results obtained in the
328 present study. A balanced pro and anti-inflammatory response in the skin is linked to an
329 increased inflammatory response at the moment of parasite attachment, and gives lower
330 parasite levels in Atlantic salmon infected with sea lice [66]. Indeed, our PCO analysis showed a
331 higher effect of cMOS in skin, relative to MOS, mainly due to upregulation of AMPs (hepcidin,
332 defensin, piscidin), MUC-2, TNFα, Mx Protein, IL-8, IL-10, IL-17 and IFNs as revealed by
333 PERMANOVA.

334 In studies of prebiotics, especially MOS, the gut is the main tissue where the effects of the
335 prebiotic take place. Although cMOS induced higher hepcidin, defensin, IFNγ, IL-10, IgM and

336 IgT, the stimulatory effect of MOS was equal to or even higher for IL-10, IgM and IgT and also
337 impacted piscidin, MUC-2 and IL-1β unlike cMOS. This modulation of the expression of these
338 selected genes reveals an increased cytokine response and enhanced mucus production [67,
339 26, 46]. Hence both MOS and cMOS could potentially have positive effects on resistance to gut
340 parasites and this should be explored in future studies.

341 The impact of dietary MOS was also assessed in head kidney and spleen, two important
342 systemic immune tissues in fish that play a key role in the maturation of B-cells and phagocytic
343 cells [68]. The importance of the head kidney and spleen response during parasite infections
344 has been described in many studies where systemic responses help coordinate the fight
345 against secondary infections and participate in the wound healing process [47]. Furthermore,
346 upregulation of proinflammatory cytokines such as IL-1β, IL-17 and TNFα in head kidney and
347 spleen has been associated with reductions in sea lice load in pink salmon [69, 70], akin to the
348 results found in spleen in the present study where IL-1β, IFNγ and IL-17D were increased.
349 cMOS is a more purified product than MOS, and some components of the outer cell wall of
350 *Saccharomyces cerevisiae* strains (probably β-glucans) could have been removed during the
351 production process, as suggested by Torrecillas et al [71]. Since β-glucans are potent PAMPs
352 able to trigger innate immunity [72], this would explain the higher stimulation of innate
353 immune parameters with MOS but not cMOS. On the other hand, B-cell stimulation will lead
354 to increased adaptive immunity, with Ig transcripts notably increased by dietary cMOS in the
355 present study. Indeed, the dispersion patterns seen in the head kidney PCO analysis in the
356 cMOS dietary group were explained by the increased number of Ig transcripts, which
357 separated cMOS from the other dietary groups. Tadiso et al [60] found that immunological
358 changes in spleen affected the skin response, strengthening the relationship between systemic
359 and mucosal immune responses.

360 The combination of MOS and cMOS showed similar results to the control diet group for most
361 of the genes analysed. PCO and PERMANOVA analyses typically showed an interaction
362 between MOS and cMOS, probably related to a loss of effect by overstimulation. It has been
363 reported previously that the combination of two different prebiotics, like MOS and
364 peptidoglycans, can have positive synergic effects in the immune system when suitable doses
365 are used [73]. In the case of cMOS, it is a second generation MOS, therefore the pathways of
366 action of these two prebiotics should be similar. Thus, the combination of both prebiotics likely
367 induces effects similar to using a high dietary inclusion of these prebiotics alone, and may
368 result in receptor overload or immune fatigue related to a high energy cost of continued
369 immunostimulation [74, 75, 76].

370 In conclusion, the utilization of dietary cMOS at 2 g kg^{-1} increased protection against *N. girellae*
371 after 90 days of feeding, by reducing the parasite level and parasite total length. This
372 protection was associated with up-regulation of several proinflammatory cytokines, AMPs,
373 MUC- 2 and IgT genes in skin and enhanced serum bactericidal activity. In contrast, dietary
374 MOS at 5 g kg^{-1} stimulated AMPs, IFNs and proinflammatory cytokines in head kidney and
375 spleen, but had little effect in skin and these fish had a higher parasite level compared with
376 fish fed the cMOS diet. The posterior gut also showed immune stimulation with dietary MOS
377 and cMOS, in terms of effects on expression of AMPs, proinflammatory cytokines, IgM and IgT.
378 However, the combination of MOS and cMOS appears to have delivered an over stimulation of
379 the immune system, resulting in a lack of effect.

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Table 1. Primers used for gene expression analysis by RT-qPCR in skin, gill, posterior gut, head kidney and spleen of greater amberjack juveniles (*Seriola dumerili*) fed MOS and cMOS (t = 90 days).

Gene	Name	Ann. temp. (°C)*	Product size (bp)	Forward Sequence	Reverse Sequence
Hep	Hepcidin	61	99	GATGATGCCGAATCCCGTCAGG	CAGAAACCGCAGCCCTTGTTGGC
Pis	Piscidin	58	112	ATC GTC CTG TTT CTT GTG TTG TCA C	CGC TGT GGA TCA TTT TTC CAA TGT GAA A
Def	Defensin	60	133	ATGAGGCTGCATCCTTTCCATG	AGAAAATGAGATACGCAACACAAGAAGCC
iNOS	Inducible Nitric oxyde synthase	60	151	TGTTTGGCCTTGGCTCCAGGG	GCCCAAGTTCTGAATGACTCCTCCTG
TNFα	Tumor necrosis factor α	62	212	GAAAACGCTTCATGCCTCTC	GTTGGTTTCCGTCCACAGTT
MX Prot	Interferon-inducible Mx protein	61	211	GGCTACATGATTGTGAAGTGCAGGG	CTTCCAGTCGAGGCAGAGATTTCTCAATGT
IFN ɣ	Interferon γ	59	163	AACTTGGTTTCACGGTGCAG	TCACAACACCGAGAAAGTCCT
IFN d	Interferon type I	59	111	GTCAGGGTGCAGCTGAGTTA	ACAGAAACGGCAGCTCAAAC
MUC-2	Mucin-2	62	342	ATT GAG TTT GGC AAC AAA CAG AAA GCC C	TAC AGC ACA GAA CTG AGG TGT CCT C
IL-1β	Interleukin 1β	62	205	TGATGGAGAACATGGTGGAA	GTCGACATGGTCAGATGCAC
IL-8	Interleukin 8	58	164	GAAGCCTGGGAGTAGAGCTG	GGGGTCTAGGCAGACCTCTT
IL-10	Interleukin 10	58	134	CTC AAG AGT GAT GTC ACC AAA TGT AGA AAC T	AGC AAA TCC AGC TCG CCC ATT
IL-17F	Interleukin 17F	62	120	GGTGGCCCCAGAGGATCTCC	GGAGGACCAAAACCTGGTAGTAGATGG
IL-17D	Interleukin 17D	62	111	CGGTCTACGCTCCCTCCGTG	GCGGCACACAGGTGCATCCC
IL-22	Interleukin 22	61	146	GCC AAC ATC CTC GAC TTC TAC CTG AAC	TGG TCG TGG TAG TGA GTC ACA TTG C
IgM	Immunoglobulin M	58	148	CTCTTTGATAGGAATACCGGAGGAGAG	CAACTAGCCAAGACACGAAAACCC
IgT	Immunoglobulin T	59	196	TGGACCAGTCGCCATCTGAG	GGGAAACGGCTTTGAAAGGA
β-Actin	β-Actin	61	212	TCT GGT GGG GCA ATG ATC TTG ATC TT	CCT TCC TTC CTC GGT ATG GAG TCC
EF1 α	Elongation factor 1α	60	194	TGC CAT ACT GCT CAC ATC GCC TG	ATT ACA GCG AAA CGA CCA AGA GGA G

*Ann. temp: anneling temperature

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Table 2. Growth performance, serum and skin mucus immunological parameters (lysozyme activity and bactericidal activity) and parasite data of greater amberjack juveniles (*Seriola dumerili*) after 90 days on the feeding trial.

	DIETARY TREATMENTS				TWO WAY ANOVA		
	C	MOS	cMOS	MOS + cMOS	MOS	cMOS	MOS*CMOS
Growth performance							
Final Weight (g)	1046.75 ±129.61	1024 ±161.17	1090.37 ±135.49	1036.55 ±126.88	NS	NS	NS
SGR (%)	1.09 ±0.04	1.09 ±0.06	1.13 ±0.09	1.08 ±0.07	NS	NS	NS
Feed efficiency	0.654 ±0.06	0.656 ±0.01	0.698 ±0.04	0.704 ±0.08	NS	NS	NS
Skin mucus							
Lysozyme activity (U/ml)	103.92 ±17.64	114.25 ±28.1	124.55 ±31.64	121.9 ±11.97	NS	NS	NS
Bactericidal activity (%)	3.72 ±1.86	5.03 ±1.21	6.54 ±0.89	5.22 ±2.61	NS	NS	NS
Serum							
Lysozyme activity (U/ml)	301.61 ±42	348.76 ±52.1	253.88 ±25.86	287.69 ±39.04	NS	NS	NS
Bactericidal activity (%)	4.89 ±1.06	5.91 ±1.70	8.27 ±1.05	9.51 ±1.27	P=0.04 F=6.68	P=0.02 F=17.56	NS
Parasite challenge							
Parasitation level (range)	2-3	2	1-2	1-2	NS	P=0.01 F=6.17	NS
Parasite total length (mm)	4.44 ±0.31	3.9 ±0.43	3.32 ±0.40	3.56 ±0.43	NS	P=0.01 F=15.47	NS
Nº parasites / fish surface (cm^2)	0.101 ±0.01	0.087 ±0.02	0.015 ±0.01	0.042 ±0.01	NS	P=0.01 F=52.36	NS

Diet C (control diet, non-supplemented), MOS (MOS supplemented diet), cMOS (cMOS supplemented diet), MOS + cMOS (combined MOS and cMOS supplemented diet). Values expressed in mean ± SD (n = 3 tanks/diet). Two-way ANOVA comparation (P<0.05). SGR: Specific growth rate; parasitation level: ranged among 1 (lower) to 3 (higher).

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Table 3. RT-qPCR gene expression in skin of *Seriola dumerili* juveniles after 90 days on the feeding trial.

	Dietary treatments				Two-way ANOVA		
Gene	**Diet C**	**MOS**	**cMOS**	**MOS+cMOS**	**MOS**	**cMOS**	**MOS*cMOS**
Hep	3.05 ±1.16	3.67 ±1.26	6.11 ±2.25	2.01 ±0.34	NS	P= 0.04, F= 2.13	NS
Pis	507.47 ±184.49	1825.94 ± 992.81	2961.56 ±969.37	3448.44 ±657.29	NS	NS	NS
Def	181.69 ±85.59	422.65 ±179.34	472.89 ±215.85	285.93 ±79.93	NS	NS	NS
iNOS	354.02±132.51	56.34±15.48	514.35±208.57	83.7±31.8	P=0.01, F=9.34	NS	NS
TNFα	10.78±2.50	10.88±0.97	18.65±4.72	8.21±2.67	NS	NS	NS
MX prot	571.15±279.59	362±272.29	805.57±460.93	112.64±38.44	NS	NS	NS
IFN γ	31.25 ±5.57	100.13 ±46.91	130.18 ±66.74	41.97 ±16.39	NS	NS	P= 0.01, F= 3.89
IFN d	9.83 ±2.01	29.20 ±10.77	44.04 ±21.61	12.71 ±3.11	NS	NS	P= 0.01, F= 2.35
MUC-2	9.96 ±4.18	8.48 ±2.85	24.74 ±6.08	8.94 ±5.51	NS	P= 0.04, F= 3.27	NS
IL-1β	4.32 ±0.87	4.48 ±1.34	9.52 ±5.36	2.08 ±0.39	NS	P= 0.02, F= 5.52	NS
IL-8	10.50 ±2.73	9.85 ±3.16	21.04 ±2.50	11.99 ±4.48	NS	NS	NS
IL-10	1468.17 ±398.19	1521.55 ±364.18	2724.73 ±812.56	231.37 ±167.44	NS	P= 0.01. F=9.52	P= 0.01, F= 4.81
IL-17F	25.37 ±7.1	13.36 ±2.58	29.66 ±9.83	10.69 ±2.78	NS	NS	NS
IL-17D	6.19 ±1.52	10.65 ±3.77	65.30 ±36.34	4.63 ±1.50	NS	NS	P= 0.01, F= 5.23
IL-22	2.26 ±0.9	4.72 ±3.98	3.29 ±2.57	1.56 ±0.75	NS	NS	NS
IgM	1008.43 ±246.86	1074.15 ±502.02	921.66 ±545.95	2155.97 ±835.60	NS	NS	NS
IgT	5.25 ±0.97	6.32 ±1.40	12.14 ±3.51	3.05 ±0.87	NS	P= 0.04, F= 3.27	P= 0.01, F= 2.23

Diets: C (control diet), MOS (5 g kg^{-1}), cMOS (2 g kg^{-1}), MOS+cMOS (5 g kg^{-1} of MOS and 2 g $kg^{-1}$of cMOS). Data are presented as means ± SD. N=3 tanks/diet. Two-way ANOVA analyses are presented when P<0.05. NS= Not significant.

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Table 4. RT-qPCR gene expression in gills of *Seriola dumerili* juveniles after 90 days on the feeding trial.

	Dietary treatments				Two-way ANOVA		
Gene	**Diet C**	**MOS**	**cMOS**	**MOS+cMOS**	**MOS**	**cMOS**	**MOS*cMOS**
Hep	3.83 ±1.09	8.07 ±3.58	20.74 ±6.40	3.05 ±0.63	NS	P= 0.01, F= 3.22	NS
Pis	123559.34 ±57885.09	49101.56 ±20481.84	145655.85 ±39802.15	220796.87 ±115335.48	NS	NS	NS
Def	154.83 ±57.91	269.99 ±117.38	1538.51 ±560.83	352.82 ±181.47	NS	P= 0.01, F=7.48	P= 0.03, F= 2.59
iNOS	22.57 ±13.59	287.96 ±200.29	591.18 ±261.70	41.52 ±26.58	NS	NS	NS
TNFα	30.65 ±12.60	68.12 ±20.59	91.23 ±32.13	32.95 ±4.90	NS	NS	NS
MX prot	24.50 ±5.55	366.77 ±244.42	1125.22 ±336.94	49.10 ±33.21	NS	P= 0.02, F= 2.27	P= 0.03, F= 4.37
IFN γ	57.11 ±9.1	118.43 ±54.38	220.41 ±52.02	69.04 ±18.96	NS	P= 0.03, F= 3.86	NS
IFN d	18.63 ±8.95	43.70 ±19.93	102.95 ±21.84	20.83 ±3.73	NS	NS	NS
MUC-2	1138.31 ±250.05	1149.28 ±633.21	522.40 ±212.30	592.53 ±226.38	NS	NS	NS
IL-1β	12.90 ±6.21	16.20 ±6.23	15.92 ±4.82	5.92 ±1.79	NS	NS	NS
IL-8	17 ±2.92	27.21 ±5.20	74.62 ±28.04	31.26 ±12.15	NS	NS	NS
IL-10	2347.06 ±824.85	2373.74 ±203.84	5078.92 ±2726	4073.95 ±2180.10	NS	NS	NS
IL-17F	7.47 ±3.96	11.69 ±4.62	21.20 ±8.31	4.78 ±1.64	NS	NS	NS
IL-17D	18.19 ±4.07	69.54 ±28.57	63.85 ±15.36	16.28 ±5.56	NS	NS	NS
IL-22	15.51 ±5.79	19.75 ±6.01	21.95 ±4.96	10.18 ±2.65	NS	NS	NS
IgM	65941.75 ±43329.10	16665.56 ±5287.38	1575.48 ±1071.64	144185.46 ±56001.67	NS	NS	NS
IgT	8.72 ±2.17	14.04 ±4.26	20.80 ±5.31	7.98 ±2.12	NS	NS	P= 0.01, F= 9.88

Diets: C (control diet), MOS (5 g kg^{-1}), cMOS (2 g kg^{-1}), MOS+cMOS (5 g kg^{-1} of MOS and 2 g $kg^{-1}$of cMOS). Data are presented as means ± SD. N=3tanks/diet. Two-way ANOVA analyses are presented when $P<0.05$. NS= Not significant.

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Table 5. RT-qPCR gene expression in posterior gut of *Seriola dumerili* juveniles after 90 days on the feeding trial.

	Dietary treatments				Two-way ANOVA		
Gene	**Diet C**	**MOS**	**cMOS**	**MOS+cMOS**	**MOS**	**cMOS**	**MOS*cMOS**
Hep	3.77 ±1.05	9.43 ±0.86	103.78 ±44.61	12.18 ±2.88	NS	P= 0.02, F= 6.23	NS
Pis	18159.99 ±6184.25	70552.71 ±20631.15	16733.78 ±2690.02	100257.10 ±47228.58	P= 0.03, F= 7.35	NS	NS
Def	103.35 ±60.43	526.85 ±272.52	5909.07 ±2592.71	1721.73 ±483.36	NS	P= 0.03, F=2.98	NS
iNOS	352.72 ±56.94	699.99 ±361.29	615.43 ±278.11	1183.26 ±418.29	NS	NS	NS
TNFα	32.27 ±12.53	64.93 ±19.44	108.79 ±43.23	71.82 ±17.04	NS	NS	NS
MX prot	410.19 ±47.28	831.96 ±175.50	955.28 ±867.09	1962.94 ±909.99	NS	NS	NS
IFN γ	34.21 ±16.26	427.99 ±193.55	1241.66 ±542.39	284.67 ±41.87	NS	P= 0.04, F= 3.32	P= 0.02, F= 1.09
IFN d	7.49 ±3.48	57.75 ±19.91	81.64 ±30.78	38.93 ±9.07	NS	NS	NS
MUC-2	2800.02 ±511.50	6819 ±1350.56	3375.84 ±993.78	2701.72 ±810.98	P= 0.04, F= 17.72	NS	NS
IL-1β	6.73 ±1.08	66.97 ±28.67	14.06 ±8.09	18.65 ±6.65	P= 0.02, F= 3.52	NS	NS
IL-8	20.75 ±6.67	53.22 ±12.95	191.49 ±99.69	75.67 ±14.98	NS	NS	NS
IL-10	1578.98 ±194.29	9495.37 ±4244.02	107128.07 ±45885.12	17241.35 ±6641.88	P=0.03, F=9.48	P= 0.02, F=2.79	NS
IL-17F	8.53 ±5.53	21.20 ±8.05	11.73 ±4.99	7.20 ±1.49	NS	NS	NS
IL-17D	31.43 ±10.75	71.53 ±15.36	74.54 ±28.76	64.69 ±23.07	NS	NS	P= 0.01, F= 5.23
IL-22	19.61 ±7.73	22.75 ±9.25	13.19 ±3.38	13.42 ±3.26	NS	NS	NS
IgM	73788.21 ±41586.91	$29.2 \times 10^5 \pm 14.2 \times 10^5$	$22.3 \times 10^5 \pm 80.3 \times 10^4$	573173.42 ±319410.84	P= 0.01, F=8.24	P= 0.02, F= 6.14	P= 0.02, F=2.41
IgT	18.47 ±13.64	70.28 ±16.82	56.15 ±19.18	34.52 ±13.48	P= 0.01, F=2.78	P= 0.02, F= 3.11	NS

Diets: C (control diet), MOS (5 g kg^{-1}), cMOS (2 g kg^{-1}), MOS+cMOS (5 g kg^{-1} of MOS and 2 g $kg^{-1}$of cMOS). Data are presented as means ± SD. N=3tanks/diet. Two-way ANOVA analyses are presented when P<0.05. NS= Not significant.

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Table 6. RT-qPCR gene expression in head kidney of *Seriola dumerili* juveniles after 90 days on the feeding trial.

	Dietary treatments				Two-way ANOVA		
Gene	**Diet C**	**MOS**	**cMOS**	**MOS+cMOS**	**MOS**	**cMOS**	**MOS*cMOS**
Hep	8.38 ±3.65	11.90 ±3.86	23.23 ±5.85	31.40 ±6.99	NS	P= 0.01, F= 10.96	NS
Pis	113233.81 ±40305.31	107678.83 ±50906.83	224992.61 ±92470.72	102237.60 ±19950.65	NS	NS	NS
Def	187.37 ±71.37	3198.20 ±1666.94	2407.93 ±1279.35	1518.66 ±793.53	NS	NS	P= 0.03, F= 2.19
iNOS	1809.69 ±689.22	12437.49 ±2634.66	1164.43 ±150.86	4101.22 ±791.51	P= 0.02, F= 8.32	NS	NS
TNFα	57.46 ±30.26	84.34 ±22.70	115.74 ±60	45.05 ±8.84	NS	NS	NS
MX prot	727.39 ±183.65	10088.19 ±1439.72	1252.17 ±62.92	4399.24 ±1623.50	P= 0.01, F= 8.48	NS	P= 0.03, F= 3.65
IFN γ	216.77 ±108.53	355.11 ±101.34	294.81 ±213.67	352.18 ±102.91	NS	NS	NS
IFN d	29.62 ±6.46	71.28 ±10.82	62.30 ±15.11	55.16 ±18.84	P= 0.02, F= 4.23	P= 0.02, F= 7.15	NS
IL-1β	52.81 ±29.24	262.49 ±117.76	121.57 ±63.93	62.41 ±9.84	NS	NS	NS
IL-8	8.05 ±1.99	32.80 ±9.16	22.41 ±9.72	105.56 ±48.61	NS	NS	NS
IL-10	852.98 ±203.37	5077.33 ±2249.74	3090.39 ±1025.95	577.80 ±117.01	P=0.02, F=5.28	P= 0.02, F=7.68	P= 0.01, F= 9.51
IL-17F	7.22 ±2.41	21.74 ±2.96	25.56 ±8.95	14.47 ±3.03	NS	NS	NS
IL-17D	36 ±18.68	139.82 ±32.31	15.47 ±2.31	58.98 ±16.09	P=0.04, F=1.67	NS	NS
IL-22	4.79 ±0.79	43.93 ±9.44	36.33 ±10.45	24.68 ±8.25	P=0.03, F=4.89	P=0.02, F=9.93	NS
IgM	18166.59 ±386.67	44482.19 ±18652.10	298249.40 ±112084.40	99464.83 ±24438.90	NS	NS	NS
IgT	48.93 ±27.35	60.15 ±32.54	80.64 ±38.20	62.13 ±15.32	NS	NS	NS

Diets: C (control diet), MOS (5 g kg-1), cMOS (2 g kg-1), MOS+cMOS (5 g kg-1 of MOS and 2 g kg-1of cMOS). Data are presented as means ± SD. N=3 tanks/diet. Two-way ANOVA analyses are presented when P<0.05. NS= Not significant.

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Table 7. RT-qPCR gene expression in spleen of *Seriola dumerili* juveniles after 90 days on the feeding trial.

	Dietary treatments				Two-way ANOVA		
Gene	**Diet C**	**MOS**	**cMOS**	**MOS+cMOS**	**MOS**	**cMOS**	**MOS*cMOS**
Hep	61.72 ±19.67	64.87 ±13.07	33.35 ±6.53	147.64 ±48.89	NS	P= 0.01, F= 7.39	NS
Pis	165899.47 ±86928.35	326203.01 ±92385.94	68108.33 ±27208.51	313455 ±159603.02	P= 0.01, F= 9.95	NS	NS
Def	276.29 ±82.03	1416.65 ±289.94	450.51 ±207.30	2815.82 ±1277.64	P= 0.01, F=8.61	NS	P= 0.01, F=7.35
iNOS	1830.64 ±504.84	2494.56 ±945.27	1089.47 ±407.30	778.71 ±279.99	NS	NS	NS
TNFα	411.98 ±82.99	925.46 ±257.80	203.52 ±63.54	429.05 ±146.70	NS	NS	NS
MX prot	1021.71 ±307.35	2182.75 ±821.69	860.82 ±350.61	439.08 ±155.54	NS	NS	NS
IFN γ	405.34 ±107.19	870 ±130.56	300.82 ±62.79	434.63 ±140.21	P= 0.02, F=3.29	NS	NS
IFN d	30.54 ±3.73	75.70 ±21.16	27.95 ±6.56	84.80 ±27.22	NS	NS	NS

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IL-1β	18.91 ±5.84	43.14 ±7.19	12 ±1.45	19.54 ±2.35	P= 0.01, F=14.36	NS	NS
IL-8	23.68 ±5.68	61.37 ±23.18	19.51 ±7.61	44.89 ±16.51	NS	NS	NS
IL-10	2268.78 ±944.39	5478.43 ±2040.92	2305.29 ±1080.20	6791.58 ±2267.62	NS	NS	NS
IL-17F	9.75 ±2.30	15.45 ±4.67	3.76 ±0.88	11.38 ±6.08	NS	NS	NS
IL-17D	14.42 ±4.08	40.39 ±12.23	14.12 ±3.22	43.94 ±22.28	P= 0.04, F=1.36	NS	NS
IL-22	6.15 ±1.53	26.11 ±10.74	6.46 ±1.91	19.35 ±7.50	NS	NS	NS
IgM	152198.50 ±42526.77	28665.69 ±6833.63	104560.74 ±35002.44	51173.12 ±15474.28	NS	NS	NS
IgT	26.17 ±10.84	63.59 ±14.76	16.13 ±3.46	38.16 ±10.21	NS	NS	NS

Diets: C (control diet), MOS (5 g kg-1), cMOS (2 g kg-1), MOS+cMOS (5 g kg-1 of MOS and 2 g kg-1of cMOS). Data are presented as means ± SD. N=3tanks/diet. Two-way ANOVA analyses are presented when P<0.05. NS= Not significant.

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Highlights

-Dietary cMOS utilization during 90 days increased protection against *Neobenedenia girellae* in greater amberjack juveniles
-Dietary cMOS focused the immune stimulation in mucosal tissues, meanwhile MOS focused in systemic immune tissues.
-The combination of both prebiotics showed a lack of effect for 90 days of dietary use.

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