MS. MARIA DOLORES GARRIDO (Orcid ID : 0000-0002-8004-0998)

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Effect of the dietary supplementation based on essential oils on the quality of gilthead seabream.

Celia Iniesta[1], Elvira Zapata[1], Joana P. Firmino[2,3], Irene Peñaranda[1], Macarena Egea[1], M. Belén Linares[1], M. Dolores Garrido[1]

[1]*Food Technology, Nutrition and Bromatology Department, Faculty of Veterinary, University of Murcia, 30100 Murcia, Spain*

[2] *TECNOVIT–FARMFAES, S.L. Pol. Ind. Les Sorts, Alforja, Spain*

[3] *IRTA, Centre de Sant Carles de la Ràpita (IRTA-SCR), Aquaculture Program, Sant Carles de la Ràpita, Spain.*

Abstract

The effectiveness of using essential oils against fish parasites and pathogenic bacteria as environmentally friendly phytotherapeutic agents in the aquaculture industry is demonstrated. A commercial additive composed of garlic essential oil, thymol and carvacrol (AROTEC-G®) was added to a diet for gilthead seabream (*Sparus aurata*) as a protection strategy against *Sparicotyle chrysophrii*. The intense aromatic properties of these essential oils might lead to the need of a suppression time to reverse possible changes in the organoleptic quality of the fish. Two experimental groups were set up: an experimental group fed a commercial feed supplemented with AROTEC-G® (diet A) during the first 4 weeks of the nutritional assay, and after which the treatment was

suspended for 14, 21 and 28 days after a first sampling performed (day 0); and a control group, fed the same basal diet without AROTEC-G® supplementation (diet C). A physicochemical evaluation measured the pH, colour and texture, and a descriptive quantitative sensory analysis (QDA) was carried out using a trained panel for gilthead seabream fillets. Overall, the findings showed that the use of dietary essential oils did not result in significant sensory differences after day 0 of the study, although slight differences were observed in some of the physicochemical parameters analysed.

Keywords: Aquaculture, gilthead seabream, essential oils, diet supplement, QDA, physiochemical.

1. Introduction

Many parasites can affect fish (Espinosa de los Monteros, & Labarta, 1988), while their presence in cultured fish will depend to a large extent on the culture conditions, the origin of the fish and the life cycle modality of the parasites. Their importance in the production process is clear particularly in the farm conditions. It is well known that parasitic infections in fish increase when they are artificially maintained due to the high densities involved that are rarely observed in natural conditions. As a result, epizootics may occur, frequently accompanied by high mortality, especially in the case of direct cycle parasites, such as certain protozoa or monogenean.

One such disease is the sparicotylosis produced by the polyopisthocotylean gill ectoparasite S*paricotyle chrysophrii* (Sitjà-Bobadilla, de Felipe, & Alvárez-Pellitero, 2006), which may increase mortality in sea cages during spring and summer. The growth of this parasite is favoured by high densities and the biofouling of nets, while symptoms

of the disease include lethargy due to hypoxia, emaciation, histopathological damage, and severe anaemia.

To control *S. chrysophrii*, on-site cage treatments that include formalin baths and other chemicals are used in routine disinfections, as well as the removal and cleaning of nets. In addition, different chemicals can be used in routine disinfections. However, neither de efficacy of chemotherapeutants nor of disinfectants has been monitored, due to the drawbacks involved in bathing infected fish with certain compounds, especially in sea cages, means that administering parasiticides in the feed is considered more convenient. Indeed, the dietary administration of herbal products to prevent bacterial and parasitic diseases is considered a promising sustainable solution for conventional and antibiotic-free animal nutrition. In their review, Dawood et al. (2021) demonstrated the effect of essential oils against fish parasites and pathogenic bacteria as an environmentally friendly phytotherapeutic in the aquaculture industry. The hydrophobic compounds of essential oils can penetrate the bacterial and parasitic cells and cause cell deformities and organelle dysfunctions. Dietary supplementation with essential oils may also modulate growth, immunity, and infectious disease resistance in several aquaculture-relevant species (Firmino et al., 2021). Other reports have also demonstrated the effectiveness of essential oils against *Ichthyophthirius multifiliis, Gyrodactylus sp., Euclinostomum heterostomum*, and other parasites both *in vivo* and *in vitro*.

In this context, a commercial additive composed of microencapsulated garlic essential oil, thymol and carvacrol (AROTEC-G®) was used by Firmino et al. (2020) to formulate a diet for gilthead seabream (*Sparus aurata*) as a prophylactic strategy against the *S. chrysophrii*, finding a significant decrease in the ectoparasite prevalence in fish fed

AROTEC-G®.However, it is unclear whether the intense aromatic properties of garlic essential oil, thymol and carvacrol can alter the organoleptic quality of fish after harvest, representing a potential limiting factor to its dietary supplementation. Therefore, an assay based on the analysis of the fish fillet quality was designed in order to determine possible physicochemical and sensory changes that may derive from the use of these compounds in seabream's diet. Moreover, the need for a suppression period of AROTEC-G® supplementation, and its optimal time length, were also evaluated to reverse possible changes in the organoleptic quality of gilthead seabream.

Overall, the aim of this work was to evaluate the inclusion of AROTEC-G® as a functional feed additive for farmed gilthead seabream (*Sparus aurata*), assessing its effect upon the physicochemical properties and sensory quality of cooked seabream fillets, at different suppression time points.

2. Materials and Methods

2.1. Diets

The fish were assigned to two different groups depending on the type of diet administrated: one group was fed a diet supplemented with 0.5% of a microencapsulated functional additive containing synthetic garlic essential oil, carvacrol, and thymol (AROTEC-G®, TECNOVIT-FARMFAES, S.L., Spain) in a vegetable fat matrix (diet A), as previously tested by Firmino et al. 2020, during the first 4 weeks of the nutritional assay; and a control group was fed the same basal diet but without AROTEC-G® supplementation (diet C). As it is not considered a medicine, the use of AROTEC-G® does not require a phase-out period or a veterinary prescription.

2.2. Fish rearing conditions and sampling

Eighty specimens of on-growing gilthead seabream (*Sparus aurata*) from the Institute of Agrifood Research and Technology, San Carle de La Ràpita centre (IRTA-SCR, Cataluña, Spain) were used. The fish were randomly distributed into two 2000 L tanks (N=40 per tank) and kept in rearing conditions similar to those described in Firmino et al. (2019). During the first 4 weeks of the study, the fish were fed the two corresponding diets (A and C). After this 4 weeks period, the AROTEC-G® supplementation was suspended in the case of diet A (initiation of the suppression period) and the first sampling was performed (day 0) for both groups. Ten fish from each dietary group, were sacrificed directly in ice slurry (hypothermia). After this first sampling, both groups were fed the control diet exclusively, and later sampled on days 14, 21 and 28 of suppression (Fig. 1). In each sampling point, commercial sized gilthead seabream specimens were weighted for body weight (BW, g) and measured for standard length (SL, cm), being the initial weight and standard length at the beginning and at the end of the feeding test : BWi-control = 398.4 ± 50.0 vs. BWi- Diet A = 387.7 ± 39.8 g; SLi-control = 24.2 ± 1.0 vs. SLi-Diet A = 24.0 ± 0.8 mm,: BWf-control = 477.4 ± 73.6 vs. BWf-Diet A = 464.8 ± 33.5 g; SLf-control = 25.3 ± 1.4 vs. SLf-Diet A = 25.0 ± 0.6 mm (Fig. 2). Then, the fishes were filleted (± 100 g each fillet) and vacuum packed in polythene bags (300 x 500 mm, 150 microns). Fillets were immediately frozen at – 18 ºC and then delivered in sealed flake ice filled in polystyrene cases to the Department of Food Technology, University of Murcia (Spain). All the samples were stored at – 18 ºC during two weeks (for each day suppression period) for further analysis

2.3. Physical chemical evaluation

pH

The pH was determined with a portable water-resistant pH meter (pH /Tª) with a glass penetration electrode. After calibrating against a pH 4 buffer and pH 7 buffer, two pH measurements were made on six raw thawed fillets from each group.

Colour

Colour was determined in raw thawed fish fillets by means of a Minolta colorimeter (Chroma meter CR300), using the CIE L * a * b * scale, after calibration against a white standard. Three measurements were made in each of the six fillets from each group.

Texture

A texture profile analysis (TPA) was made in cooked fillet pieces (4 cm x 3 cm) (see below for sample preparation section) at the same time as the sensory analysis, following the procedure described by Bourne (1978) using a CT 3 texture analyser (Brookfield). The method was as follows: two compressions, 25 kg load cell, 10 mm diameter cylindrical probe (TA-10), trigger force= 5 g, strain = 50%, speed = 5 mm/s and pause time between cycles = 5 s.

Through the TPA analysis, the values for hardness, deformation, adhesiveness, cohesiveness, springiness, gumminess and chewiness were determined. The measurements were made in the centre of eight samples from each group.

2.4. Sensory evaluation

Panel

148 The tasting panel was selected and trained in accordance with ISO 8586-2 (2012). The
149 panel consisted of eight members of the Food Science and Technology research group
150 of Murcia University. The panellists used an unstructured continuous line scale to score
151 the intensity of the sensory attributes. A Quantitative Descriptive Analysis (QDA) was
152 carried out for sensory analysis. A total of 11 attributes were agreed upon and defined
153 for fish odour, aromatic odour, anomalous odour, meat colour, brightness, fish flavour,
154 aromatic flavour, anomalous flavour, juiciness, firmness and chewiness.
155 *Sample preparation*
156 Twenty-four hours before the tasting session, the vacuum packed gilthead seabream
157 fillets were thawed in a cold store at a temperature of between 4 and 6 ºC. The area
158 near the head, sides, spine area and tail were removed from the fillets, so that only the
159 parts corresponding to the central fillet with skin were used (4 x 3 cm.)
160 The portions were wrapped in aluminium foil coded with a three-digit number on the
161 back of the wrapping, and eight wrapped and coded samples were steam coked in a
162 Thermomix model TM31 until a temperature of 72 ºC was reached in the centre
163 (approximately 4 minutes of cooking).
164
165 *Sensory evaluation*
166 Sensory analysis was performed in accordance with UNE-ISO 4121:2006 and UNE-EN-
167 ISO 8589:2010. Tasting sessions (four in total) were held in the morning and each
168 panellist tasted three samples of fish taken from each dietary group at day 0 and after
169 each suppression period (day 14, 21 and 28). This meant that 24 samples were tasted
170 for each group. Panellists were provided with mineral water (ALIADA, Madrid, Spain)

and unsalted toasted breadsticks (ALIADA, Madrid, Spain), to clean the oral cavity between samples.

2.5. Statistical analysis

The mean, standard deviation, and P-value from each group of data were calculated. One-way analysis of variance (ANOVA) was used to evaluate the effect of AROTEC-G® on the sensory parameters of each sample. In the case of differences, a multiple comparison analysis was made using a Tukey test. All data were analysed using the statistical program SPSS Statistical Software System ver. 25.0. The level of statistical significance was set at 5% for all analyses.

3. Results and discussion

pH and Colour

A significant effect of the analysis day on the pH values of the fish receiving the control diet was observed (Table 1), the highest value obtained on day 0 (5.38) probably being due to the fish size. This finding coincides with that observed by Suárez et al. (2010) in *Sparus aurata*, where fish of low weight had a higher pH than larger ones. In the present study, the same effect was also observed in fish receiving diet A. However, another explanation could be the storage temperature and time since Lakshmanan, Varma, Iyer, & Gopakumar (1990) found a slight drop in the pH of fish samples between week 4 and 8 of storage at -20 ºC, which is similar to that observed in our study. In addition, they pointed out that this decrease in pH would affect the water binding capacity of muscle proteins and hence increase hardness. The results pointed to no significant differences

195 in pH values between the control and A diets for any time of suppression, strongly
196 suggesting that supplementation with AROTEC-G® does not imply modification of the
197 pH values.
198 Although, in general, the diet did not lead to significant differences regarding colour,
199 there was a slightly significant difference ($p<0.05$) in the L * parameter on day 14
200 between the fish receiving the control diet (56.58 ± 1.44) and those receiving diet A
201 (54.79 ± 0.89) and, in the case of diet A, between the values measured on day 21 (56.35
202 ± 1.69) and on day 28 (53.59 ± 0.67) ($p<0.05$) (Table 1). Lightness was higher in the case
203 of the control diet and the value lower in larger fish (day 28) and those that had been in
204 frozen storage for the longest time. The colour of the muscle varied considerably from
205 one fish to another, reflecting the observations of Choubert, Blanc, & Vallée (1997), who
206 observed highly significant variations in muscle colour between individuals.
207 In terms of redness (a*), in general, older fish (days 21 and 28) for both diets tended to
208 present lower values. A study conducted by Martínez-Llorens et al., (2008), which
209 examined the effect of a haemoglobin rich meal diet, the parameter a * was not found
210 to be dependent on the diet. Once again, any differences could be explained by fish
211 inter-variability.
212
213 *Texture*
214 After the TPA analysis of the samples (Table 2), significant inter- or intragroup
215 differences were found in the case of Hardness 1, Hardness 2, Adhesiveness and
216 Gumminess.
217 The hardness 1 values were generally higher in the case of diet A with respect to the
218 control diet. There is little in the bibliography regarding the effect of feeding fish with

219 essential oils on the hardness parameters. In a study carried out on salmon burgers in
220 which essential oils of thyme and oregano were incorporated in the fish feed (Dolea et
221 al., 2018) a slight increase in hardness was observed in the case of hamburgers with
222 essential oils but without significant differences.
223 Regarding adhesiveness, a slight but significant difference was observed for diet A,
224 producing slightly higher values than diet C. This was also observed in a study carried
225 out in rainbow trout fillets (Santos et al., 2019), in which adhesiveness was also
226 significantly affected by the enrichment of feed with essential oils. These authors
227 observed a particular inhibition of protein oxidation, especially notable in the
228 myofibrillar fraction, and a protection of the loss of protein solubility, together
229 preventing flesh changes in adhesiveness.
230 Finally, there was an upward trend in the case of gumminess, this parameter increasing
231 with time, although only statistically significant in the case of diet A. Santos et al., (2019)
232 found that gumminess in rainbow trout fillets increased during the first weeks of storage
233 and then remained constant during the rest of frozen storage.
234
235 *Sensory evaluation*
236 Table 3 shows the average values (mean ± standard deviation, SD) for the sensory
237 analysis attributes, comparing the flesh parameters obtained with the control diet and
238 diet A in relation with each suppression period. The attributes aromatic odour,
239 anomalous odour, aromatic flavour, and anomalous flavour were not detected and are
240 therefore not included. This can be regarded as a positive result since one of the
241 drawbacks of using essential oils in the diet is their potential of giving rise to anomalous
242 odours and flavours in the final product.

243 Most studies into the effect of diets supplemented with essential oils carried out in fish
244 species during their storage mention an increase in shelf-life when these active
245 compounds are used (Álvarez et al., 2012; Hernández, García, Jordán, & Hernández,
246 2014; Rezanejad et al., 2019). However, although in these studies shelf-life is increased
247 using these plants that have a strong aromatic effect, no significant sensory differences
248 appear in the fish with the use of essential oils, which is in accordance with the present
249 study. In the case of fish odour (FO) and fish flavour (FF), there were no significant
250 differences between the control and the supplemented group regardless of the different
251 suppression times, confirming that the feed additive did not interfere with the odour of
252 fresh fish. Other studies obtained similar results; for example, Johnsen, & Dupree (1991)
253 concluded that the desirable flavours of farmed catfish are produced by the
254 biochemistry of the fish themselves, rather than by the feed consumed. Similar results
255 were described by Gajardo (2007), who carried out sensory analyses of fish fed a
256 functional feed based on essential oils. The above study using a diet composed of
257 fishmeal, α-tocopherol, fish oil and rosemary extract was administered to salmon and
258 no significant differences were observed in any of the attributes analysed.
259 On the other hand, regarding the appearance of the flesh, while there were no
260 significant differences in colour or brightness, a small variation was observed in the case
261 of diet with AROTEC-G®.The colour was slightly whiter in the gilthead seabream fillets
262 obtained from the fish fed the functional feed than from the control. This result
263 coincides with that observed by Hernández, (2018) in gilthead seabream fed extracts of
264 thyme and oregano. This effect would be due to the capacity of carvacrol and thymol to
265 reduce lipid oxidation, which would provide a whiter colour. Regarding the texture
266 parameters, there were no significant differences between the fish that received the

267 control diet and those that were fed the diet containing AROTEC-G®. A study carried
268 out by Cai et al., (2015) describes a sensory analysis of *Sciaenops ocellatus* fillets, in
269 which an improved texture was observed with the use of natural compounds, as they
270 would reduce the action of endogenous enzymes and microbial activity in fillets,
271 resulting in a decrease in protein degradation and, therefore, a better texture
272 (Mahmoud et al., 2004).
273 If we compare each control according to the sampling time, there were significant
274 differences in fish flavour between day 28 with respect to days 0 and 14. This would be
275 related with the longer frozen storage of 0 and 14 days samples than the 28 days
276 suppression time**,** since fish flavour could have diminished during storage. Although the
277 difference in storage time between day 0 and day 28 was only about a month and a half,
278 similar results were found by other authors, who described how the intensity of the
279 characteristic odour and fresh flavour gradually decreased during storage of seabass
280 (*Dicentrarchus labrax*) (Di Turi et al., 2009). According to Farmer et al. (1997), fish flavour
281 is related with the amount of the fatter the fish contain, the higher the flavour intensity.
282 However, in our study, the fat content of the fish of was not determined in either group.
283 Chewiness was significantly higher at day 28 than at day 21. Although there are not
284 many days of difference between one and the other, this could be due to the difference
285 in size between fish, since there is an inverse relationship between water and lipid
286 content in fish as they increase in size (Hyldig, & Nielsen, 2001; Rasmussen, 2001).
287 Furthermore, other authors, including López-Albors et al. (2005), showed that the larger
288 the fish, the greater the density of muscle fibres. In addition, it is important to consider
289 that the size of muscle fibres is one of the main determining factors in texture. As a
290 general rule, it should be considered that the higher the fibre density, the greater the

291 firmness of the fillet. In this sense, Periago et al. (2005) verified that fibre density can
292 explain up to 46% of the variations in chewiness found in raw fillets of farmed and wild
293 seabass. According to Montero, & Borderias (1990), increases in chewiness with frozen
294 storage time are due to a toughening of the muscle induced by both myofibrillar
295 proteins and collagens aggregate. Although proteins are known to denature during
296 freezing, this fact alone would not be sufficient to cause the toughening. Howgate (1977)
297 suggested that the sarcoplasmic reticulum degrades and then acts like cement to hold
298 the individual myofibrils together. Regarding the AROTEC-G® diet groups, and unlike
299 the control diet results, none of the attributes showed significant differences with the
300 suppression period. It is known that garlic, carvacrol and thymol essential oils have a
301 positive effect on fish shelf-life (Hernández et al., 2014), which would explain the fact
302 that no significant differences of the attribute derived from storage time were showed.
303 Some studies have investigated the ability of essential oils to preserve the product.
304 Thymol and carvacrol, in particular, are highly hydrophobic compounds that accumulate
305 in the plasma membrane of bacterial cells, where they destabilize the membrane,
306 causing the loss of intracellular components and a change in the membrane potential,
307 leading to the inhibition of spoilage (Shapiro, & Guggenheim, 1995). Therefore, the
308 inclusion in the diet of garlic essential oil, carvacrol and thymol, in the amounts applied
309 in the present study, not only demonstrate no significant effect on the sensory quality
310 of gilthead seabream, but according to some studies, might have a beneficial
311 antimicrobial effect, thus increasing the shelf life of the final product.
312
313 **4. Conclusions**

Our results indicate that the microencapsulated essential oils present in the commercial feed additive AROTEC-G®, which is used to improve the sanitary status of *Sparus aurata*, do not affect the sensory characteristics of cooked fish, although slight differences were observed in some of the physicochemical parameters analysed in raw fish, mostly associated to storage time.

The inclusion of AROTEC-G® in gilthead seabream feed does not require a period of suppression since the sensory characteristics of the fish were not affected from day 0 of feed additive suppression onwards.

It would be advisable to carry out another study to assess whether, in addition to the effect that AROTEC-G® has on the parasite *Sparycotyle chrysophrii* in *Sparus Aurata*, it has a positive effect on the shelf-life of refrigerated fish fillets.

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Data Availability Statement.

The data used in this work have been obtained by the authors from the experimental work carried out and are in the possession of the corresponding author. No data from any repository has been shared or used.

ORCID

Celia María Iniesta Adán https://orcid.org/0000-0002-8928-1616

Elvira Zapata Galián https://orcid.org/0000-0002-9153-4627

Joana P. Firmino https://orcid.org/0000-0002-7694-0416

Irene Peñaranda Verdú https://orcid.org/0000-0001-8661-8677

Macarena Egea Clemenz https://orcid.org/0000-0002-8164-5868

Mª Belén Linares Padierna https://orcid.org/0000-0003-4903-2436

Mª Dolores Garrido Fernández https://orcid.org/0000-0002-8004-0998

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