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Research Article

Effect of polyunsatured aldehyde (A3) as an antiparasitary ingredient of *Caligus rogercresseyi* in the feed of Atlantic salmon, *Salmo salar*

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ABSTRACT. Some polyunsaturated aldehydes (PUAs) such as 2-trans, 4-trans decadenial (A3) have a detrimental effect on the development of copepod sharmful to the salmon industrysuch as *Caligus rogercresseyi* (sea lice). The purpose of this study was to evaluate the potential use of A3 as a salmon feed ingredient in order to reduce reproduction of *Caligus rogercresseyi* in infested Atlantic salmon *(Salmo salar*). The toxicity of A3 was assessed histopathologically for 7 days, using intra-peritoneal injections of different doses (0, 0.24, 0.47, 2.37, 11.86, and 23.71 mg kg^{-1}) in brain, intestine, skin, liver, and muscletissues of *Salmosalar* at the end of each treatment. The effect of A3 on sea lice was evaluated using 250 Atlantic salmon in an open-flow system of seawater (~13°C). The effect of the dosage in the fish diet was evaluated at two levels (9 mg kg^{-1}, 18 mg kg^{-1}) and considering a control (without A3) for 4, 8, and 12 days, once the sea lice had reached maturity. As a complement, the persistent effect of diluted A3 in sea water (0.5 g L^{-1}) was evaluated at 10, 12, and 14°C for 0, 1, 3, 6, 10, and 15 days; and the maximum persistence was found at 10 days at 10°C. The results showed that the dosages over 0.47 mg kg^{-1} had no toxic effect on Atlantic salmon, but induced a detrimental effect on *Caligus rogercresseyi* (reduction of 15% of mature females with a dose of 18 mg kg^{-1}), which could be attributed to alterations in the embryonic development of the sea lice. A3 is a potential supplement in the diet of salmon. However, studies ofits mechanism of action should be undertaken prior to its use.

Keywords: *Caligus rogercresseyi*, sea lice, PUA, reproduction, A3, infestation, *Salmo salar*, aquaculture, Chile.

Efecto del aldehído poli-insaturado (A3) como ingrediente antiparasitario de *Caligus rogercresseyi* en la alimentación de salmón del atlántico, *Salmo salar*

RESUMEN. Algunos aldehídos poli-insaturados (PUAs) tales como 2-trans, 4-trans decadenal (A3) tienen efecto perjudicial para el desarrollo de copépodos perjudiciales para la industria del salmón, tales como *Caligus rogercresseyi* (piojo de mar). El objetivo del presente estudio fue evaluar el uso potencial de A3 en alimentación de salmones, de forma de reducir la reproducción de *Caligus rogercresseyi*, en salmón del Atlántico (*Salmo salar*) infestado. Se evaluó en forma histopatológica la toxicidad de A3 durante siete días, a través de distintas dosis (0, 0,24, 0,47, 2,37, 11,86 y 23,71 mg kg^{-1}) inyectadas por vía intra-peritoneal en *Salmo salar*, en tejidos de cerebro, intestino, piel, hígado y músculo, al final de cada tratamiento, mientras que el efecto de A3 contra los piojos de mar fue evaluado usando 250 salmones del Atlántico en un sistema de flujo abierto de agua de mar (~13°C). El efecto de la dosis en la dieta de los peces se evaluó a dos niveles (9 mg kg^{-1} y 18 mg kg^{-1}) y con una muestra control (sin A3), por 4, 8 y 12 días, una vez alcanzado el estado maduro del piojo de mar. En forma complementaria, se evaluó el efecto de la persistencia de A3 diluido en agua marina (0,5 g L^{-1}) a 10, 12 y 14°C, durante 0, 1, 3, 6, 10 y 15 días, donde la máxima persistencia fue de 10 días a 10°C. Los resultados permitieron concluir que las dosis de prueba sobre 0,47 mg kg^{-1} no tuvieron efecto tóxico en el salmón del Atlántico, pero indujeron un efecto perjudicial sobre *Caligus rogercresseyi* (reducción del 15% de hembras maduras para una dosis de 18 mg kg^{-1}), lo cual podría atribuirse a alteraciones en el desarrollo embrionario del piojo de mar. A3 puede ser un potencial complemento en la alimentación de salmones, empero estudios sobre su mecanismo de acción deben realizarse previo a su uso.

Palabras clave: *Caligus rogercresseyi*, piojo de mar, PUA, reproducción, A3, infestación, *Salmo salar*, acuicultura, Chile.

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INTRODUCTION

Sea lice infestation (*Caligus rogercresseyi*) is one of the most important health challenges for the Chilean salmon industry. This external parasite impairs production efficiency; causes stress to the fish and it is a possible vector for other pathogens like ISAv and *Piscirickettsia salmonis* (J. Gonzalez, *pers. comm.*). In the last three years, infestation rate has increased in all three species produced in Chile, including Atlantic salmon (*Salmo salar*), Rainbow trouts (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*), probably due to a combination factors like resistence to Emamectin Benzoate (Report N°08-08) and the proximity between different companies that sharing the same production area. As a consequence, since 2007 new antiparasite drugs have been approved to be used in the Chilean salmon farming industry, including deltamethrin (used as a bath treatment and mostly at adult stage of lice, but with reduced efficacy in the last time (increasing the frequency of the treatment (Bravo *et al*., 2008)) and diflubenzuron (dfb, EWOSMR) whose effects are mediated by disruption of the chitin synthesis, preventing the lice to molt at the juvenile stages of lice development. However, resistance to another arthropodos like house fly (*Musca domestica*) has been reported by Pimprikar & Georghiou (1979).

Many terrestrial and marine plant extracts have been assessed for antibacterial, antifungal, antiviral and antioxidant activity for humans and animals (Bakkali *et al*., 2008). Moreover, it has been reported that aldehyde production and their release from terrestrial plants is a mechanism of defense against pathogens and insects (Rosahl, 1996; Pohnert & Boland, 2002). In this sence, recent research have reported certain marine microalgaes, including *Thalassiosira weissloggi*, *Thalassiosira rotula* and *Skeletonema costatum* produces some polyunsaturated aldehydes (PUAs) as secondary metabolites like 2-trans, 4-trans decadenial (A3). This metabolite stops partially the embryogenesis in copepods and sea urchin (Miralto *et al*., 1999; Ianora *et al*., 1999; Leflaive & Ten-Hage, 2007). In fact, it have been showed the effects of A3 in copepods like *Calanus helgolandicus* at concentrations >5 μg mL^{-1} are structural modifications in the cellular chromatin, due to fragmentations on DNA, followed by disrupting the RNA translation during embryogenesis (Romano *et al*., 2003).

Since the aldehyde 2-trans, 4-trans decadenial is available in pure chemical form and as a flavor ingredient (Adams *et al*., 2008) and taking into account the above; the A3 molecules in copepods could have use as antiparasitic. However, there are no reports in the scientific literature assessing the potential use of 2-trans, 4-trans decadenial against sea lice reproduction or over other copepod parasites, so the objective of this research was to assess the effectiveness of A3 in salmon feeds and their potential using to reduce sea lice reproduction when parasite infests in Atlantic salmon, taking into account their stability in sea water too, because no previous information is available in order to assess the potential persistence of the compound in the aquatic environment, as a base line of its potential negative effect in marine species.

MATERIALS AND METHODS

All the experimental methods were performed with sea water and in the species *Salmo salar* and *Caligus rogercresseyi* during 56 days, according with the following details:

Persistance of 2-trans, 4-trans decadenial (A3) in sea water

Trial was conducted at the Chemical Ecology Laboratory at Universidad de Temuco. A3 was diluted in sea water (32 psu) at 0,5 g L^{-1} in 9 different 250 mL flasks. The flasks were maintained in an incubator at 10, 12 and 14°C, using three replicate per temperature. The flasks were kept for 15 days at the respective temperatures. During this period, samples of 6 mL were taken from each flask at days 0, 1, 3, 6, 10 and 15. Air was continuously pumped into the flasks and samples taken from the flasks were processed according with the following protocol.

Solid extraction

The solid extraction was used to isolate the A3 compound diluted in water, according with the procedure described by Pino-Marambio *et al*. (2007). The compound was extracted in an EFS column (Biotage, Uppsala, Sweden) using a glass cartridge of

6 mL ((layer C2 (500 mg) over ENV+ (200 mg)) using one per each replica. Cartridges were previously prepared with 2 mL of HPLC grade methanol and then removed with HPLC grade water (2 mL). After the extraction of A3, impurities were again removed with HPLC grade water (2 mL). The chemical compounds absorbed in both phases (C2 and ENV+) were eluted with 2 mL of HPLC grade ethanol.

Distillation with vacuum

Volatile and non- volatile fractions of A3 compound were obtained through distillation with vacuum. Distillation was performed during 24 h with pressure at 0.04 torr and at environment temperature (average 20°C).

Liquid to liquid extraction

The volatile fraction of the distilled was obtained through liquid-liquid extraction, using dietylether (20 mL) and distilled ether (3 x 20 mL). Organic phases were mixed and dried with $CaSO_4$. The extract obtained was concentrated until 100 µL, stored in vials (-18°C) and then were sent to the Laboratory of Chemistry of the Universidad de La Frontera for A3 GC-EM analysis

Evaluation of the toxicity of A3 injected in Atlantic salmon

In order to perform sub lethal toxicity test, one hundred and thirty eight (138) fishes (~200 g) were pit-tagged and allocated in six tanks (23 fishes/tank), during April 29th of 2009, each one with a total capacity of 350 L. After 7 days of acclimatization, the fish were injected intraperitoneally (i.p.) with increasing doses of A3: 0.24, 0.47, 2.37, 11.86 and 23.71 mg kg^{-1}. Non-injected fishes were used as a control at Fundación Chile facilities (Quillaipe, X Region). Environmental variables were recorded daily, including water temperature (°C) and oxygen saturation.

After injection, fishes were monitored for mortality during 7 days in starving conditions and Probit Analysis was tested. Three fishes were euthanized per tank. Tissue samples were taken from brain, intestine, skin, liver and muscle (three pieces per tissue): one piece was sent for histopathological evaluation at Biovac Diagnostic Laboratory (Puerto Montt, Chile) and the other two pieces were pooled and were sent to the Chemical Ecology Laboratory of the Universidad de la Frontera (Temuco, Chile) for chemical analysis of A3 by GC-MS.

Effect of dietary A3 against sea lice (*Caligus rogercresseyi*) infestation and embryogenesis

The purpose of this trial was to investigate the possible effect of A3 over lice embryogenesis and fish infestation when added in the feed. The efficacy trial was conducted between middle of March to April, 2009.The 2-trans, 4-trans decadenial (A3) used in this trial were acquired from SIGMA Aldrich (85% of purity). Different solutions of A3 were prepared by dilution with methanol and then was added in the feed to reach different concentrations of the compound, mixing Danish fish oil with A3 compound. The dietary levels of A3 tested in this experiment were 0.0009% and 0.0018%, equivalent to 0.9 and 1.8 g ton^{-1} of feed, using a manual sprinkler for 4, 8 and 12 days according to a factorial experimental design, with five tanks of replicates. Pellets extruded were coated with the mixture of Danish fish oil and A3 compound, using a vacuum system.

Three hundred Atlantic salmons (150 g average weigh), were infested with copepodites of *Caligus rogercresseyi* (80 sea lice/fish) in a 1.5 m^3 tank with open sea water flow system at 13°C (average temperature). When 80% of sea lice infesting the fish reached adult stages, 250 fish were selected and randomly allocated in 25 tanks of 350 L each (10 fish/tank), with same conditions. A mesh (80 µm) was placed in the outlet of each one of the tank for controlling larvae losses. The meshes were cleaned daily and larvae were collected, fixed with formaldehyde (5%) and then counted. The 25 tanks were assigned to the three experimental diets with different levels of A3. The different life stages of sea lice were counted in 25 fish per tank at the end of each time period.

At the end of each time, the following lice parameters were recorded for analyses:

a) N° of mature females (females with mature eggs in the egg strings).
b) N° of female with eggs strings (females without mature eggs in the egg strings).
c) N° of total embryos per each egg string (embryos in different stages of development in the egg strings).
d) N° of total larvae, as the sum of viable and non viable larvae in each tank.
e) N° of non-viable larvae (deformed larvae in the limbs or cephalotorax).
f) N° of nauplius II.

Ten females with egg strings were collected from each tank of fish fed the control and the diet with 0.0018% A3 and incubated (14°C) for 2 days with filtered (1 µm) sea water. After the larvae were hatched and developed into copepod stage, they were collected and used in the *in vitro* method to evaluate formation of frontal filament as indicator of viability. This methodology can be pointed as follows:

a) Mucus was obtained directly from Atlantic salmon previously anaesthetized with AQUÍ-S (Bayer®) in a bath for 5 min approximately. Under the tail, it was placed a plastic glass (10 mL) to receive the mucus of each fish. Samples were frozen in liquid nitrogen container and finally kept in a freezer (-80°C). Mucus was used to prepare a stock (1 L) of agar and mucus solution. *Salmo salar* filtrated mucus (100 mL) was defrosted at room temperature.

b) It was prepared a solid agar with 1 L of distilled water and 30 g of agar, heating at 35°C.

c) When temperature of agar was 35ºC, it was added mucus defrosted.

d) The solution of agar and mucus were distributed in each Petri dish (~60 mL each), waiting to become in gel. Two Petri dishes were allocated per each plastic container (2.5 L) with microfiltrated (5 µm), aerated and sterilized seawater (UV light at 70 µW cm^{-3}).

e) Finally, a minimum of 100 copepodids were deposited in each container and then incubated (14°C) in an incubator chamber for 48 h.

After 48 h, all the copepods of each container were extracted and then fixed with a mixture of alcohol, distilled water and glycerol (45, 45 and 10% respectively in a volume of 0.5 mL). Fixed copepods were dehydrated at constant temperature (35°C) for 1 day. Measurement of the presence/absence of the frontal filament in the copepods was performed with an optical microscope (40x).

Finally, the specific feeding rate percentage (SFR) in *Salmo salar* fed with or without A3, was calculated. In addition, a generalized linear model with Poisson distribution was used for the analysis for prediction of the *C. rogercresseyi* embryonic development .In this sence, host-parasite counts are often found to be over-disperse and this was taken into account for the analysis. This was achieved by using a quasi-Poisson distribution allowing for a scale parameter instead of the ordinary Poisson distribution.

According with Myers & Montgomery (1995), the factors (dose and exposure time) were scaled and coded as follows: -1 is the low level, +1 is the high level and 0 is the center point. This coding makes factor and interaction estimates independent from each other. The linear predictor model fitted was thus:

$$\eta i = p0 + p1Di + p2Ti + p12DiTi$$

where: ηi are the observed counts after applying the link function, Di the doses, Ti the exposure times at the respective doses, and $p1$- $p12$ estimated parameters describing the effects of the predictors. The link function for the Poisson model is the log. All statistical modeling was conducted with the R language and its corresponding packages (R Core Team, 2008).

RESULTS

Persistance of 2-trans, 4-trans decadenial (A3) in sea water

Persistency of A3 in water is an important indicator of potential time of exposure of animals in the water environment to this compound. A higher exposure time to A3, it means higher toxicity risk in aquatic animals. After 10 days of exposure, it was not detected A3 in the different solutions and/or temperatures tested (Fig. 1). However, at 10°C, aldehyde concentrations were higher at 3 and 6 days of exposure, indicating a higher stability of the aldehyde as the temperature of the water decreased. According to the analyses, the persistence of A3 in sea water at 10°C was between 6 to 10 days.

Effect of dietary A3 against sea lice (*Caligus rogercresseyi*) infestation and embryogenesis

In all the tanks, the percentage of specific feeding rate (SFR) of the fish increased along the time during the feeding period of 10 days (Fig. 2). However, there were no differences ($P > 0.05$) in the feed consumed among the different doses of A3 added in diets. The highest average SFR (1.3%) was recorded at the seventh day of feeding for all treatments, while the lowest SFR (0.57%) was recorded at the initiation of the feeding period.

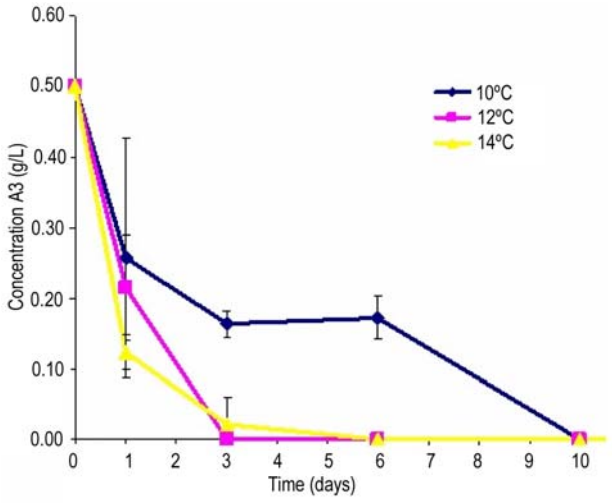

Figure 1. Concentration of A3 maintained in sea water for 15 days at three different temperatures.

Figura 1. Concentración de A3 en agua de mar durante 15 días a tres temperaturas distintas.

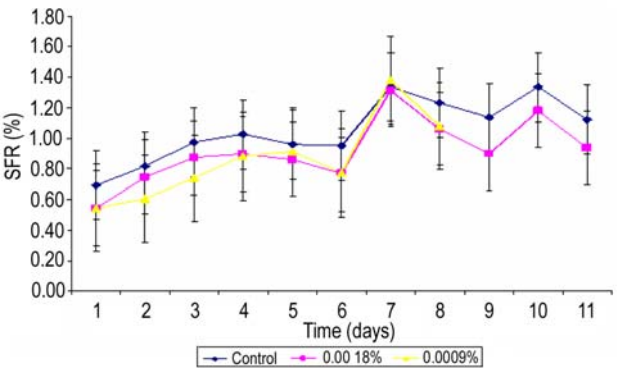

Figure 2. Percentage specific feeding rate (SFR) of fishes fed with different dietary doses of A3.

Figura 2. Porcentaje específico de tasa de alimentación (SFR) en peces alimentados con distintas dosis de A3.

As it was expected, the time effect was significant over the egg counts of females, indicating that the number of eggs increased with time. Interestingly, increasing dose of A3 significantly decreased the total egg counts. In addition, the number of gravid females was not significantly affected by either time or dose, neither a significant interaction was observed for this variable ($P > 0.05$). There is a trend for a reduction in the number of gravid females with exposure time. This can be explained because with time, eggs become mature and hatch, reducing the number of gravid females (Table 1).

Both dose and exposure time showed a detrimental effect over the number of mature females, but there was no significant interaction between them. This means effects are additive. Both increasing dose and increasing exposure time, decreased the count of mature females, where increasing the time was about twice as effective. Based on the model, increasing the dose from zero to 0.0018% reduced the number of mature females from about 99 to 85 (-15%). Similarly, increasing the exposure time from 4 to 12 h at dietary 0.0018% A3, reduced the number from about 98 females to 73 females (-25%). This model could help to optimize the dose (A3 added in feed) and time of exposure to reach the maximum effect in terms of mature female reduction.

The A3 dose did not influence the total larvae counts. However, with time these parameters increased significantly. This may be explained by the constant release of larvae from the egg strings and their accumulation in the different larvae life stages (*nauplius* I, *nauplius* II and copepodites). Same situation was observed here, in comparison with total larvae count. The A3 dose did not show any direct effect in non-viable larvae but time showed an important effect, which means in morphology terms, quality of larvae became worst in last emission from the eggs. The results for the number of *nauplius* II are similar to the response observed for total larvae with a non significant effect of the dose of dietary A3 or interaction between time and dose. As expected, with time the number of *nauplius* II increased significantly.

Formation of the frontal filament

The results showed that the copepods hatched from females taken from the fish fed with a diet supplemented with 0.0018%, A3 had a significantly lower capacity ($P \leq 0.05$) to develop the frontal filament when compared to the copepods hatched from females taken from the fish fed with the control (Fig. 3).

Toxicity of A3 injected in Atlantic salmon

During the 7-days period after the fish were injected with different doses of A3, no mortality was recorded in *S. salar*. However, different histopathological changes were observed in the analyzed tissues. The findings were classified from mild to severe damage with a score ranging from 0 (no damage or changes) to 4 (severe). Table 2 shows this classification with the respective damage tissues.

There were histopathological changes found in brain and liver (included a mild meningitis, eosinophilic cytoplasm in liver cells, hepatitis and necrotic area in the liver). Moreover, in the intestine

Table 1. Estimated coefficients according linear model for embryonic stage development in *Caligus rogercresseyi.*

Tabla 1. Coeficientes estimados según modelo lineal para estado de desarrollo embrionario en *Caligus rogercresseyi.*

	Total eggs	Gravid females	Mature females	Total larvae	Non viable larvae	Nauplius II
Intercept (p_0)	8.81± 0.03	1.94 ± 0.12	4.52 ± 0.03	6.09 ± 0.16	2.24 ± 0.3	5.65 ± 0.14
C dose (p_1)	-0.08 ± 0.03	0.00 ± 0.13	-0.08 ± 0.03	0.07 ± 0.19	-0.10 ± 0.41	0.05 ± 0.17
C time (p_2)	0.19 ± 0.03	-0.23 ± 0.13	-0.15 ± 0.03	0.96 ± 0.17	1.88 ± 0.31	0.90 ± 0.15
C dose:time (p_{12})	-0.03 ± 0.03	-0.13 ± 0.13	-0.02 ± 0.03	-0.10 ± 0.19	-0.03 ± 0.41	-0.14 ± 0.17

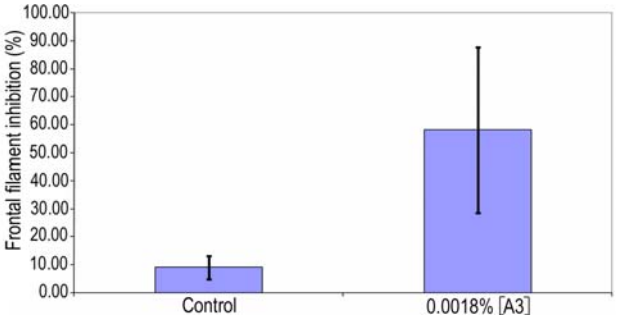

Figure 3. Percentage of inhibition of the formation of the frontal filament in *C. rogercresseyi* hatched from females taken from fish fed the control feed and the diet supplemented with 0.0018% of (A3). Values differ significantly ($P < 0.05$).

Figura 3. Porcentaje de inhibición en formación de filamento frontal en *C. rogercresseyi* eclosionados de hembras tomadas de peces alimentados con el alimento control y con 0,0018% de A3. Los valores difieren significativamente ($P < 0,05$).

an inflammatory reaction in peripherical cells and Giant cells with empty vacuoles was observed. There were no evidences of histopathological changes in the skin and muscle tissues. The most severe damage found was a necrotic area in the liver of one fish with a dose of 11.86 mg kg^{-1} weight of A3 (Fig. 4a).

The prevalence and severity of the histopathological changes in the more affected tissues of *S. salar* injected with different doses of A3 are shown in (Table 3). Only the liver and intestine showed histopathological changes with scores 2 and 3. However, except for one sample on liver showing a score 3, all the other changes were classified as score 2. In both tissues, there is a trend to have a higher prevalence of damage with increasing doses of A3. It is also worth noting that in the intestine, there was a high prevalence of an inflammatory reaction in peripherical cells with doses equal or above 0.47 mg kg^{-1} of A3 (Table 3, Fig. 4b). Only one case, classified as score 1, was observed in the brain of a control fish which corresponded to mild meningitis (33% prevalence) (Table 3, Fig. 4c).

The same tissues evaluated for histopathological changes were analyzed too, for the concentrations of 2-trans, 4-trans decadenial (A3). The results of these analyses are shown in (Table 4*)*. The compound A3 was found in all the tissues analyzed (liver, brain, intestine, skin and muscle). Higher concentrations of A3 were observed in the intestinal tissue. Although there appears to be some relationship between the injected dose of A3 and its concentration, for intestine, skin and muscle, there was no detection of A3 in any of the analyzed tissues at the highest dose (23.72 mg kg^{-1}) with the exception of the intestine. No content of A3 were detected in the tissues of the control fish.

DISCUSSION

According with the results obtained in this study, it is possible to indicate persistence of A3 in sea water at 10°C is between 6 to 10 days. The results of this study

Table 2. Description and grading of the histopathological changes observed in the different tissues of fish injected with A3.

Tabla 2. Descripción y clasificación de los cambios histopatológicos observados en los diferentes tejidos de peces inyectados con A3.

Findings histopathology	Brain	Tissue liver	Intestine
No damage	–	–	–
Low damage in tissue without deleterious effects for the organ.	Mild meningitis	Cells with eosinophilic cytoplasm and eccentric nuclei	–
Damage in tissue with potential deleterious effects in functionality of the organ. However, this damage is not permanent and the tissue can recover its functionality.	–	Hepatitis	Inflammatory reaction in peripherical cells. Giant cells with empty vacuoles.
Damage in tissue with deleterious effects in functionality of the organ.	–	Necrotic area	–
Morphological severe damage of tissue. Endanger life.	–	–	–

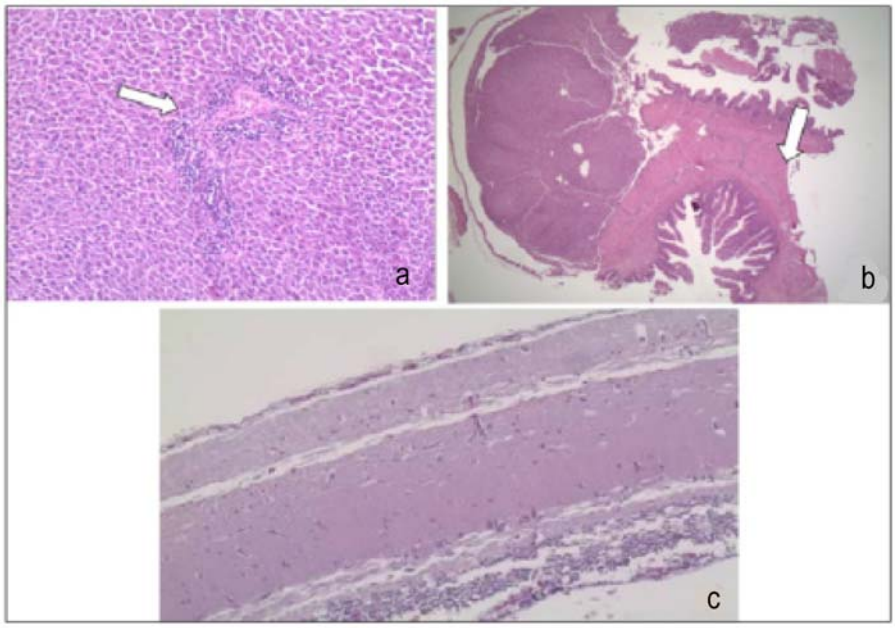

Figure 4. Histopathological changes found in different tissues: a) Sample of liver (20x) from a fish injected with 11.86 mg kg^{-1} of A3, showing an area with necrotic tissue indicated by the arrow, b) sample of intestine (2,5x) form a fish injected with 0.47 mg kg^{-1} of A3 showing inflammatory response in the peripherical cells indicated by the arrow, c) sample of brain tissue (10x) of a control fish showing a mild meningitis.

Figura 4. Cambios histopatológicos encontrados en distintos tejidos: a) Muestra de hígado (20x) de un pescado inyectado con 11,86 mg kg^{-1} de A3, mostrando un área de tejido necrótico indicado por la flecha, b) muestra de intestino (2,5x) de un pescado inyectado con 0,47mg kg^{-1} de A3, mostrando respuesta inflamatoria en las células periféricas indicadas por la flecha, c) muestra de tejido cerebral (10x) de un pescado control, mostrando una leve meningitis.

Table 3. Prevalence of histopathological changes observed in the different tissues of *S. salar* injected with different doses A3.

Tabla 3. Prevalencia de cambios histopatológicos observados en distintos tejidos de *S. salar* inyectado con distintas dosis A3.

Tissue	A3 dose mg kg^{-1}	1	2	3
Liver	0.00	33%	-	-
	0.24	-	-	-
	0.47	-	33%	-
	2.37	-	-	-
	11.86	-	33%	33%
	23.72	-	67%	-
Intestine	0.00	-	33%	-
	0.24	-	-	-
	0.47	-	100%	-
	2.37	-	100%	-
	11.86	-	67%	-
	23.72	-	100%	-

also showed that the persistence of A3 was higher at lower temperatures. Refsgaard *et al.* (2000) indicated that molecules, like unsaturated aldehydes, are unstable and can make a covalent bond with núcleophilic compounds. This may explain why the persistence of this compound is short but extended at lower temperatures.

The aldehydes type α - β were described as toxic for marine organism, principally invertebrates *e.g*: *Nereis virens* (Lewis *et al*., 2004), *Calanus helgolandicus* (Pohnert *et al*., 2002), *Temora stylifera* (Ceballos & Ianora, 2003), *Asteria rubens* (Caldwell *et al*., 2002), stopping reproduction process. These compounds also showed to be toxic for rats (Adams *et al*., 2008) and human lung cells (Chang & Lin, 2008). According to a previous research, (Ponhert *et al.,* 2002), it´s production in nature is due to fatty acid oxidation by the stress in some diatoms like *Phaeodactylum tricornutum, Phaeocystis pouchetii, Skeletonema costatum and Alexandrium formosa* in presence of free grazer copepods (Hansen *et al.*, 2004).

Sublethal essay showed not severe histopathological changes observed in tissues treated with the unsaturated aldehydes. The tissue presented higher frequency of histological damages was the intestine, mainly in the doses of A3 injected between 0.47 mg kg^{-1} to 23.72 mg kg^{-1}. It is related with the average

Table 4. Concentration of A3 in tissue samples of fishes injected with different dose of A3[1].
Tabla 4. Concentración de A3 en muestras de tejidos de peces inyectados con distintas dosis de A3[1].

Tissue	Injected dose of A3 mg kg^{-1}	Minimum	Maximum
Liver	0.00	0	0
	0.24	0	0
	0.47	0	0.03
	2.37	0	0.01
	11.86	0	0.04
	23.72	0	0
Brain	0.00	0	0
	0.24	0	0.00
	0.47	0	0
	2.37	0	0
	11.86	0	0.02
	23.72	0	0
Intestine	0.00	0	0
	0.24	0	0
	0.47	0	0.04
	2.37	0	0.03
	11.86	0	0.05
	23.72	0.02	0.03
Skin	0.00	0	0
	0.24	0	0.03
	0.47	0	0.01
	2.37	0	0.03
	11.86	0	0
	23.72	0	0
Muscle	0.00	0	0
	0.24	0	0
	0.47	0	0.01
	2.37	0	0.04
	11.86	0	0
	23.72	0	0

[1]Minimum and maximum concentration of A3 were calculated from three samples analyzed per each dose injected in each tissue.

concentration of A3 obtained in the intestine tissues, (0.01 to 0.03 mg kg^{-1}) at the same doses of A3 injected. Similar damages observed for intestine tissue of *S. salar* were reported for rats, with inflammation and edema in the forestomach, when the animals were treated with 200 to 800 mg kg^{-1} body weight/day of A3. However, when lower doses of A3 were tested (3.39 to 33.9 mg kg^{-1} body weight/day), no damages in tissues were observed (Adams *et al*., 2008).

The 2-trans, 4-trans decadenial has been recognized by FEMA (Flavor and Extract Manu-facturers Association) as a safe product (GRAS) for it uses as a flavoring in human foods, at doses lower than 33.9 mg kg^{-1} BW/d (Adams *et al.*, 2008). This dose is significantly higher than the dietary doses of A3 used in this study to treat *Salmo salar* against sea lice, where a maximum of 0.27 mg A3/kg BW/d was used in the efficacy trail. In addition, the fact founding A3

in the tissues at low concentrations, and especially in the skin (~0.01 mg kg^{-1}), make this product a potential candidate to be added in the feed as an oral treatment for salmons against sea lice infestation, in low doses. However, further studies will be required to understand the potential toxicity, lethal and sub-lethal effects, when fish will be treated more than once with A3 added in feed. Also it is important to study the detoxification and metabolization mechanisms for A3 in the salmon, in order to optimize the A3 doses in feed.

Regarding the effects of dietary A3 over sea lice infestation and embryogenesis, a significant reduction in the number of mature females and eggs of *Caligus rogercresseyi* as increasing doses of A3 in the feed could be observed. However, no effect of A3 was observed over gravid females or the number of different larvae stages of lice. This suggests through A3 exposure time, the compound could have a detrimental effect over the females, turning maturation and the new generations of eggs produced during the assay. In addition, sea lice larvae could be already hatched, explaining why the number of larvae increased with time. Therefore, one theory that can be drawn from these observations is A3 might affect the normal development of the lice embryos. Similar results were obtained for embryos of the copepods *Calanus helgolandicus*, when treated with A3 (Romano *et al.*, 2003; Ianora *et al.*, 2004). Moreover, formation of Frontal Filament Test showed sea lice grown from larvae hatched from females (obtained from fish fed with 0.0018 A3 doses) had a significantly lower capacity to develop the frontal filament. These lice could suffer an alteration during its embryogenesis which explains their further impossibility to develop the frontal filament. Several authors have described deformities or inhibitions in the development of the limbs of copepods embryos and larvaes exposed to different doses of this unsaturated aldehyde (Caldwell *et al*., 2002; Pohnert *et al*., 2002; Romano *et al*., 2003; Ianora *et al*., 2004; Lewis *et al.*, 2004; Taylor *et al*., 2005).

The following conclusions could be obtained from this research:

1. The persistence of 2-trans, 4-trans decadenial dissolved in seawater is maximum 10 days with temperature of 10°C.
2. At the injected doses tested in the present study (up to 0.47 mg kg^{-1} BW), 2-trans, 4-trans decadenial did not induce a toxic effect in Atlantic salmon (*Salmo salar*).
3. 2-trans, 4-trans decadenial induced a detrimental effect over *C. rogercresseyi* when added in the feed of Atlantic salmon (*Salmo salar*). Its effect is likely related to alterations in the normal development of lice embryos, like formation of frontal filaments, during infestation.

Concluding remarks: A3 could be a novel alternative as *Salmo salar* feeding additive. This molecule affects sea lice embryogenesis, having potential as a complementary control strategy. However, it is necessary to study LD_{50} in *S. salar* and better knowledge about the ultimate mechanisms of action of 2-trans, 4-trans decadenial over lice development to evaluate its true potential as a new feed additive alternative

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