PJ-00051 Palbio 50 salmon Chile 12/11/21 PROJECT CLOSURE

Sergi Segarra

R&D Bioiberica SAU 12/11/2021





PJ-00051 Palbio 50 salmon Chile

| Title: | Palbio 50 salmon Chile | | |
|-----------------------|------------------------|--|--|
| Project number: | PJ-00051 | | |
| Preceding Idea: | IDEA-0184 | | |
| Business Unit: | Animal nutrition | | |
| Project Leader: | Sergi Segarra | | |
| Responsible | Xavier Córdoba | | |
| Manager: | | | |
| Type of Project: | Product Support | | |
| Subfamily: | 421 - PALBIO 50 | | |
| Portfolio: | 208 - PALBIO | | |
| Start date | 21/10/2019 | | |

OBJECTIVES:

The purpose of this project is to obtain scientific evidence to support the use of Palbio50 as a high-quality protein source for salmon and thus increase the sales of this product in the LATAM aquaculture market.

PJ-00051 Palbio 50 salmon Chile: SCOPE & MPP/TPP

SCOPE:

- This project involves the performance of a study to evaluate performance parameters and intestinal health in salmon in which fish meal has been partially replaced by Palbio50.
- ➤ If we confirm that Palbio50 can be used as an adequate source of protein in such animals, this would open the door to new potential applications of this product for the animal nutrition business unit in the aquaculture industry, and especially in the LATAM market.



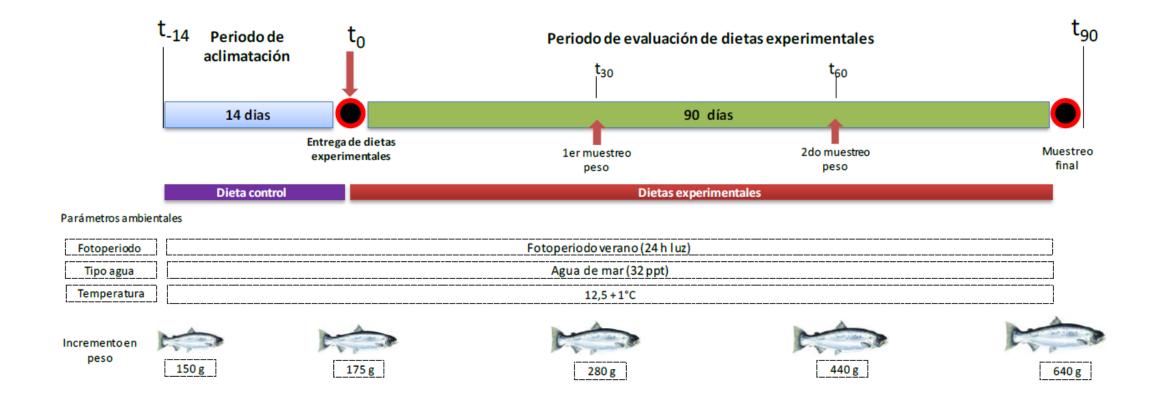
MPP/TPP:

- **Minimum product profile:** The use of Palbio50 in salmon diets, as partial replacement of fish meal, allows a reduction in the diet cost and leads to non-inferiority in terms of performance.
- **Target product profile:** The use of Palbio50 in salmon diets, as partial replacement of fish meal, allows a reduction in the diet cost and leads to a significant superiority in terms of performance.



PJ-00051 Palbio 50 salmon Chile - STUDY

Experimental design:



PJ-00051 Palbio 50 salmon Chile - DIETS

- **DIET 1**: representative of the commercial diets commonly used in salmon production in Chile, including regular costs and levels of inclusion of fish meal, corn, soy and omega6/3 ratio.
- **DIET 2**: Formulated from DIET 1, by including Palbio50 at 2.5% as substitutive for fish meal and adjusting the rest of the formula.
- **DIET 3**: Formulated from DIET 1, by including Palbio50 at 5% as substitutive for fish meal and adjusting the rest of the formula
- All diets must be iso-proteic, iso-electrolytic and iso-energetic.
- In all 3 diets, an adequate omega6/3 will be maintained, and this ratio will be similar between the 3 diets.
- Soy levels will be adequate in all 3 diets, and within an acceptable range according to the inclusion levels routinely used in diets for salmon production in Chile.
- Corn gluten meal levels will be the same in all diets, and without exceeding the maximum acceptable in salmon to avoid excessive pigmentation (10%).
- Costs of the study diets will be provided once formulated. These calculations will be made considering the current prices of the ingredients that are used when designing the formulations for the study diets.
- Diets 2 and 3 must be cheaper than diet 1. More specifically, the ideal target is that diets including Plabio50 should be around 20€/MT cheaper than diet 1. However, other options might be considered as long as they are cheaper than diet 1.



PJ-00051 Palbio 50 salmon Chile - DIETS

DIETS: MILESTONE-0010K

| Formulación de dietas (%) | | | | | |
|--------------------------------|---------------|---------|---------|---------|--|
| Ingrediente | Costo (\$/MT) | Dieta 1 | Dieta 2 | Dieta 3 | |
| Palbio50 | 1.348 | | 2,5 | 5,0 | |
| Fish meal | 1.510 | 20,0 | 17,5 | 15,0 | |
| Fish oil | 1.521 | 8,0 | 8,1 | 8,3 | |
| Blood meal | 1.720 | 5,0 | 4,8 | 4,8 | |
| Canola oil | 934 | 9,8 | 9,4 | 8,9 | |
| Corn gluten meal (Empyreal 75) | 670 | 10,0 | 10,0 | 10,0 | |
| Feather meal | 750 | 5,0 | 6,5 | 7,0 | |
| Lupine meal | 490 | 0,6 | | | |
| Poultry by meal (66% Prot) | 953 | 10,0 | 13,5 | 17,2 | |
| Premix vit+min | 4.574 | 1,0 | 1,0 | 1,0 | |
| Soy protein concentrate | 843 | 10,0 | 7,7 | 3,8 | |
| Soybean meal | 422 | 5,0 | 5,0 | 5,0 | |
| Wheat gluten | 2.064 | 1,7 | | | |
| Wheat meal | 410 | 12,0 | 12,0 | 12,0 | |
| Bio-Lys | 842 | 0,7 | 0,8 | 0,9 | |
| CaHPO4 | 393 | 0,9 | 0,9 | 0,9 | |
| MetAmino | 2.505 | 0,2 | 0,2 | 0,2 | |

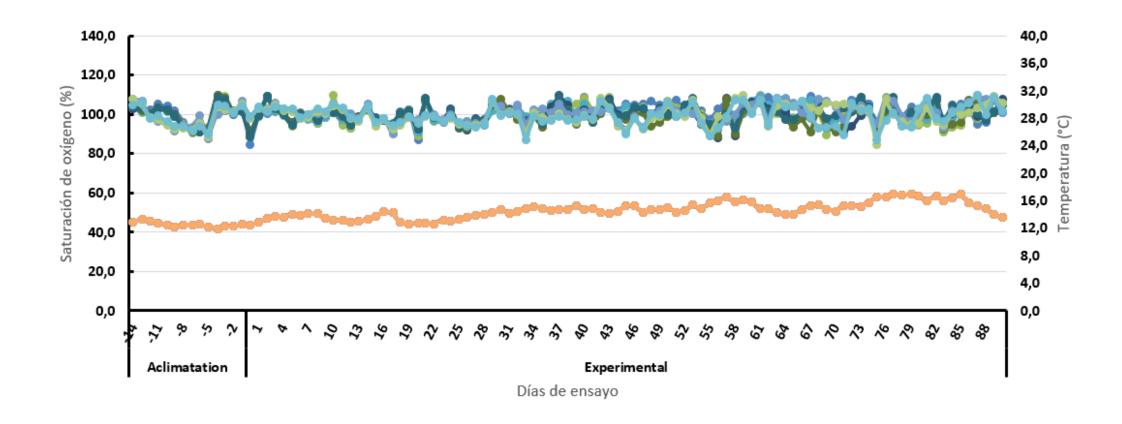
| Total | 100 | 100 | 100 |
|---------------------------------------|-------|-------|-------|
| Costo (\$/MT) | 1.055 | 1.034 | 1.034 |
| Costo (€/MT) | 960 | 941 | 941 |
| Ahorro (€/MT), con respecto a Dieta l | - | 19 - | 19 |

| Com | posición proximal teórica de la | s dietas | | |
|-------------------------|---------------------------------|----------|---------|---------|
| Nutriente | Unidades | Dieta 1 | Dieta 2 | Dieta 3 |
| Proteína cruda | % | 47,5 | 47,5 | 47,5 |
| Proteína digestible | % | 39,2 | 38,9 | 38,8 |
| Humedad | % | 7,3 | 7,2 | 7,0 |
| Cenizas | % | 6,1 | 6,5 | 6,9 |
| Lípidos | % | 22,0 | 22,0 | 22,0 |
| Fibra | % | 1,10 | 1,01 | 0,87 |
| Extracto no nitrogenado | % | 16,0 | 15,8 | 15,7 |
| W6/W3 | | 0,6 | 0,6 | 0,6 |
| Metionina total | % | 1,2 | 1,2 | 1,2 |
| Metionina digestible | % | 1,1 | 1,1 | 1,1 |
| Lisina total | % | 3,3 | 3,3 | 3,3 |
| Lisina digestible | % | 2,8 | 2,8 | 2,8 |
| Energía bruta | MJ/kg | 22,8 | 22,7 | 22,7 |
| Energía digestible | MJ/kg | 18,7 | 18,6 | 18,6 |





PJ-00051 Palbio 50 salmon Chile - RESULTS



No significant variations overtime or between groups



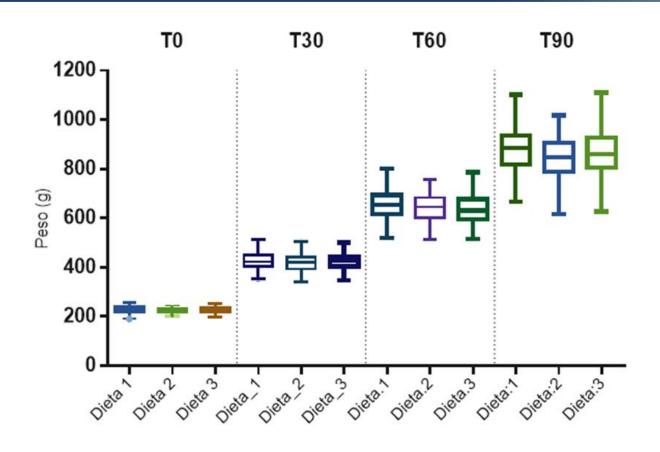
| Estanque | Dieta | N° peces | Mínimo (g) | Máximo (g) | Promedio (g) | Desviación estandar (g) | Coef. Variación |
|----------|---------|----------|------------|------------|--------------|-------------------------|-----------------|
| 1 | Dieta 3 | 45 | 198,4 | 247,8 | 228,6 | 11,7 | 5,1% |
| 2 | Dieta 1 | 45 | 199,2 | 254,8 | 227,2 | 11,9 | 5,2% |
| 3 | Dieta 2 | 45 | 202,6 | 246,4 | 224,0 | 9,9 | 4,4% |
| 4 | Dieta 1 | 45 | 196,8 | 252,2 | 227,0 | 13,0 | 5,7% |
| 5 | Dieta 2 | 45 | 201,2 | 259,0 | 229,1 | 12,4 | 5,4% |
| 6 | Dieta 3 | 45 | 203,6 | 250,2 | 227,7 | 11,8 | 5,2% |
| 7 | Dieta 2 | 45 | 196,8 | 247,8 | 225,5 | 11,2 | 5,0% |
| 8 | Dieta 3 | 45 | 207,0 | 251,8 | 227,3 | 9,8 | 4,3% |
| 9 | Dieta 1 | 45 | 190,6 | 256,0 | 228,7 | 15,4 | 6,7% |

No significant differences at baseline





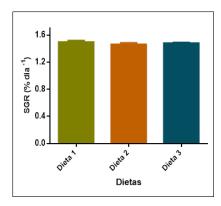


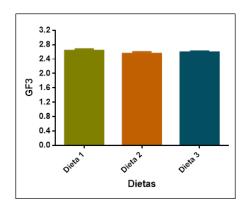


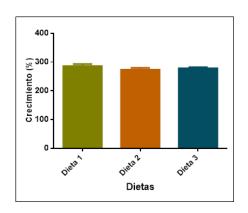


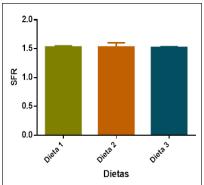
Tiempo de muestreos

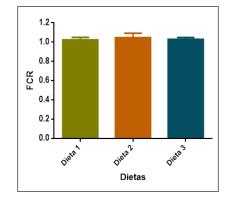
Weight: No significant differences











- Specific Growth Rate (SGR) incremento porcentual de peso del pez por día
- Growth Factor Three (GF3): relaciona crecimiento con temperatura del agua
- Factor de conversión de alimento (FCR): cuántos kilos de alimento se necesitan para producir un kilo de salmón
- Specific Feeding Rate (SFR) cantidad de alimento ingerida por día y expresado como porcentaje de peso corporal del pez
- Crecimiento (%): incremento porcentual de peso corporal de un pez o una población finita (jaula), en un periodo de tiempo determinado (diario, semanal, o mensual).

SGR, GF3, FCR, SFR & growth: No significant differences



Mortality: no remarkable effects



Registro de Mortalidad_Ensayo Bioiberica

Volver a indice

| Nº pez | Fecha | Estanque | Peso (g) | Longitud (cm) | Índice de condición (K) | Observaciones |
|--------|------------|----------|----------|---------------|----------------------------|-----------------------|
| 1 | 25/11/2019 | 7 | 274,7 | 29,5 | 1,1 | Sin causa aparente |
| 2 | 07/01/2020 | 5 | 635,3 | 37,0 | 1,3 | Post manejo tiempo 60 |
| 3 | 07/01/2020 | 9 | 505,5 | 35,0 | 1,2 | Post manejo tiempo 60 |

MILESTONE 2:

End of performance study

Go/no go: YES

Due date: 24/04/20.

Deliverable: Performance study data from research center: Reporte final

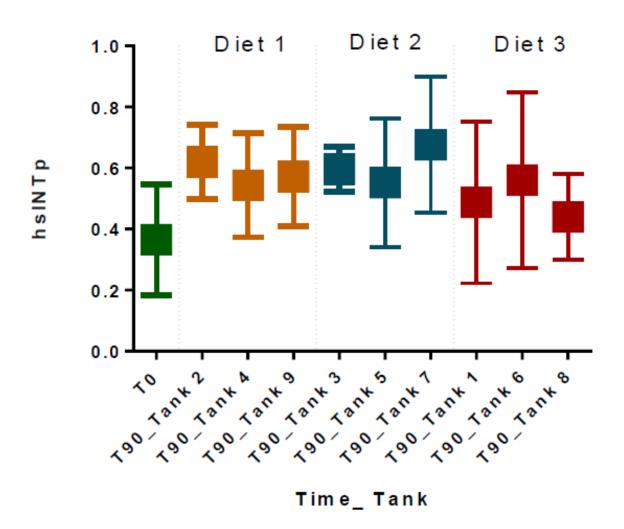
Cost: 48,801 €.

Acceptance: Results should be, at least, in accordance with the minimum product profile:

"The use of Palbio50 in salmon diets, as partial replacement of fish meal, allows a reduction in the diet cost and leads to non-inferiority in terms of performance."

Actions: Based on the results obtained, a decision on whether to perform histology and gene expression or not will be made. If the MPP has not been achieved based on performance results, histology and gene expression will not be performed.

PJ-00051 Palbio 50 salmon Chile - HISTOLOGY



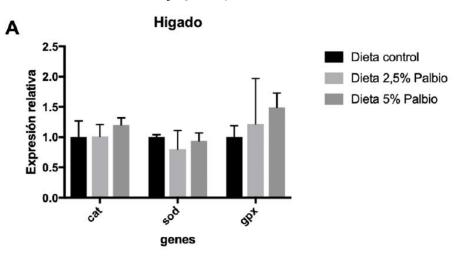
No relevat pathological alterations and no significant differences between study groups

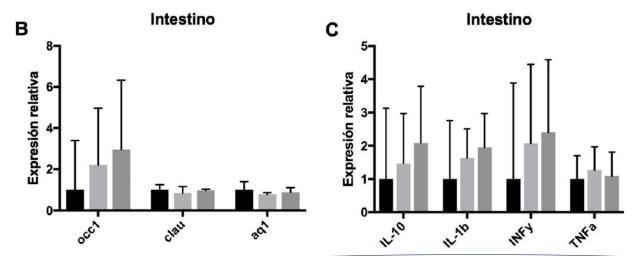
No negative impact

PJ-00051 Palbio 50 salmon Chile - GENE EXPRESSION

No significant differences between study groups

No negative impact





| cat | catalasa | Respuesta oxidativa hepática, descomposición especies reactivas de oxígeno |
|-------|----------------------------------|--|
| sod | Superóxido dismutasa | Respuesta oxidativa hepática, descomposición especies reactivas de oxígeno |
| gpx | Glutatión peroxidasa | Respuesta oxidativa hepática, descomposición especies reactivas de oxígeno |
| occ1 | Occludina | Integridad estructural intestinal, absorción |
| clau1 | Claudina 25b | Integridad estructural intestinal, absorción |
| aqp1 | Acuaporina 8 | Integridad estructural intestinal, absorción |
| TNF α | Factor de necrosis tumoral | Respuesta inmune e inflamación intestinal. |
| IL-1β | Interleuquina 1 beta | Respuesta inmune e inflamación intestinal. |
| IL-10 | Interleuquina 10 | Respuesta inmune e inflamación intestinal. |
| ΙΝΕγ | Interferón gama | Respuesta inmune, inflamación |



PJ-00051 Palbio 50 salmon Chile - CONCLUSIONS

The use of Palbio50 in salmon diets, as partial replacement of fish meal (2.5 – 5%), allows a reduction in the diet cost (around 20€/MT) & leads to non-inferiority in performance (no significant differences vs control commercial diet)



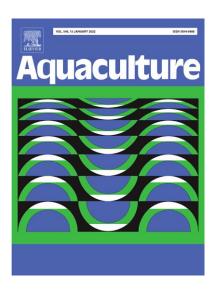


Lessons learned:

In future research projects with a similar approach, during the planification, an additional task will have to be included to make sure that the nutritional assessment of diets for the study is adequately performed and that diet formulation are optimal in order to achieve our efficacy targets

SCIENTIFIC COMMUNICATION

- **Abstract Aquaculture 2022** ACCEPTED (March 2022)
- **Abstract World Aquaculture 2020** ACCEPTED (November 2022)
- Paper Aquaculture?



SUITABILITY OF A PORCINE HYDROLYSED INTESTINAL MUCOSA PROTEIN SOURCE FOR AOUACULTURE SPECIES BASED ON AMINO ACID PROFILES AND FEED REQUIREMENTS

Sergi Segarra*, Jesús Cabañas and Daniel Martínez-Puig R&D Bioiberica SAU, Barcelona, Spain, ssegarra@bioiberica.com

Aquaculture ongoing growth requires new efficient, safe, and sustainable protein sources. The use of plant-based protein sources, especially from soy, as partial replacement for fish meal is common but limited due to the presence of antinutrients. Palbio is a high-quality, sustainable, protein obtained from porcine intestinal mucosa as a byproduct of the heparin manufacturing process following a circular economy approach. It contains bioactive peptides and leads to health benefits in animal species, especially in swine. Its use in aquaculture might allow lower inclusion levels of plant-based protein sources. The essential amino acids profile is a key parameter to evaluate the suitability of a protein source for animal feed, and protein quality can be estimated by calculating the contribution of each amino acid to the ideal pattern. We evaluated the suitability of Palbio by analyzing its amino acid profile and comparing it with that of soy and with the requirements of Atlantic salmon (Salmo salar) and pig (Sus scrofa) using the ideal protein approach.

The amino acid content in three different Palbio HP industrial batches (20/0001, 21/0001, 21/0072; Bioiberica SAU, Palafolls, Spain) was measured with high-performance liquid chromatography with fluorescence detection (HPLC/FLD). Pig and salmon muscle amino acid profile were obtained from Bahelka et al., 2020 and Wilson & Cowey et al., 1985, respectively; soybean meal data from AmiPig (2000); and nutritional requirements from publicly available defined nutritional requirements (NRC of swine 2012, and NRC of fish and shrimp 2011).

Palbio HP results are shown in Table 1, while Figure 1 depicts a comparison and degree of overlap between the different amino acid profiles and nutritional requirements. Our analyses showed a high degree of overlap between Palbio HP and salmon muscle and nutritional requirements, and similar to those of pig. Conversely, soybean meal, as a reference plant-based protein source, shows a lower degree of overlap representing lower theoretical nutritional adequacy for salmon diets.



FIG.1. Palbio and soybean meal AA content (%) vs pig and salmon muscle (A) and their nutritional requirements (B). Given the suitability and the reported health benefits of Palbio as protein source for pigs and based on the herein reported suitability also for salmon, Palbio could serve as an adequate protein source to maximize growth and feed utilization in salmon and provide health benefits as well.

EVALUATION OF ATLANTIC SALMON Salmo salar PERFORMANCE FOLLOWING PARTIAL REPLACEMENT OF FISH MEAL BY PORCINE HYDROLYSED PROTEIN. Sergi Segarra*, Nicole Acevedo, Christian Correa.

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Fish meal (FM) is a protein source commonly used in feeds for atlantic salmon (Salmo salar), but it is gradually becoming a more expensive and less sustainable resource. The use of alternative plant-based protein sources, especially from soy, as partial replacement for FM is currently common practice but limited due to the presence of antinutrients that negatively affect function and health of the gut of fish. Palbio 50 (Bioiberica SAU, Barcelona, Spain) is a high-quality protein source obtained from porcine intestinal mucosa as byproduct of the heparin manufacturing process. which makes it very sustainable. Palbio 50 is also a source of bioactive peptides. Its inclusion in diets for salmon could allow lower inclusion levels of plant-based protein sources, hence sparing their associated undesired effects, as well as providing economic savings. The objective of the present study was to evaluate the effects of partially replacing FM by Palbio50 on diet costs and performance parameters in salmon which are fed plant-based protein sources as alternative to FM.

Fish were allocated in 1000-L tanks and received different diets (in triplicate) as shown in Fig. 1. All diets used in the study were formulated so that they were isoprotein, isoelectrolytic and isoenergetic. In our study, including Palbio 50 allowed lower diet costs, compared to the control commercial diet. After 90 days, fish experienced a significant weight increase (863 vs 228 g; p=0.0091). Replacing FM by Palbio 50 did not affect fish performance, as there were no significant differences (p>0.05) between groups in weight, SGR, GF3, FCR and SFR (Fig. 2). No significant changes in water temperature or oxygen saturation occurred (around 12.5 °C and 99.5 %).

