Enteritis Inducida por la Harina de Soya en las Dietas de Peces Marinos: Efectos Sobre la Integridad del Intestino Distal y la Respuesta Inmune en *Totoaba macdonaldi*

José Pablo Fuentes-Quesada[1], Artur N. Rombenso[2], Yanet Guerrero-Rentería[1], María Teresa Viana[2], Juan Pablo Lazo[1], José A. Mata-Sotres[3*].

[1] CICESE, Ensenada, Baja California, Mexico, 22860

[2] Nutrición y Fisiología Digestiva. Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California (UABC), Baja California, México, 22860

[3] CONACYT - Nutrición y Fisiología Digestiva. Instituto de Investigaciones Oceanológicas UABC. E--mail: mata@uabc.edu.mx

Resumen

El objetivo del presente estudio fue caracterizar los efectos perjudiciales en el intestino de juveniles de *Totoaba macdonaldi* producidos por el incremento en los niveles de inclusion de una mezcla de harinas de soya (SBM) en dieta en la inducción de enteritis. Cuatro dietas isoproteícas (48%) e isolipídicas (8,6%) fueron formuladas incluyendo niveles crecientes de SBM, 0%, 22%, 44% y 64% de la dieta con 1% de taurina adicionada. Al final del experimento (i.e., 8 semanas), se observó una marcada respuesta dosis-dependiente en el crecimiento producida por la inclusión de SBM. Basándose en las alteraciones histológicas del intestino distal, se observó una severa enteritis al incluir SBM por encima del 22%. Además, se observó un aumento en la exfoliación epitelial del borde de cepillo intestinal en relación con los niveles de inclusión de SBM. Los niveles de expresión de la interleucina (*il-8*) mostraron una clara respuesta inflamatoria en presencia de SBM en la dieta lo que sugiere un estado de estrés crónico cuo se presentan niveles más altos de inclusión de SBM (i.e., 44% y 64%). Los resultados muestran un efecto perjudicial por parte de SBM sobre la fisiología digestiva de totoaba en niveles de inclusión superiores al 22%. Por lo tanto, se sugiere que se utilice con cautela SBM al momento de formular alimentos para totoaba.

Palabras clave: *Totoaba macdonaldi*, harina de soya, expresión de interleucina, enteritis.

Fuentes-Quesada, J., Rombens, A., Guerrero, Y., Viana, M., Lazo, J., Mata, J. *. 2017.Enteritis inducida por la harina de soya en las dietas de peces marinos: efectos sobre la integridad del intestino distal y la respuesta inmune en *Totoaba macdonaldi*. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., López Acuña, L.M. y Galaviz-Espinoza, M. . (Eds), Investigación y Desarrollo en Nutrición Acuícola Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, pp. 560-569. ISBN 978-607-27-0822-8.

Abstract

The objective of the present study was to identify the detrimental effects produced in the posterior intestine in juvenile *Totoaba macdonaldi* due to increasing levels of dietary soybean meal (SBM) in the induction of enteritis. Four isoproteic (48%) isolipidic (8.6%) diets were formulated to include increasing levels of a mixture of soybean meals (SBM) at 0%, 22%, 44% 64% in a diet containing 1% taurine. At the end of the experiment (8 weeks), a marked dose-dependent response produced by SBM inclusion was observed in growth performance. Based in histological alterations in distal intestine, a severe enteritis response was found when SBM was included above 22%. Furthermore, an increase in epithelial exfoliation from the intestinal brush border was observed in relation with SBM inclusion levels. Interleukin (*il-8*) expression levels clearly showed an inflammatory response in the presence of dietary SBM suggesting a state of chronic stress in treatments containing higher levels of SBM (i.e., 44% 64%). Results indicate the disruptive effects of SBM on digestive physiology of totoaba at inclusion levels above 22%. Thus, suggesting that SBM should be cautiously used in totoaba feed formulations.

Key words: *Totoaba macdonaldi*, soybean meal, interleukin expression, enteritis.

1. Introduction

The increasing dem of seafood has led to a sustained rise in aquaculture production (FAO, 2014). Availability of raw materials for aquafeed manufacturing is one limitation towards this continued expansion. Although the so-called “reduction fisheries” that produce fishmeal (FM) fish oil are considered stable, dems for FM increases as aquaculture exps. Aquaculture annually consumes close to 70% of the FM produced worldwide, with diets for carnivorous fish species consuming the higher proportion. Therefore, the search for new protein sources to substitute FM is still of great concern in aquaculture research. Alternative vegetable protein sources, in particular soybean meal (SBM), which contains one of the highest protein levels amongst the vegetable sources, has been pointed out as one of the most promising alternative protein sources. Not surprisingly, the use of SBM has been increasingly implemented adapted into husbry nutrition of several species over the last years. In aquaculture, defatted-SBM has been considered a viable alternative to replace at least part of FM in marine fish feeds due to high availability in the market a high crude protein content (40-48%) with a constant profile of amino acids at low cost (Gatlin *et al*. 2007). However, inclusion of high levels of SBM in carnivorous fish diets has been associated to the occurrence of intestinal enteritis (Bakke-McKellep *et al*. 2000; Krogdahl *et al*. 2003), which is defined as non-infectious inflammation of distal intestine (DI) (Baeverfjord & Krogdahl, 1996).

Additionally, SBM contains a high level of antinutritional factors for fish, apart from trypsin inhibitors (saponins, lectins, phytic acid, alkaloids, oligosaccharides, antigens), that are associated with damage of mucosal integrity, decreased pancreatic and mucosal enzymes, loss of dietary nitrogen in the faeces, thyroid hormone suppressors, lower mineral absorption, reduced palatability and suppression of the immune system (Francis *et al*. 2001; Krogdahl *et al*. 2010). Chemokines are a group of structurally related cytokines that are able to attract and activate specific types of leucocytes to the site of inflammation or injury. Therefore, the relative expression levels of interleukin (*il-8*) is a good indicator of an inflammatory reaction (Li & Yao, 2013).

Fuentes-Quesada, J., Rombens, A., Guerrero, Y., Viana, M., Lazo, J., Mata, J. *. 2017.Enteritis inducida por la harina de soya en las dietas de peces marinos: efectos sobre la integridad del intestino distal y la respuesta inmune en ***Totoaba macdonaldi*****.** En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., López Acuña, L.M. y Galaviz-Espinoza, M. . (Eds), Investigación y Desarrollo en Nutrición Acuícola Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, pp. 560-569. ISBN 978-607-27-0822-8.

Totoaba macdonaldi is the largest Sciaenidae from the Gulf of California, highlighted as an endangered species with high commercial value. Its culture is of great importance not only to stock enhancement programs, but also to stimulate its aquaculture. Under commercial conditions totoaba can reach 2-3 kg in 18 months (Juarez *et al*. 2016) and it is considered a candidate for commercial aquaculture in the Baja California region. Totoaba is a carnivorous species with a high protein demand of >50% (Rueda-López *et al*. 2011), and therefore, there is an interest to reduce dietary FM inclusion. In the present study, we evaluated the effects of increasing inclusion levels (0, 22, 44 and 64%) of a mixture of soybean meals (soy meal + soy concentrate) with a constant taurine (1%) level in the induction of enteritis in totoaba juveniles (ca. 70g) fed for 56 days and using intestinal histology and interleukin (*il-8*) expression levels as response variables.

2. Results

2.1. Distal intestine morphology

Histological analysis of the DI revealed that SBM inclusion in the diet decreased the Mucosal Fold (MF) length of the intestine with increasing coalescence of the intestinal folds (Fig. 1.B). The width of Lamina Propia (LP) and Sub-epithelial Mucosa (SM) increased with a few infiltrations of eosinophilic granulocytes with increasing content of SMB in the diet. Likewise, Goblet cells (GC) increased in number and compact groups of these structures are observed towards the apexes of MF (Fig. 1. B to D). Supranuclear Vacuoles (SV) in 22% SBM diet showed some vacuolization (Fig. 1.B) but in the 44 and 64% SBM treatments SV decreased to almost none-existing (Fig. 1. C and D). At 56 days, the same trend of shortening of the MF length with increasing inclusion of SBM in the diet was observed. Moreover, an increase width of LP, the infiltration of Eosinophilic Granulocytes (EG) into SM and LP, the coalescence of the folds and disruption of the tissue of the MF was observed with increasing dietary SMB content. Furthermore, some samples resulted in enterocyte vacuolization with high variation in vacuole size (Fig. 1. F and H). In the most severe cases, the MF was generally shorter with a wider lamina propia and a disordered cellular arrangement (i.e., few GC and MF without the presence of SNV) (Fig. 1. G and H).

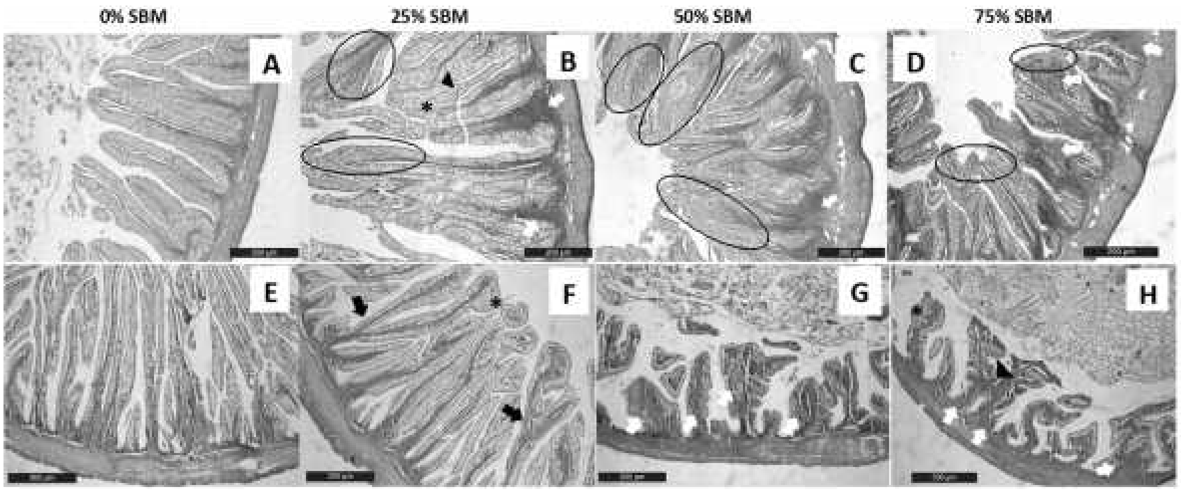

Fig.1. Histomorphological changes associated an inflammatory process of the distal intestine of *T. macdonaldi* fed diets with 0% SBM (A, E), 22% SBM (B, F), 44% SBM (C, G) and 64% SBM (D, H) during 28 days (A - D) and 56 days (E - H). Bar = 200 µm.

2.2. Gene expression

Relative expression patterns of Interleukin (*il8*) are shown in Fig. 2. At 28 days relative *il8* expression exhibited a peak at 22% SBM diet (2.00), whereas the expression level of 0%, 44% and 64% SBM diets remained with significantly lower expression levels (0.87, 0.75, and 1.02, respectively). At 56 days, the highest expression level was found at 44% SBM diet

(1.10). Nevertheless, no significant differences were found (P>0.05) among SBM inclusion levels (0.93, 82.46 and 0.54 for 0%, 22% and 64%, respectively).

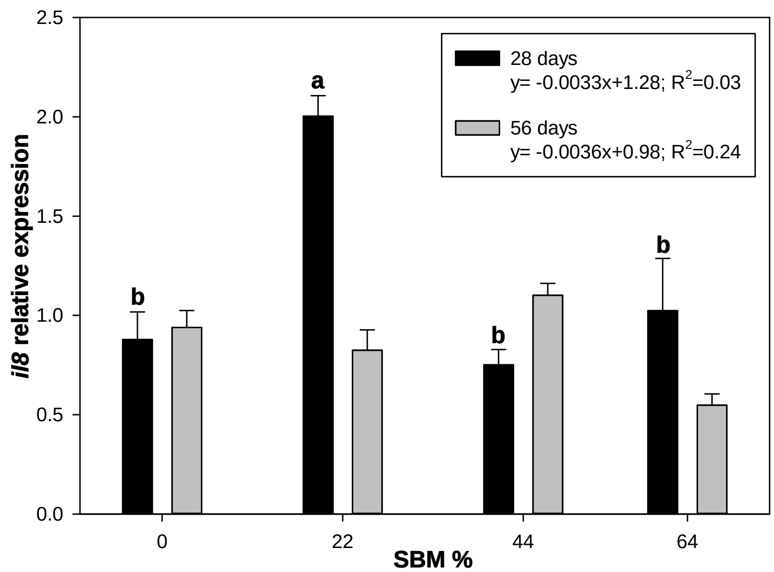

Fig. 3. Interleukin (*il8*) relative expression in *T. macdonaldi* at 28 and 56 days fed diets containing different SBM levels. Different letters represent significantly different values ($P<0.05$) within the same day (n=9). r^2 and equation of the regression are shown in the box.

4. Discussion

Research effort to evaluate enteritis induction by dietary alternative protein sources, including SBM, is considerable. There are plenty of reports regarding the use of SBM in carnivorous fish with contradicting results; for example some researchers confirm that SBM could be used at high levels without negative effects (Bonaldo *et al*. 2008; Trejo-Escamilla *et al*. 2016), while most reports demonstrate negative performance at intermediate or high inclusion levels (Bakke-McKellep *et al*. 2007; Chikwati *et al*. 2013; Ferrara *et al*. 2015; Gu *et al*. 2016; Urán *et al*. 2008). The present work corroborates with the majority of the literature highlighting that dietary SBM affected the overall digestive physiological performance and health status of juvenile totoaba at the intermediate or high inclusion levels.

The histological results highlighted a clear pathological detrimental inflammatory damage of the tissues analyzed (distal intestine and liver) related to dietary SBM. The integrity of the mucosal barrier is crucial to maintain tissue homeostasis against pathogens and feed antigens (Sahlmann *et al*. 2013). The mucus secreted from GC provides the first layer of intestinal protection and the integrity of mucosal barrier depends on cellular proliferation to replace damaged cells (Sahlmann *et al*. 2013).

Based on the histopathological alterations previously reported for the DI in the literature, our findings suggest that totoaba suffered a DI inflammation. This DI inflation was dose-dependent as dietary SBM increased, resulting in a morphological change of the intestinal mucosa. The most severe cases were found in fish fed the 44 and 64% SBM diets with a clear unorganized cellular arrangement, with markedly tissue disruption, increase in coalescence of the folds and high presence of EG in the SM with migration into the LP. This is evidence of a clear process of chronic inflammation of the intestine with an important reduction of the absorption surface of the intestine reflected in the reduction of the ISI clearly documented at 56 days. These morphological changes have been suggested to be related to a cellular turnover with a concomitant increase in cell migration and apoptosis (Sahlmann *et al*. 2015).

With respect to the molecular expression levels of the immune response, interleukin-8 (*il-8*) is one of the main immune-relevant citokines produced during an inflammatory reaction to induce wound healing during an inflammatory process (Li & Yao, 2013; Lilleeng *et al*., 2009). As expected and in agreement with the literature, we observed an increase in *il-8* expression in fish fed SBM-based diets, including at the lower inclusion level (22% SBM), indicating a response in the posterior intestine (Bonaldo *et al*. 2015; Perera & Yúfera, 2017; Urán *et al*. 2008). The reduction in expression levels of *il-8*, demonstrate the negative effect of dietary SBM, producing a constant stress reaction, regardless of the inclusion level. Nonetheless, the reduction in expression levels, with relation to the higher SBM inclusion level, could be a consequence of the degree of damage in the DI, as verified through histological analysis. It is possible that the reduction in *il-8* expression within the DI could be a result of the increased epithelial exfoliation from the intestinal brush border as reported for many species (Bakke-McKellep *et al*. 2007; Gu *et al*. 2016; Sahlmann *et al.* 2013).

In conclusion, the present study characterizes the limitations of SBM inclusion in *T. macdonaldi* diets, noticeably affecting the structure and physiology of distal intestine (i.e. at histological and molecular level. Additionally, our findings demonstrated a state of chronic stress in totoaba caused by high dietary SBM inclusion levels. Therefore, the present work directly suggests that SBM should be cautiously used in totoaba feeds.

References

Baeverfjord, G., Krogdahl, Å., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. J. Fish Dis. 19, 375–387.

Bakke-McKellep, A.M., McL Press, C., Baeverfjord, G., Krogdahl, Å., Landsverk, T., 2000. Changes in immune and enzyme histochemical phenotypes of cells in the intestinal mucosa of Atlantic salmon, *Salmo salar* L., with soybean meal-induced enteritis. J. Fish Dis. 23, 115–127.

Bakke-McKellep, A.M., Penn, M.H., Salas, P.M., Refstie, S., Sperstad, S., Landsverk, T., Ringø, E., Krogdahl, Å., 2007. Effects of dietary soyabean meal, inulin and oxytetracycline on intestinal microbiota and epithelial cell stress, apoptosis and proliferation in the teleost Atlantic salmon *(Salmo salar* L.). Br. J. Nutr. 97, 699.

Bonaldo, A., Roem, A.J., Fagioli, P., Pecchini, A., Cipollini, I., Gatta, P.P., 2008. Influence of dietary levels of soybean meal on the performance and gut histology of gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.). Aquac. Res. 39, 970–978.

Chikwati, E.M., Gu, J., Penn, M.H., Bakke, A.M., Krogdahl, Å., 2013. Intestinal epithelial cell proliferation and migration in Atlantic salmon, *Salmo salar* L.: Effects of temperature and inflammation. Cell Tissue Res. 353, 123–137.

FAO, 2014. The State of World Fisheries and Aquaculture 2014, Food and Agriculture Organization of the United Nations.

Ferrara, E., Gustinelli, A., Fioravanti, M.L., Restucci, B., Quaglio, F., Marono, S., Piccolo, G., 2015. Histological and micro-/macro-morphological evaluation of intestine in sharpsnout seabream (*Diplodus puntazzo*) fed soybean meal-based diets added with MOS and inulin as prebiotics. Aquac. Int. 23, 1525–1537.

Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197–227.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G.,Hardy, R.W.,Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust,M., Sealey,W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. 38, 551–579.

Gu, M., Bai, N., Zhang, Y., Krogdahl, A., 2016. Soybean meal induces enteritis in turbot *Scophthalmus maximus* at high supplementation levels. Aquaculture 464, 286–295.

Juarez, L.M., Konietzko, P.A., Schwarz M.H., 2016. Totoaba Aquaculture and Conservation: Hope for an Endangered Fish from Mexico's Sea of Cortez. World Aquaculture Magazine, December.

Krogdahl, Å., Bakke-McKellep, A.M., Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo solar L*.). Aquac. Nutr. 9, 361–371.

Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S., Bakke, A.M., 2010. Important antinutrients in plant feedstuffs for aquaculture: An update on recent findings regarding responses in salmonids. Aquac. Res. 41, 333–344. doi:10.1111/j.1365-2109.2009.02426.x

Li, C., Yao, C.L., 2013. Molecular and expression characterizations of interleukin-8 gene in large yellow croaker (*Larimichthys crocea*). Fish Shellfish Immunol. 34, 799–809.

Rueda-López, S., Lazo, J.P., Reyes, G.C., Viana, M.T., 2011. Effect of dietary protein and energy levels on growth, survival and body composition of juvenile *Totoaba macdonaldi*. Aquaculture 319, 385–390.

Sahlmann, C., Gu, J., Kortner, T.M., Lein, I., Krogdahl, Å., Bakke, A.M., 2015. Ontogeny of the Digestive System of Atlantic Salmon (*Salmo salar* L.) and Effects of Soybean Meal from Start-Feeding. PLoS One 10, e0124179.

Sahlmann, C., Sutherland, B.J.G., Kortner, T.M., Koop, B.F., Krogdahl, Å., Bakke, A.M., 2013. Early response of gene expression in the distal intestine of Atlantic salmon (*Salmo salar* L.) during the development of soybean meal induced enteritis. Fish Shellfish Immunol. 34, 599–609.

Trejo-Escamilla, I., Galaviz, M.A., Flores-Ibarra, M., Álvarez González, C.A., López, L.M., 2016. Replacement of fishmeal by soya protein concentrate in the diets of *Totoaba macdonaldi* (Gilbert, 1890) juveniles: Effect on the growth performance, in vitro digestibility, digestive enzymes and the haematological and biochemistry parameters. Aquac. Res. 1–20.

Urán, P.A., Gonçalves, A.A., Taverne-Thiele, J.J., Schrama, J.W., Verreth, J.A.J., Rombout, J.H.W.M., 2008. Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.). Fish Shellfish Immunol. 25, 751–760.