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Enteritis induction by soybean meal in *Totoaba macdonaldi* diets: Effects on growth performance, digestive capacity, immune response and distal intestine integrity

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ABSTRACT

The aim of the present study was to investigate the effects of increasing levels of dietary soybean meal (SBM) with constant taurine supply in the induction of enteritis in juvenile *Totoaba macdonaldi*. Four isoproteic (48.5%) and isolipidic (8.6%) diets were formulated to include increasing levels of a mixture of soybean meals (SBM); (soy protein concentrate and soybean meal at a ratio of 1:4) at 0%, 22%, 44% and 64% replacing fishmeal in a diet containing 1% taurine. Upon completion of the 56-day feeding trial, SBM caused marked dose-dependent responses in growth performance and digestive physiology processes. Severe enteritis symptoms in the distal intestine and liver were found when SBM was included above 22%. SBM dose-dependent impairments in digestive functions were found in digestive enzyme activity for trypsin, chymotrypsin, L-aminopeptidase, total alkaline proteases, and amylase. Interleukin (*il-8*) expression patterns showed an inflammatory response during the first four weeks in the presence of the higher levels of SBM (44% and 64%) suggesting an impaired immunological response. However, after 8 weeks no immunological inflammatory response was observed, but a severe atrophy of the intestine could still be revealed. Results indicate a detrimental status of the digestive physiology of totoaba fed SBM-based diets at inclusion levels above 22%. Thus, suggesting that SBM should be cautiously used in totoaba feed formulations.

1. Introduction

Fish meal (FM) sparing and replacement is still a great concern in aquaculture research. Alternative vegetable sources, in particular soybean meal (SBM), which has the highest protein content among the plant-based ingredients, has been pointed out as one of the most promising alternative protein sources. Not surprisingly, the use of SBM has been implemented and adapted into husbandry nutrition of several species over the years. In the aquaculture industry, defatted-SBM has been considered a viable alternative to replace at least part of FM in marine fish feeds due to high availability and 40–48% crude protein with a constant amino acid profile at low cost (Gatlin et al., 2007). However, increase in SBM in certain carnivorous fish diet is related to the occurrence of enteritis (Bakke-McKellep et al., 2000; Krogdahl et al., 2003), defined as non-infectious inflammation of distal intestine (Baeverfjord and Krogdahl, 1996) which can be reversed by eliminating dietary SBM (Bakke et al., 2010).

Additionally, SBM contains a high level of antinutritional factors for fish (i.e. protease inhibitors, saponins, lectins, phytic acid, alkaloids, oligosaccharides, glucosinolates, antigens), associated with a damage of the mucosal integrity, decreased pancreatic and brush-border enzymes, loss 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; Krogdahl and Bakke-McKellep, 2015). From all, saponins were suggested to induce enteritis in Atlantic Salmon *Salmo salar* (Krogdahl et al., 2015). Distal intestine associated with enteritis exhibited the following histological features: shortening of mucosal folds (MF), reduction in the number of

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supranuclear vacuoles (SNV) of the enterocytes, thickening of the lamina propria (LP), enlargement of the connective tissue, increased number of goblets cells (GC) in the epithelium and infiltration of inflammatory cells in connective tissue and LP (van den Ingh et al., 1991; Baeverfjord and Krogdahl, 1996).

There is a lack of nutritional studies evaluating soy products in diets of *Totoaba macdonaldi,* which is a marine carnivorous species with high potential of commercial aquaculture (Juárez et al., 2016). A previous study investigating dietary soybean protein concentrate (SPC) reported improved growth and feed efficiency with acceptable haematological parameters in totoaba fed diets containing 30 to 60% of SPC (López et al., 2015). Despite, a hepatic damage was reported in fish fed SPC-based diets, regardless SPC dietary level. Likewise, Bañuelos-Vargas et al. (2014) concluded that since taurine is an important modulator of the intermediate metabolism of the liver, when it is present at 1% in SPC-based diets no damages of enteritis could be noticed. In agreement, Trejo-Escamilla et al. (2016) reported that up to 34% of SPC could be included in totoaba diets containing 1.5% taurine without affecting growth. However, to our knowledge no data has been published evaluating the overall performance of the fish, the effect on digestive physiology and immunological responses.

As SBM is a lower-cost protein alternative to FM, in the present work we evaluate the effect of graded levels of SBM on growth performance, digestive capacity, molecular expression, distal intestine and liver integrity in juveniles of *Totoaba macdonaldi*, that may result in enteritis with detrimental outcomes in fish of commercial size.

2. Materials and methods

2.1. Diet formulation

Four isoproteic (485 g crude protein (CP) kg^{-1} diet) and isolipidic (86 g crude lipid (CL) kg^{-1} diet) diets were formulated to replace FM (69% protein content, Maz Industrial SA de CV, Mazatlán Sinaloa, México) protein at 0, 25, 50 and 75%, with a mixture of SPC and soybean meal (SBM) at 1:4 ratio, Alimentos COLPAC, Sonora, México and NutriVance™, Midwest Ag Enterprices, Inc. MN, USA and referred as SBM from this point forward. Soybean meal contained 48% CP. Soy protein concentrate contained 60% protein. Additionally, all diets included 1 g kg^{-1} of krill oil (Biogrow, ProAqua, México) as attractant and 10 g kg^{-1} of taurine (Insumos Nubiot, SA de CV, México) (Table 1). Diets were mixed (Robot-Coupe, model R10, USA), pelleted at 5 mm in a meat grinder (Tor-Rey, Model M32–5, Mexico) and dried at 60 °C in a forced air oven for 24 h. Once dried, diets were packaged and stored at −20 °C until used for the feeding trial. Four essential amino acids (lysine, methionine, threonine and arginine; EVONIK, Degussa, México) were supplemented to reach equal levels found in the control diet (0% SBM). Although dietary treatments were formulated to replace FM protein at 0, 25, 50 and 75% with a mix of soybean meals, the actual SBM mixture content for each treatment was 0, 22, 44 and 64% (Table 1).

2.2. Experimental design, animals and facilities

Juvenile totoaba were reared from eggs, of hormone-induced spawns, at the Marine Fish Culture Laboratory at the Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Mexico. Forty-eight fish (71.7 ± 35.7 g; mean ± SE) were randomly stocked into twelve 450-L cylindrical blue fiberglass tanks (four fish per tank) connected to a closed recirculation system composed of a compacted bead bed filter coupled with a fluidized biofilter (media kaldnes) and a heat pump (Titan 1 1/2 hp. Aqualogic, USA) with a daily water renewal of 5%. Water quality was monitored daily, with mean values for temperature = 23.3 ± 1.1 °C, dissolved oxygen = 5.5 ± 0.4 mg L^{-1} with an oxygen saturation up to 80%, salinity = 35.2 ± 1.0‰ and a water flow of 2.5 L min^{-1}. Every three days the ammonia, nitrite and nitrate levels were measured (Api Pharmaceutic Aquarium Kit) to keep values < 1.0 mg L^{-1}, 0.5 mg L^{-1} and < 80 mg L^{-1}, respectively. Fish were kept under natural photoperiod between September and November of 2016 (31°87 N, 116°66 W).

Table 1
Ingredient formulation (g kg^{-1}) and proximate composition of diets used to fed *T. macdonaldi* containing increasing levels of SBM. Dietary formulation is presented as fed basis and proximate composition in g kg^{-1} on a dry matter basis.

Experimental diets	0% SBM	22% SBM	44% SBM	64% SBM
Ingredients (g kg^{-1} DM)				
Sardine meal (69%CP)[a]	619.2	464.4	309.6	154.8
Soybean meal (48%CP)[b]	0.0	163.4	327.0	490.5
Soy protein concentrate (60%CP)[c]	0.0	54.5	109.0	163.5
Starch	218.2	146.8	73.9	0.4
Sardine oil[a]	52.0	58.8	65.7	72.5
Gelatin	60.0	60.0	60.0	60.0
Rovimix for carnivorous fish[d]	25.0	25.0	25.0	25.0
Stay-C[d]	4.0	4.0	4.0	4.0
Taurine[e]	10.0	10.0	10.0	10.0
Methionine[f]	2.7	3.9	5.1	6.3
Lysine[f]	0.0	2.6	5.3	7.9
Arginine[f]	2.9	0.9	0.0	0.0
Threonine[f]	1.9	1.6	1.3	1.0
Attractant (krill oil)[g]	1.0	1.0	1.0	1.0
Sodium benzoate	2.0	2.0	2.0	2.0
Choline chloride	1.0	1.0	1.0	1.0
BHT	0.1	0.1	0.1	0.1
Proximate composition (g kg^{-1} DM)				
Dry matter	989 ± 1.0	991 ± 0.4	975 ± 1.6	988 ± 1.0
Crude protein	488 ± 1.7	486 ± 3.4	488 ± 4.0	484 ± 6.4
Crude fat	88 ± 1.3	87 ± 1.3	85 ± 0.4	84 ± 0.9
Ash	147 ± 1.1	131 ± 0.7	118 ± 0.2	96 ± 0.9
NFE[h]	277 ± 3.7	296 ± 3.2	309 ± 4.0	336 ± 5.7

[a] Maz Industrial SA de CV, Mazatlán, Sinaloa, México.
[b] Alimentos COLPAC, Sonora, México.
[c] NutriVance™ Midwest Ag Enterprises, Inc. MN, USA (U.S. Soybean Export Council).
[d] Rovimix; Stay-C DSM, Guadalajara, México.
[e] Insumos NUBIOT SA de CV, México.
[f] Free aminoacids donated by EVONIK, Degussa, México.
[g] Biogrow, Proveedora de Insumos Acuícolas, SA de CV, Mazatlán, Sinaloa, México.
[h] Nitrogen free extract; NFE (%) = 100 − (% crude protein + % total lipid + % ash).

Each dietary treatment was randomly assigned into triplicate experimental units. Fish were hand-fed daily to apparent satiation at 08:30, 12:00 and 16:00 h during 8 weeks. Daily, all uneaten feed was removed within an hour of feeding and dry weighed to determine the most accurate feed consumption rates possible.

2.3. Sampling

All fish were measured (mm, SL) and weighted (g) at the beginning of the feeding trial and then every fifteen days. Mean individual weight of each experimental unit was determined dividing the bulk weight by the number of individuals, performance response indices and somatic indices were calculated as follows:

Thermal growth coefficient (TGC) = [(final weight ⅓ − initial weight ⅓) / ($T_{°C}$ × D_{days})] × 1000.
Feed Conversion Ratio (FCR) = total feed consumed/ wet weight gained.
Condition Factor (CF) = final body weight × (body length)^3 × 100 (Hardy and Barrows, 2002).
Protein Efficiency Ratio (PER) = weight gain/ protein intake.
Feed Intake = FI (% day^{-1}) = 100 × (total amount of the feed