

SUSTAINABLE TILAPIA PRODUCTION IN A BACKYARD SYSTEM FOR RURAL AREAS USING FISH BY-PRODUCTS IN FORMULATED DIETS

[PRODUCCIÓN SUSTENTABLE DE TILAPIA EN SISTEMAS RURALES DE TRASPATIO EMPLEANDO SUBPRODUCTOS DE PESCADO PARA LA FORMULACIÓN DE RACIONES]

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SUMMARY

The aim of the present study was to demonstrate the feasibility of producing Tilapia (Oreochromis niloticuss. L.) with formulated diets containing acid fish silage made with Hancock (Pterigoplichthys *multiradiatus*) by-products in substitution of fishmeal. Thus four treatments containing increasing levels (0, 5, 10 and 15%) of acid fish silage to substitute fishmeal were used to feed tilapia juveniles in triplicate groups (average 1.5±0.7 g and 4.3±1.0 cm). Fish silage was prepared by adding citric and phosphoric acids at 2.6% each resulting in a liquefaction of the chop by-product mixture. After two weeks, the diets were prepared to contain 32-35% protein content. After 50 days of experimental period a total growth increase up to 827% was observed without significant differences among treatments. A specific growth rate from 3.70 to 4.10 and a survival rate from 96 to 99% was observed. The cost analysis shows a saving on production cost up to 23.87% using the highest incorporation level of fish silage. The use of Aquaculture Recirculation System made with components normally found in a hardware store in rural or semi urban areas is discussed. It is concluded that fish offal shows a promising activity that favors the increase in fish protein production to resolve the demand of cheap protein.

Keywords: Tilapia; Backyard System ratio; by-products diets; Recirculating Systems.

RESUMEN

El objetivo del presente trabajo fue demostrar la factibilidad de la producción de tilapia (Oreochromis niloticuss, L.) mediante el empleo de dietas conteniendo ensilado de subproductos de pescado (Pterigoplichthys multiradiatus) en reemplazo de la harina de pescado. Se emplearon cuatro tratamientos conteniendo niveles crecientes (0, 5, 10 y 15%) de sustitución de harina de pescado por ensilado para la alimentación de tilapias juveniles (promedio de 1.5±0.7 g y 4.3±1.0 cm). El ensilado se preparó mediante la adición de ácido citrico y ácido fosfórico al 2.6% c.u. resultando en la licuefacción de subproducto de pescado. Después de dos semanas, las dietas fueron preparadas y contenían 32-35% de proteína. Después de 50 d de experiment se registró un crecimiento de 827% y no se encontraron diferencias significativas entre tratamientos. Se observe una tasa específia de crecimiento de 3-7 a 4.1 y una tasa de sobrevivencias de 86 a 99%. El análisis de costo mostró un ahorro de hasta 23.87% al máximo nivel de incorporación del ensilado. El uso de un sistema de recirculación acuícola elaborado con componentes que se encuentran normalmente en el medio rural o áreas peri-urbanas es discutido. Se concluye que el aprovechamiento de los residuos de pescado es una actividad promisoria para incrementar la producción de proteína de pescado y resolver la demanda de proteína barata.

Palabras clave: Tilapia; Sistema de traspatio; subproductos; sistemas de recirculación.

INTRODUCTION

Food production has been a worrying subject along the world history where programs or funds are oriented according to the different policies adopted for the different economies around the world like the Backyard Aquaculture. The FAO has foresee that up to year 2030 even if globally there will be enough food for a growing world population, hundreds of millions of people in developing countries will remain hungry and many of the environmental problems caused by agriculture will remain serious. Most of the poor people is located in rural areas from the third world countries, thus promoting the growth of food products in rural areas by giving rural people better access to land, water, credit, health and education, is essential to alleviate poverty and hunger. However, interestingly, FAO ("World agriculture: towards 2015/2030") states that aquaculture will be the fastest growing activity, where efforts on new species domestication and species improvements within traditional breeding. chromosome manipulation and hybridization will be of importance, a subject that somehow have already given significant contributions.

Nevertheless, aquaculture industry will grow only if sustainable practices will take place. For instance, how to optimize the use of water, either for food production or for direct human needs. The idea of reusing the water from agriculture or aquaculture is not a new idea (Nduwimana *et al.*, 2007). In such case, in order to be competitive, lowering production cost besides the intensification of fish production will be the key to success, and food or feeds in one of them.

In Mexico since the 80's several attempts has been made to encourage fish protein consumption in rural areas by promoting the aquaculture. However this activity has been growing very slow apart from those areas where larger facilities have been developed throughout the private sector inversion. The reason can be explained due to the lack of effective rural programs concerning the necessary inversion, and the requirements to undertake a proper transfer technology. Backyard animal rearing systems on smallholdings in the rural areas in Mexico has been for centuries, where livestock have been an integral part of family production resulting in the main source of protein intake. Low cost backyard aquaculture has been already reported (Szyper, 1989), but a proper transfer technology is needed in rural areas in Mexico.

In a recirculation system, the water is optimized by reusing it several times using filters and pumps with a refill of water to compensate the evaporation. Then, the water is treated internally and the sludge from the discharge can be concentrated and eliminated or used as fertilizer. Ammonia can be also reduced by biofilters. This system has been widely described by several authors (Timmons *et al.*, 2001; Gutierrez-Wing and Malone, 2006) and named as Recirculation Aquaculture System (RAS). The equipment is operated by electricity pumps where often a high production costs are experienced and technical skills are needed to fully operate the system.

Tilapia is an excellent fish to promote backyard Aquaculture like resistant to rough handle, rapid growth under a wide range of temperatures (24 to 32°C) and intermediate water quality. Their nutrition has been thoroughly described (El-Sayed, 1999) being well adapted and fed with a variety of ingredients and their protein requirement is close to 35% (Goddard *et al.*, 2008).

Besides, the increasing demand and progressive scarcity of fishmeal in the international market has led the prices to boost and therefore the need to search for protein optimization is obliged. In rural and semi urban areas a great amount of protein is throw away throughout the fish filleting industry, waste that usually becomes an environmental problem that could be used in feeds throughout fish silage. Fish waste can efficiently be used as a feed ingredient by chopping the fish and preserve it either by fermentation or acid preserved. This last is easier to control by lowering the pH preventing the proliferation of bacteria and mold, whereas by fermentation a strict control of anaerobic conditions is needed besides adding soluble sugar and a source of fiber to promote lactic acid bacteria to hold the mixture at pH around 4-5, process that if is not appropriated controlled easily brings the fermentation to putrefaction.

The *Pterigoplichthys multiradiatus* (Hancok) a native fish from Orinoc River from tropical areas of the South America was introduced to Mexico for the aquarist as a cleaner fish for home aquariums, in Mexico known as "Pez Diablo or Armour catfifh". Today has become an invasive species accidentally introduced in lakes and rivers along the Mexico natural areas. Here it constitutes an environmental problem, and due to their aggressive reproduction behavior has led a large competition in the capturing gill nets, inside the natural reservoirs with concomitant commercial production problems. Therefore the government has made a called to researchers to investigate all possible uses of this fish to an overall production in the Michoacán, Mexico in the Adolfo López Mateos Dam (Presa infiernillo). This fish has show to have excellent meat properties but their aspect makes this product to be considered as trash. However, their hard skin and low proportion to meat has made the process to recover the meat protein cost effective and difficult to be implemented for direct consumption. However, the use of acids to promote the autolysis to produce acid fish silage could be an alternative to preserve fish protein to prepare formulated diets for fish aquaculture in substitution of fishmeal without reducing their performance. Therefore the aim of the present work is the use of fish silage from the *Pterigoplichthys multiradiatus* by products used to formulate diets with different levels to substitute fishmeal for tilapia feeds, reducing the costs of tilapia production using a backyard Recirculation Aquaculture System model.

MATERIALS AND METHODS

Diet preparation

Four experimental diets were formulated to contain 35% protein (Table 1) with four levels of fish silage incorporation in dry weight basis (0, 5, 10 and 15%) in substitution of fishmeal. Primary sources of protein were fishmeal and soybean meal (42% CP). Silage was made as described by Viana *et al.* (1993). In summary, chopped fish (P. *multiradiatus*) (heads, skin and bones) was mixed with 2.6 % phosphoric acid, 2.6 % citric acid. The mixture was blended to obtain a homogenate with a pH around 4 to 4.5 and left in buckets at 20°C. After 2 weeks the fish silage was incorporated into the diets as follows.

All ingredients were blended in a mixer (Robot Coupe, USA, model R10), to produce a homogeneous mixture. The water content was controlled to achieve moisture levels not higher to 60%. The dough-like diets were cold extruded through a meat grinder (Toro Rey, Mexico, model M32-5) into 0.5×0.7 mm pellets, and then dried in a convection oven for 12 hr at 60 °C. The dry pellets were placed in sealed plastic bags and stored at -40°C until use.

Analytical Methods

Proximate composition of diets was determined according to standard procedures (AOAC, 1990), in summary, moisture content of each diet was calculated from triplicate samples (4-5g) that were dried to constant weight at 60°C. Mean total nitrogen content was determined by the micro-Kjeldahl method, and percent crude protein was then calculated as % N x 6.25. Mean total dietary crude lipid was determined by extraction using chloroformmethanol (2:1 V/V) following the method of Folch *et al.* (1957). Ash content was determined by heating samples to 550° C for 4 h. Nitrogen free extract was calculated by difference as follow: NFE = 100 - (% crude protein + % crude lipid + % ash).

To and Barris	TREATMENTS			
Ingredients	T_1	T ₂	T ₃	T_4
Fishmeal ^a	250	200	150	100
Fish silage ^b	0	50	100	150
Soybean meal ^c	250	250	250	250
Wheat bran	150	150	150	150
Wheat flour	145	145	145	145
Cornstarch	88	88	88	88
Gelatin	50	50	50	50
Corn oil	30	30	30	30
Fish oil	20	20	20	20
Vitamin and mineral premix ^d	15	15	15	15
Stay C	2	2	2	2

Table 1. Ingredient (g kg⁻¹ dry weight) of four experimental diets at different levels of fishmeal replaced by fish silage made from *Pterigoplichthys multiradiatus* (Hancok).

^a From Concentrados California (Ensenada, Mexico) 68% crude protein.

^b Made from *Pterigoplichthys multiradiatus* (Hancok) according to Viana *et al.*, 1993

^c From (42% CP).

^d Vitamin mixture (1.3 %), Stay–C (0.4 %) and minerals mixture (3.3%), kindly donated by DSM, Guadalajara, Mexico.

Fish management

The fry of Nile Tilapias (*Oreochromis niloticus, L.*) were acquired from Sistemas Acuícolas Integrales S. de R.L. de C.V. (Ensenada, Baja California, México). The fish fry's were maintained in an economic outdoor Recirculation Aquaculture System, consisting in a one 6.5 Ft³ "POLYGEISER" Pneumatic Drop Bead Filter (Aquaculture Systems Technologies, L.L.C., New Orleans, LA, USA); a 2,000-L water container (like sump), twelve 550-L water containers; a homemade wood structure; one centrifugal ³/₄ Hp water pump and pipe and connections of PVC.

Fry Nile Tilapias (660) with an average weight of 1.8 \pm 0.8 g were randomly distributed is similar batch groups (P>0.5) among twelve circular plastic tanks 550-L (commercial water containers) at a stocking density of 55 fry per tank in triplicate groups. The dietary treatments were hand fed at apparent satiation three times per day (800, 1400 and 2000 h). The experiment started in June 2010 with a photoperiod of 14:10 (light/dark) and lasted after 50 days or up to a total growth higher than 400% according to the National Research Council (NRC, 2011). Every 15 days all fishes from each experimental unit were individually weighed (g) and measured (mm), and the following parameters were calculated as follows:

The total growth increment (%) was calculated as follow:

TGI = (Final body weight *100) / Initial body weight.

The growth rate (GR, mg day⁻¹) was calculated as follow:

GR = (Final body weight – Initial body weight) / Time in days.

The Specific Growth Rate (SGR) was calculated as follow:

SGR = ((ln(Final body weight) – ln(Initial body weight)) / Time in days)) X 100.

The Feed Conversion Ratio (FCA) was calculated as follow:

FCA = Dry weight of feed intake (g) / Gain in body wet weight (g).

Water temperature was recorder every hour, for the 50 experimental days, with a Tidbit temperature data logger (Onset HOBO Data Logger, USA). Dissolved oxygen from each experimental tank was measurement every day with an YSI ® D.O. Meter Y55 series, whereas ammonia, nitrite and alkalinity were measurement every week with an Aquarium

colorimeter kit (API Aquarium Pharmaceutical Kit). The pH of water was recorder every week with an YSI ® EcoSense ® pH/Temp Pen.

Cost-Benefit Analysis

The costs of diets were calculated considering the ingredients, fish silage process, labor and energy expenditure. The analysis of the cost-benefit was calculated multiplying the costs of the diets by the feed conversion ratio.

The cost of production of Tilapia for each diet was calculated as follow:

Cost Kg Tilapia Tn = Feed Cost Tn * FCA Tn.

The saving for each diet (Tn) was calculated as follow:

Save Tn = Cost Kg Tilapia T1 - Cost Tn.

Statistical Analysis

All data were analyzed by one-way ANOVA to detect potential differences at the start and the end of the experiment among the experimental treatments. The normality of the samples was tested with the Kolmogorov-Smirnoff test. When significant differences were found (P<0.05), a Duncan's Multiple Test was used. Statistical analyses were made using Sigma-Stat software for Windows. All data was presented as mean \pm SEM.

RESULTS

All diets made with the inclusion from 0 to 15% fish waste silage (dry weight basis) resulted in a significant similarity in protein (32.96 to 37.63%) and lipid content (4.43 to 5.07%) (F=3.86; P=0.056 and P>0.05, respectively) (Table 2). However differences were found in ash content where levels from 8 to 10% ash where registered along T1 to T4 treatments.

Water quality from fish tanks was under acceptable parameters with a register values under of: 0.25 mg I^{-1} for NH₄ and NO₂; levels of dissolved oxygen ranged over up a 6.5 mg I⁻¹. Mean temperature values was recorder at 24.23±1.42 °C and pH values of 8.2±0.2. The alkalinity of the systems was adjusted every week at 140 mg I⁻¹, with Sodium Bicarbonate industrial grade to keep the balance of carbonate in the system.

Growth was significant similar among treatments (Figure 1), with a growth rate in length of 0.66 to 0.75 mm day⁻¹ and 166.70 to 196.94 mg day⁻¹ in weight (Table 3). The SGR and FCA resulted in significant similarities among treatments, with values between

640 and 827 % and 1.73 to 1.88, respectively. No differences were observed in survival; however from 0.6 to 3.7% organism died during the 50 days experimental procedure (Table 3).

Diet cost analysis (Table 4) shows that cost of diets decreases from 1.54 to 1.27 US\$ kg-1, with fishmeal replacement with fish silage (5, 10 and 15%). The production costs decreases proportionally with the fishmeal replacing, generating from 11.76 to 23.87%.

 Table 2. Proximate composition (%) of four experimental diets at different levels of fishmeal replaced by fish silage made from *Pterigoplichthys multiradiatus* (Hancok).

	TREATMENTS				
	T ₁	T_2	T_3	T_4	
Crude protein (%)	37.63±0.00 a	33.25±2.32 a	33.54±1.82 a	32.96±2.53 a	
Total lipids (%)	4.43±0.19 a	4.65±0.29 a	4.92±0.95 a	5.07±0.11 a	
Ash (%)	7.56±0.05 d	8.43±0.20 c	9.36±0.15 b	10.13±0.15 a	
NFE ^a	47.79	50.87	50.10	49.79	

^aNFE = 100 - (% Crude protein + % Total lipids + % Ash).

Values in the same row with different superscripts are statistically different p<0.05.

INDICES	TREATMENTS				
	T1	T2	T3	T4	
Initial Length (cm)	4.23±0.77	4.20 ± 0.75	4.26 ± 1.01	4.31±0.72	
Final Length (cm)	8.06 ± 1.47	8.12±1.26	8.13±1.55	8.14 ± 1.42	
Length growth rate (mm/day)	0.71±0.04	0.75 ± 0.02	0.71±0.05	0.66 ± 0.03	
Initial Weight (gr)	1.53±0.69	1.39±0.72	1.77±1.63	1.53 ± 0.70	
Final weight (gr)	11.43±1.52	11.25±1.34	11.30±1.54	11.41±1.47	
Growth rate (mg day ^{-1})	186.40 ± 14.20	196.94±19.78	178.93±3.52	166.70±10.29	
TGI (%)	757.73±126.05	827.30±184.91	640.46±95.61	751.37±78.04	
SGR (%)	4.03±0.35	4.19±0.44	3.70±0.29	4.03±0.21	
FCA	1.88±0.55	1.76 ± 0.05	1.80 ± 0.08	1.73±0.16	
Survival (%)	99.41±1.03	98.25±1.75	96.33±4.91	98.25±1.75	
Mortality (%)	0.59±1.03	1.75 ± 1.75	3.67±4.91	1.75 ± 1.75	

Table 3. Biological indices obtained for juvenile of Tilapia (*Oreochromis niloticuss, L.*), feed four levels diets of fish silage replacement for fishmeal.

Standard errors are given.

Values in the same row with different superscripts are statistically different p<0.05.

Table 4. Cost-benefit analysis of four experimental diets at different levels of fishmeal replaced by fish silage made from *Pterigoplichthys multiradiatus* (Hancok), based on FCA and cost per kg.

	TREATMENTS			
	T1	T2	T3	T4
Feed Cost (US\$ kg-1)	1.54	1.45	1.36	1.27
FCA	1.88	1.76	1.80	1.73
Cost Kg Tilapia (US\$)	2.89	2.55	2.44	2.20
Save (US\$)		0.34	0.45	0.69
Save (%)		11.76	15.57	23.87

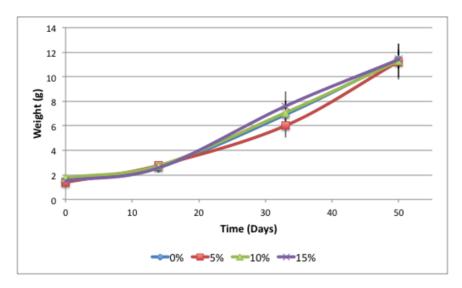


Figure 1. Growth in weight for juvenile of tilapia (*Oreochromis niloticuss*, *L*.), feed four levels diets of fish silage replacement for fishmeal. Average values and standard deviation were obtained from three independent replicates.

DISCUSSION

In the present work it was demonstrated that fish silage from Pterigoplichthys multiradiatus waste could efficiently replace fishmeal in tilapia feed formulations up to 60% protein replacement, a replacement that would be difficult to increase even more due to the high ash content from the by-products (up to 20%; NRC, 2011). Fish silage was made from by products from this invasive species found in large quantities in the Infiernillo Dam in Michoacán, Mexico. The silage was made out only from heads and skin with fins, whereas the flesh was used with a different purpose as food for human. This silage was prepared by adding a mixture of citric and phosphoric acids and kept at room temperature. Previously reports demonstrate that silage made in similar conditions can be store for several months without any deterioration (Viana et al., 1993). The use of this acid mixture has the advantage of accessibility since both are use in the food industry, compare to sulfuric and formic acids commonly used in acid fish silage (Raa and Gildberg, 1982). Citric acid is a powder that can be measured by weight or volume, whereas the phosphoric acid in their liquid form per volume without any problems for an additional 1-2% difference than that obtained by an accurate measurement. The fish silage can be then stored in 20L plastic containers with a lock in a dry place without problems to be contaminated by flies or bugs within rural areas. After 2 weeks the silage can be used by mixing with the rest of the ingredients to prepare the formulated diets in the backyard aquaculture system. The silage was added up to 60%

fishmeal replacement corresponding to a 15% from the diet considering in a dry matter content basis. This kind of preservation is cause the liquefaction of protein resulting in a liquid protein product with a content of dry matter as low as 15 to 20%, making difficult to incorporate higher amount of silage without a previous dehydration. Therefore for further inclusion of acid fish silage a previous dehydration will be needed unless the ash content is not high.

Here, all treatments resulted in an overall growth increase up to 827% during 50 days experimental procedure and even if no significant differences could be reported, a result that is considered optimal as recommended by the NRC (2011). Earlier reports claim an advantage the use of partially hydrolyze protein (Espe et al., 1992) due to a more efficient use of protein, whereas a high inclusion of fish silage leads to a high amount of free amino acids and therefore a growth reduction. However, in this study we did not found significant differences at increasing amounts of fish silage on the diets, but rather the specific growth rate was hold between 3.70 to 4.19. According to Espe et al. (1992) the rate of peptides and amino acids uptake should be in good balance in order to promote growth. Then, when a pre-digested proteins are used in feed, if the rate of uptake exceeds the anabolic capacity of the animal then an extra amount of amino acids will rapidly be absorbed are then catabolized resulting a poor protein utilization. The inclusion of acid fish silage in substitution of fishmeal, has the advantage, not only to reduce the cost from the fishmeal (to 23%), but also for using a by-product that is scarcely used and available in most

natural lakes and rivers in rural areas. According to several authors (Tacon and Metian, 2011; Tacon et al., 2012) fishmeal will not be sustainable due to overfishing and need to feed the increasing human population. A large amount of fishmeal comes from minor pelagic species with an increasing pressure to target the human market. Therefore the finding of any ingredient to substitute fishmeal will be positive to increase the possibilities to supply protein for fish feed artisanal manufacture. However, since the acid fish silage has a large amount of water, it will be interesting to dry it previously and then try a higher level of substitution if ash content is not higher than 20%. Moreover, these diets were composed with a mixture of fishmeal/fish silage and soybean meal in equal parts. As mentioned above the fish silage was added according to their dry matter content and it was directly replaced for fishmeal.

Today, one of the priority issues in any order of scientific knowledge relevant to food production is sustainability (FAO, 2012), so that food production goes according to the economic, social, cultural and of a healthy environment, without compromising the satisfaction of the same to future generations (Mojica-Sastoque et al., 2010). Tilapia production is no stranger to this concern for sustainability, where there are even a number of studies that establish the foundations of sustainability in Mexico. Vivanco-Aranda, et al (2011) addresses this issue where they study the level of modernization in Mexico among several states establishing the critical factors of operation. The automation is a necessary process for a controlled and sustainable production where formulated feeds play an important role in the production cost to increase growth rate. However, the cost of food is an issue that if not studied could have a negative effect on the production of tilapia. In a traditional culture of tilapia, regardless of the level and intensity of crop you have, the cost of feed accounts for up to 60% of the production cost. Hartley-Alcocer, et al. (2006), mentioned that the 92% of the farms in Mexico employed commercial feeds (i.e. Purina, AS, El Pedregal, Malta-Clavton and Algimex). In this paper we present a cost-benefit analysis with the idea of evaluating the replacement of fishmeal by Pterigoplichthys multiradiatus silage,s (Hancock), finding that replacing up to 15% of fishmeal gives a saving of 23.9% of the production cost. Importantly, in this order of sustainability, that this saving involves not only the product system of Tilapia, but to reduce the consumption of fish meal as this would impact on fresh sardines could be directly feeding the population.

If such results could be applied in different product tilapia system in different states of the country, is likely to crop sustainability nationwide increase causing more competitive with the product that is imported from Asian countries and increase the sardine availability for human consumption.

Water quality was measured daily or weekly showing an optimum quality as recommended by Webster and Lim (2006). The main quality parameters of water was recorded along the experiment: $\leq 0.25 \text{ mg l}^{-1}$ of NH_4 ; $\leq 0.25 \text{ mg l}^{-1}$ of NO_2 , $pH = 8.2 \pm 0.2$; Dissolved Oxygen of $\geq 6.5 \text{ mg l}^{-1}$ and alkalinity was controlled \geq 140 mg l⁻¹. The Recirculation Aquaculture System used here was loaded with a fish density necessary to achieve a final biomass of 50 Kg m⁻³ per tank, an appropriated amount to support a backyard Tilapia production. The survival rate reported in this study is considered as optimal being over 90% in all cases. Under this system a production of 400.00 to 500.00 g commercial size fish would be obtained after 6 months under same growth rate. This backyard production should be promoted in rural to semi-urban areas in Mexico, where families could use the opportunity to ingest fresh protein in small quantities without the need to refrigerate the left carcass as it has been done with poultry production.

In conclusions, the use of fish by-products from the *Pterigoplichthys multiradiatus* in fish silage in substitution of fishmeal has resulted in a similar growth rate than those with fishmeal. Their use can significantly reduce the feed cost and therefore a reduction up to 23% was observed, besides the use of fishery by-products instead of minor pelagic fish species to sustain the fishery for human consumption. Therefore the introduction of backyard aquaculture should be promoted to produce high fresh quality protein in rural areas.

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