Key Words: ***Lutjanus guttatus*** **– Spotted rose snapper, Pacific lane snapper, Nutrivance™ SPC, soy-based feed, cage culture**

SOY PROTEIN CONCENTRATE AS A PARTIAL REPLACEMENT FOR FISHMEAL IN THE DIET OF ROSE SPOTTED SNAPPER, PAQUERA, COSTA RICA 2013

TO

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Company:	Martec Industries S. A.
Location:	Paquera in front of Isla Cedro
Farm:	Experimental Site - Cage Farm
City:	Paquera (Puntarenas Province), Costa Rica
Trial Type:	Feeding demonstration in Experimental Cages
Treatments:	Experimental diet with soy protein concentrate (SPC-NutriVance) versus a fishmeal based commercial diet (50% CP/13% fat content)
Species:	*Lutjanus guttatus* (Spotted rose snapper)
Source of juveniles:	Hatchery "Rancho Chico" located in Playa Bejuco (Nandayure, Guanacaste Province)

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EXECUTIVE SUMMARY:

A cooperative feeding demonstration with spotted rose snapper was conducted in 2013 between USSEC and Martec Industries S. A. at the Martec experimental aquaculture site located at Paquera in front of Isla Cedro, Puntarenas Province, Costa Rica. The objective of the demonstration was to show the feasibility of using a diet in which a feed grade soy protein concentrate (SPC) having high protein and low oligosaccharide content partially replaced fishmeal. Results of the feeding demonstration showed that inclusion of SPC Nutrivance™ at an inclusion rate up to 26.4% in the spotted rose snapper diet did not affect snapper production parameters, and that there was no difference in fish performance with the SPC diet and a commercial diet formulated with fishmeal as the primary protein source. Hence, the SPC-Nutrivance™ appears to be a potential ingredient for manufacturing aquafeeds for marine fish species.

INTRODUCTION

The purpose of this commercial feeding demonstration was to evaluate the partial replacement of fishmeal with a SPC product "Nutrivance" ™, having high protein (55.37%) and low soy anti-nutritional (raffinose 0.2%, stachyose 0.5%, and low trypsin inhibitor activity 2.31 mg/g) content, in a diet for the spotted rose snapper (*Lutjanus guttatus*). The experimental SPC diet was formulated to contain 50% crude protein content and 13% fat content. Snapper performance was compared with the SPC diet and a standard commercial diet formulated with fishmeal as the primary protein source. The fishmeal based diet was manufactured by "Montes de Oro" Feed mill in Costa Rica. Additionally, Martec's research team separately evaluated a third diet, Otohime, imported from Japan. The Otohime diet was not included as a treatment in the feeding demonstration, but results with the Otohime diet are discussed in this report. The feeding demonstration with the SPC and fishmeal based feeds was conducted over a 75-day period.

The snapper feeding demonstration was conducted at the Martec experimental cage farm station is located in front of Isla Cedro at Paquera, Puntarenas Province, Costa Rica (Figure 1). The facility includes eight (8) cages having a volume of 343-m^3 (7m x 7m x 7m) each. Two of the cages are dedicated to brood stock and six are available for fish grow-out. The facility is used primarily for brood stock development and experimental studies related to nutrition and production technologies for the grow-out stage.

FEEDING DEMONSTRATION PROTOCOLS

Three diets were tested in the feeding demonstration. A Nutrivance SPC diet, designated Diet 1 – SPC, was formulated to contain 50% crude protein and 13% fat and was nutritionally equivalent to a fishmeal based commercial diet manufactured by the Montes de Oro Feed mill (Diet 2 – MDO) and to the Otohime diet manufactured in Japan (Diet 3 – OTO). The SPC experimental diet was manufactured by Montes de Oro feed mill on September 4, 2013. Nutrivance ™ from TechMix in the United States served a key protein source in the SPC experimental diet, with an inclusion level of 26.4%. Proximal analysis of the Nutrivance SPC

diet is shown in Table 1. Formulation of the Nutrivance SPC diet is displayed in Table 2. Proximal analysis of the SPC experimental diet is displayed in Table 3.

The feeding demonstration was conducted in 12, 1.2-m3 experimental cage units at the Martec Paquera facility in Costa Rica. The experimental test cages were placed inside the farm's two brood stock cages (cages 7 and 8), which were holding 142 and 139 organisms respectively). Six experimental cages were placed in each brood stock cage (Figures 2 and 3), with the cages oriented in the prevailing water current to maximize water exchange.

Spotted rose snapper for the demonstration were 2.39-g fish produced by Martec's hatchery at Rancho Chico, located in Playa Bejuco, Nandayure, Guanacaste Province. Fish were stocked in the experimental cages and the demonstration started on October 7, 2013. Fish were stocked at a density of 170 fish per experimental cage, which was equivalent to a biomass 0.34 kg/m^3. Fish were fed *ad-libitum*, usually four times a day. Nets from each experimental cage were cleaned every ten to twelve days. Twenty fish were sampled from each experimental unit every two weeks during the 75 days of the feeding demonstration. Individual fish weights were determined during each sampling. Fish were harvested after 75 days of feeding. Information was collected at harvest on total yield, individual fish weight, fish survival, final biomass, feed consumption, feed conversion ratio (FCR), and protein efficiency ratio (PER).

The following variables were calculated:

- Survival
  (%) = 100 x (final amount of fish)/(initial amount of fish)

- Biomass gain (g) = Final biomass - initial biomass

- Final biomass
  (Kg/m^3) = Biomass of fish harvested, Kg / Experimental unit volume, m^3

- Weight gain
  (WGR, %) = 100 x (final body weight - initial body weight) / initial body weight

- Specific growth rate
  (SGR) = 100 x ln (final weight/initial weight)/days of the experiment

- Feed conversion ratio
  (FCR) = feed fed (g, DW)/body weight gain (g)

- Protein efficiency ratio
  (PER) = body weight gain (g)/protein intake (g)

Statistical analysis was performed using One-way Analysis of Variance. Although normality and equal variance tests were appropriate (passed), the power of the test with alpha 0.05 was below the desired power of 0.800 for all the parameters evaluated, for that reason there are limitations to the generalization of this analysis.

RESULTS

Proximate composition of the experimental SPC Nutrivance™ diet was performed at the laboratory "Food Science Testing –FST" in San Jose (Costa Rica), and reported on November 6th, 2013. Proximate analysis indicated a crude protein content of 54.4% and a fat content of 11.7%, which were higher and lower, respectively, than the target formulation values of 50% for crude protein and 13% for fat content (Table 3). Proximal analysis of diets 2 (Montes de Oro-MDO) and diet 3 (Otohime – OTO) were not available. Some differences in regards to feed manufacturing were identified, specifically the presence of excessive amount of fines (small particles, broken pellets) in the experimental diet 1 – SPC, versus the commercial diets 2 and 3 (MDO and OTO, respectively) (Figure 4).

Water quality parameters during the feeding demonstration were very stable with an average temperature of 27.6±0.45 °C and dissolved oxygen of 5.38±0.94 mg/L (Table 4).

Feeding was *ad-libitum*, with a target of four feedings per day. However, climatic conditions affected feeding, and after 75-days of culture it was found that 60% of the time fish were fed four times per day, 17% of the time fish were fed three times per day, 11% of the time fish were fed once or twice daily, and 12% of the time organisms were without food (Figure 5).

A total of 2,040 spotted rose snapper were equally distributed among the twelve experimental cages, with 170 fish per cage. The initial average weight was calculated by weighing a total of 115 fish (5.6% of the studied population). After 60-days of culture one replicate from the Diet 2 – MDO, was lost. The average initial weight was 2.39±0.60-g (Coefficient of variance, CV=25.34%), and that weight was assumed for all replicates and treatments. Organisms that were fed Diet 1 - SPC (Nutrivance™ SPC) grew from 2.39 g to an average weight of 43.63 g at harvest (Table 4, Figure 6), while fish fed Diet 2 (Montes de Oro – MDO) reached an average weight of 43.88-g. Fish fed Diet 3 (Otohime – OTO) reached an average weight of 45.82-g (Table 4, Figure 6). Growth curves for this period of culture were fitted using an exponential model, as follows: Diet 1 – SPC, $y = 3.0663e^{0.0376x}$ ($R^2=0.9675$), Diet 2 – MDO, $y = 3.0904e^{0.0374x}$ ($R^2=0.9660$), and Diet 3 - OTO, $y = 3.1217e^{0.0381x}$ ($R^2=0.9642$) (Figure 6). This analysis showed a slightly better growth response for fish fed Diet 3-OTO, but it was not significantly different after evaluating all the production parameters.

Fish survival after 75-days of culture was 90.2% for snapper fed Diet 1-SPC, 98.0% for fish fed Diet 2-MDO, and 87.6% for fish fed Diet 3-OTO. There was no significant difference among those values (p=0.3151) (Table 5 and 6). Similarly, other variables such as individual final weight, biomass gain, final density, weight gain (WGR, %), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were also not significantly different among dietary treatments (Table 5 and 6).

Individual final weight ranged from 43.6-g per individual in fish fed Diet 1-SPC, up to 43.9-g per individual for fish fed Diet 2-DMO and 45.8-g per individual for fish fed Diet 3-OTO, with no significant differences among the treatments (p=0.1565) (Table 5 and 6). Final biomass per experimental cage unit averaged 5.58 kg/m^3 for the replicates fed the SPC diet, followed by 5.67

kg/m^3 for fish fed Diet 3-OTO, and 6.10 kg/m^3 for fish in units fed Diet 2-MDO. There was no significant difference (p=0.4355) among the feed treatments. Biomass gain ranged from 6.3 kg up to 6.9 kg during the feeding demonstration period.

Weight gain (WGR, %) for spotted rose snapper fed Diet 3-OTO was the highest with an average value of 1,817.2%, followed by Diet 2-MDO at 1,735.9%, and Diet 1-SPC at 1,725.5%. There were no significant differences among those values (p=0.1573) (Table 5 and 6). Similarly, specific growth rate (SGR/day) for fish fed Diet 3-OTO was the highest 5.03±0.07 %/day, followed by Diet 2-MDO with 4.97±0.04 %/day, and Diet 1-SPC with 4.96±0.03 %/day. These also did not have any significant differences (p=0.1645) (Table 5 and 6).

Feed conversion ratio (FCR) was lowest for fish fed Diet 2-MDO with an average FCR of 1.32±0.08 (CV=6.23%). Fish fed Diet 1-SPC and Diet 3-OTO coincidentally had similar FCR, with values of 1.39±0.21 (CV=14.93%), and 1.39±0.10 (CV=7.18%), respectively.

Protein Efficiency Ratio (PER) was estimated based on 54.40% crude protein content for diet 1-SPC, 52.58% crude protein content for diet 2-MDO, and 52.00% crude protein content for diet 3-OTO. The crude protein content for Diets 1-SPC and 2-MDO was based on results from the proximal analysis (Table 3). The lowest protein efficiency ratio was determined for Diet 1-SPC with an average value of 1.34±0.17 (CV=12.69%). PER for Diet 3-OTO was 1.39±0.11 (CV=7.19%), and the highest value was achieved for Diet 2-MDO with an average value of 1.44±0.09 (CV=6.25%). However, those differences were not significantly different (p=0.6331) (Table 6).

DISCUSSION

The purpose of this commercial feeding demonstration was to corroborate the feasibility of using Nutrivance™ SPC as a partial fishmeal replacement in the diet for the spotted rose snapper (*Lutjanus guttatus*).

The experiment design had three treatments with four replicates using juvenile fish 2.39-g in size to reach 50-g fish in size during 75 days of culture.

The statistical analysis of the production variables obtained at the end of this feeding demonstration indicated no significant difference among the three feed treatments. Results support the substitution of SPC as a partial replacement for fishmeal in the diet for spotted rose snapper when it is cost effective to do so. In fact, previous research from Silva-Carrillo et al. (2012) found that up to 20% of fishmeal could be substituted using regular soybean meal in spotted rose snapper diets, representing an approximately 10.5% soybean meal inclusion in the diet. In this particular feeding demonstration, an SPC inclusion of 26.4% yielded similar snapper performance to fishmeal based diets. Hence, both soybean meal and soy protein concentrate can serve as potential replacements for fishmeal without affecting growth performance.

An economical analysis for these diets requires considering an economy of scale to effectively reduce the ingredient price. The current price for the experimental Nutrivance™ SPC diet was

established at US$2.60 per kilogram of feed, which represents US$3.33 per kilogram of raised fish. Those values are slightly higher than for the commercial diet from Montes de Oro's feed mill, in which the price of feed reached US$2.32 per kilogram, and that price jumped up to US$2.90 per kilogram of fish cultured in the experimental units. The Otohime diet has a price of US$19.00 per kilogram of feed, and that price reaches US$24.70 per kilogram of fish raised during the feeding demonstration. Availability and cost of fishmeal in the future are two conditions that might shift the cost of the SPC experimental diet compared to the commercial diet. Hence, continue development of diets based on SPC and soybean meal can be considered a key aspect for having an environmental and sustainable aquaculture industry.

CONCLUSIONS & RECOMMENDATIONS

Results from this commercial feeding demonstration encourage continued development of alternative diets for marine fish species based on soybean meal and soy protein concentrate, with a goal of economic and environmental sustainability.

It is important to address some issues in the feed manufacturing, because the presence of excessive fines in the finished SPC feed may have an impact in the feed conversion ratio due to potential feed losses. Adequate proximal analysis of ingredients is critical to reduce potential gaps between formulation and actual composition of the experimental diets.

TABLE 1 – PROXIMAL ANALYSIS OF THE NUTRIVANCE™ SPC HAVING A LOW LEVEL OF SOY ANTI NUTRITIONAL FACTORS. THE NUTRIVANCE SPC PRODUCT WAS TESTED IN A DIET FOR ROSE SPOTTED GROUPER *Lutjanus guttatus* AT THE MARTEC AQUACULTURE EXPERIMENTAL FARM IN COSTA RICA.

Parameter	Nutrivance ™ SPC

Moisture (% dry matter) maximum	12.00
Crude protein (% dry matter) minimum	55.37
Crude fat (% dry matter) minimum	0.50
Crude fiber (% dry matter) maximum	4.50

TABLE 2 – FEED FORMULATION FOR THE NUTRIVANCE™ SPC DIET FOR SPOTTED ROSE SNAPPER *Lutjanus guttatus* EVALUATED IN THE MARTEC OCEAN CAGE FEEDING DEMONSTRATION AT PAQUERA, PUNTARENAS PROVINCE, COSTA RICA IN 2013.

Ingredient	SPC - 50% CP 13% CF
Soy Protein Concentrate, SPC (55.37% protein)	26.40%
Fish meal, unspecified	27.70%
Others	45.90%
TOTAL	100.00%

TABLE 3 – PROXIMATE COMPOSITION OF NUTRIVANCE™ 50/13 SPC DIET AND COMMERCIAL DIET EVALUATED IN THE SPOTTED ROSE SNAPPER *Lutjanus guttatus*

FEEDING DEMONSTRATION CONDUCTED AT THE MARTEC EXPERIMENTAL AQUACULTURE FARM AT PAQUERA, PUNTARENAS PROVINCE, COSTA RICA IN 2013.

Parameter	SPC 50/13	COMMERCIAL
Moisture (% dry matter)	5.60	6.63
Dry matter (% dry matter)	94.40	93.37
Crude protein (% dry matter)	54.40	52.58
Crude fat (% dry matter)	11.70	16.16
Ash (% dry matter)	11.50	19.78
Crude fiber (% dry matter)	5.50	1.21
Calcium (% dry matter)	2.99	6.18
Phosphorus (% dry matter)	1.80	3.62

TABLE 4 – PERIODIC SAMPLING DATA ON FISH GROWTH AND WATER QUALITY PARAMETERS DURING THE 75 DAY FEEDING DEMONSTRATION WITH SPOTTED ROSE SNAPPER *Lutjanus guttatus* IN 2013.

Treatment	Replicate	10/6/13	10/22/13	11/6/13	11/21/13	12/5/13	12/20/13
	Number of days	Stock	16	31	46	60	75
	Temperature T(ºC)		27.61 ±0.71 (n=15)	27.78 ±0.33 (n=13)	27.56 ±0.36 (n=15)	27.45 ±0.30 (n=14)	27.55 ±0.34 (n=10)
	Dissolved oxygen DO (mg/L)		4.39 ±0.69 (n=15)	4.85 ±0.70 (n=13)	5.75 ±0.77 (n=15)	6.00 ±0.50 (n=14)	6.12 ±0.57 (n=10)
1 – SPC	1	2.39	7.1	10.5	21.2	31.0	44.84
	2	2.39	6.9	11.6	18.1	29.0	42.57
	3	2.39	6.8	10.6	18.0	27.6	43.00
	4	2.39	7.2	11.1	18.6	31.4	44.10
	Average	2.39	7.0	11.0	19.0	29.7	43.63
2 – MDO	1	2.39	6.9	10.2	19.1	29.7	43.79
	2	2.39	8.0	11.5	19.5	29.7	45.06
	3	2.39	6.8	10.9	16.8	28.1	42.78
	4	2.39	6.7	11.6	21.0	---	---
	Average	2.39	7.1	11.1	19.1	29.2	43.88
3 – OTO	1	2.39	7.7	11.7	21.2	33.6	47.39
	2	2.39	7.6	11.1	18.0	30.7	45.65
	3	2.39	7.9	11.6	18.7	33.4	47.33
	4	2.39	6.3	10.9	19.2	29.8	42.92
	Average	2.39	7.4	11.4	19.3	31.9	45.82

TABLE 5 – FISH PRODUCTION DATA COLLECTED AFTER 75 DAYS OF CULTURE OF SPOTTED ROSE SNAPPER *Lutjanus guttatus* FED THREE TEST DIETS IN 2013.

Treatment	Replicate	Number of fish	Survival	Individual weight

		Initial	Final	(%)	Initial	Final
1 – SPC	A	170	158	92.9	2.39	44.84
	B	170	129	75.9	2.39	42.57
	C	170	164	96.5	2.39	43.00
	D	170	162	95.3	2.39	44.10
2 – MDO	A	170	170	100.0	2.39	43.79
	B	170	168	98.8	2.39	45.06
	C	170	162	95.3	2.39	42.78
	D	170	---	---	---	---
3 – OTO	A	170	128	75.3	2.39	47.39
	B	170	163	95.9	2.39	45.65
	C	170	143	84.1	2.39	47.33
	D	170	162	95.3	2.39	42.92

Treatment	Replicate	Weight gain (%)	Specific growth rate (SGR)	Final Biomass (kg)	Final Density (kg/m^3)	FCR
1 – SPC	A	1776.3	4.998	7.085	5.905	1.286
	B	1681.3	4.925	5.492	4.577	1.702
	C	1699.1	4.939	7.052	5.877	1.304
	D	1745.1	4.974	7.144	5.953	1.272
2 –MDO	A	1732.3	4.965	7.445	6.204	1.234
	B	1785.4	5.005	7.570	6.309	1.395
	C	1690.1	4.932	6.931	5.776	1.344
	D					
3 – OTO	A	1882.8	5.076	6.066	5.055	1.506
	B	1809.9	5.023	7.440	6.200	1.266
	C	1880.4	5.074	6.769	5.641	1.420
	D	1695.7	4.936	6.953	5.794	1.376

TABLE 6 – PRODUCTION DATA AND STATISTICAL ANALYSIS OF DATA FOR THE 75-DAY FEEDING DEMONSTRATION CONDUCTED WITH SPOTTED ROSE SNAPPER *Lutjanus guttatus* AT THE MARTEC EXPERIMENTAL AQUACULTURE FARM AT PAQUERA, PUNTARENAS PROVINCE, COSTA RICA IN 2013.

	Survival	CV	St. Error	F	P

Parameter	Treatment	(%)	(%)			
Survival (%)	1 – SPC	90.2 ± 9.62	10.67	4.261	1.3388	0.3151
	2 – MDO	98.0 ± 2.45	2.49	4.921		
	3 - OTO	87.6 ± 9.85	11.24	4.261		
Individual final weight (g)	1 – SPC	43.6 ± 1.03	2.37	0.771	2.3592	0.1565
	2 – MDO	43.9 ± 1.14	2.60	0.890		
	3 - OTO	45.8 ± 2.10	4.58	0.771		
Biomass gain (g)	1 – SPC	6287.0 ± 801.7	12.75	312.76	0.9237	0.4356
	2 – MDO	6909.0 ± 338.7	4.90	361.15		
	3 - OTO	6400.7 ± 569.4	8.90	312.76		
Final density (Kg/m^3)	1 – SPC	5.58 ± 0.67	11.98	0.261	0.9239	0.4355
	2 – MDO	6.10 ± 0.28	4.63	0.301		
	3 - OTO	5.67 ± 0.47	8.37	0.261		
Weight gain (WGR, %)	1 – SPC	1725.5 ± 43.3	2.50	32.254	2.3514	0.1573
	2 – MDO	1735.9 ± 47.7	2.75	37.243		
	3 - OTO	1817.2 ± 87.8	4.83	32.254		
Specific growth rate (SGR)	1 – SPC	4.96 ± 0.03	0.67	0.025	2.2811	0.1645
	2 – MDO	4.97 ± 0.04	0.74	0.028		
	3 - OTO	5.03 ± 0.07	1.30	0.024		
Feed conversion ratio (FCR)	1 – SPC	1.39 ± 0.21	14.93	0.0735	0.2277	0.8013
	2 – MDO	1.32 ± 0.08	6.23	0.0849		
	3 - OTO	1.39 ± 0.10	7.18	0.0735		
Protein efficiency ratio (PER)[a]	1 – SPC	1.34 ± 0.17	12.69	0.0659	0.4843	0.6331
	2 – MDO	1.44 ± 0.09	6.25	0.0761		
	3 - OTO	1.39 ± 0.10	7.19	0.0659		

[a] Protein efficiency ratio (PER): it was calculated based on 54.40% crude protein level for diet 1 (SPC), 52.58% crude protein for diet 2 (MDO), and 52.00% crude protein for diet 3 (OTO).

FIGURE 1 – EXPERIMENTAL STATION FOR THE SPOTTED ROSE SNAPPER *Lutjanus guttatus* FEEDING DEMONSTRATION WITH THREE FEEDS CONDUCTED BY MARTEC IN 2013.

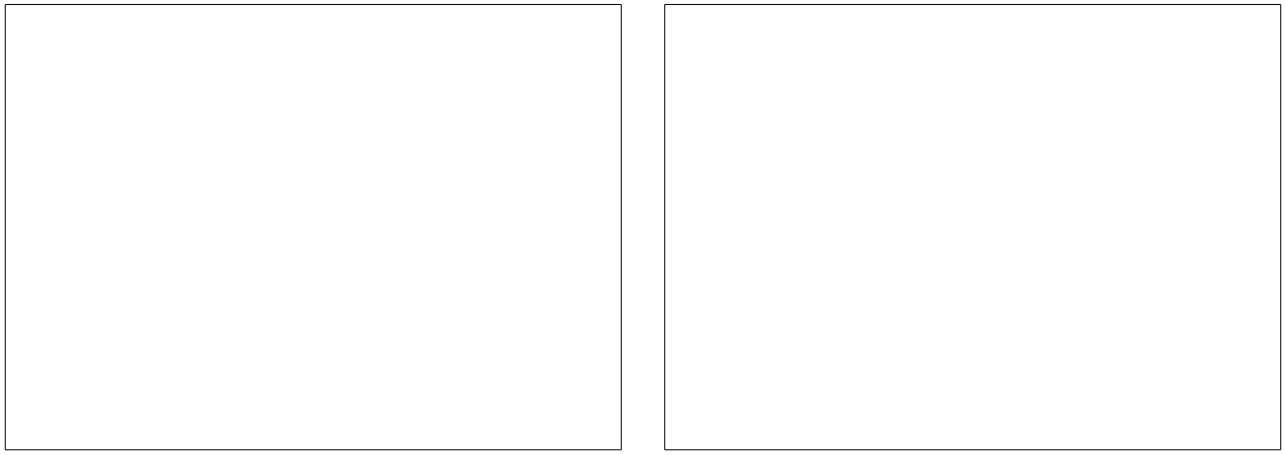


FIGURE 2 – CONFIGURATION OF THE 12 EXPERIMENTAL CAGE UNITS INSIDE THE MARTEC MARINE FISH BROOD STOCK CAGES 7 AND 8 FOR THE 2013 SPOTTED ROSE SNAPPER *Lutjanus guttatus* FEEDING DEMONSTRATION WITH THREE TEST FEEDS.

D1 - SPC: Experimental feed with high inclusion of Nutrivance™ SPC
D2 - MDO: Commercial feed manufactured by Montes de Oro feed mill
D3 - OTO: Otohime feed imported from Japan

FIGURE 3 – EXPERIMENTAL UNITS INSIDE CAGES 7 AND 8 DURING A FEEDING DEMONSTRATION FOR SPOTTED ROSE SNAPPER *Lutjanus guttatus* USING AN SPC NUTRIVANCE™ 50/13 DIET AND TWO COMMERCIAL DIETS AT PAQUERA, PUNTARENAS PROVINCE, COSTA RICA in 2013

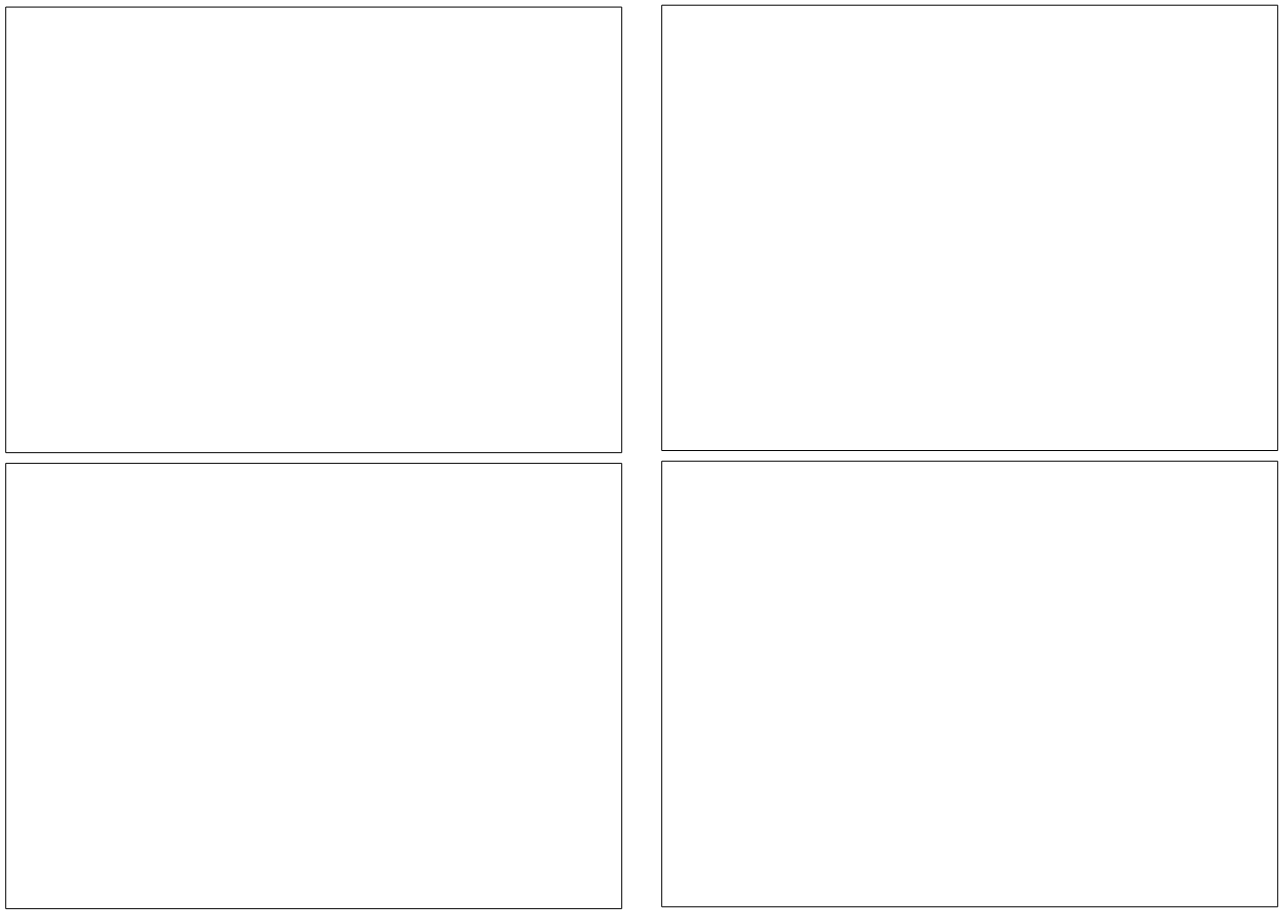
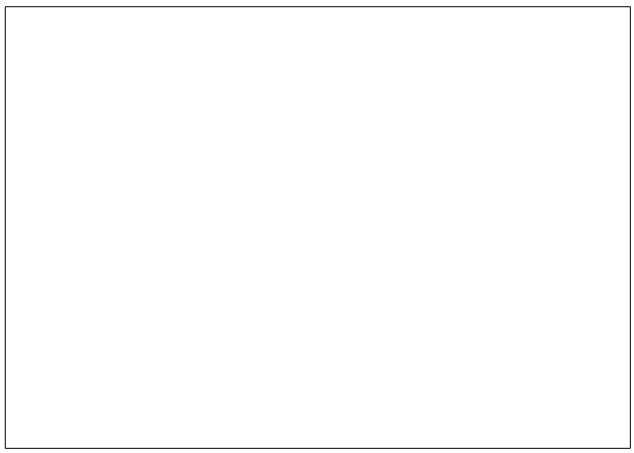
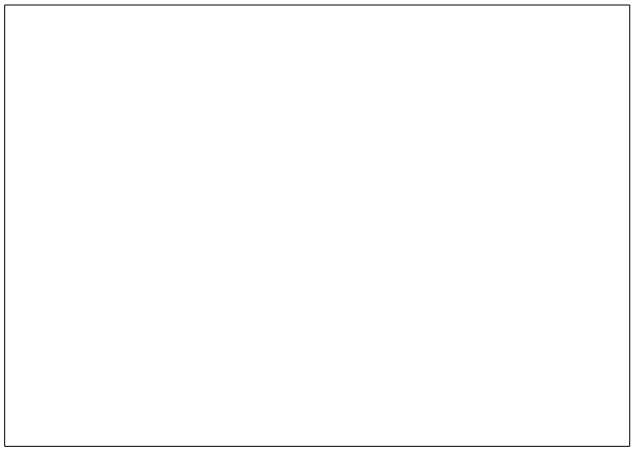


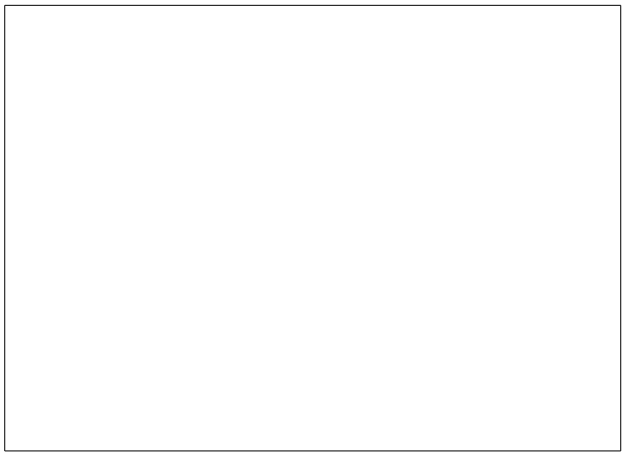
FIGURE 4A, B, C & D – EXPERIMENTAL FEEDS USED DURING A FEEDING DEMONSTRATION FOR SPOTTED ROSE SNAPPER *Lutjanus guttatus* IN 2013.

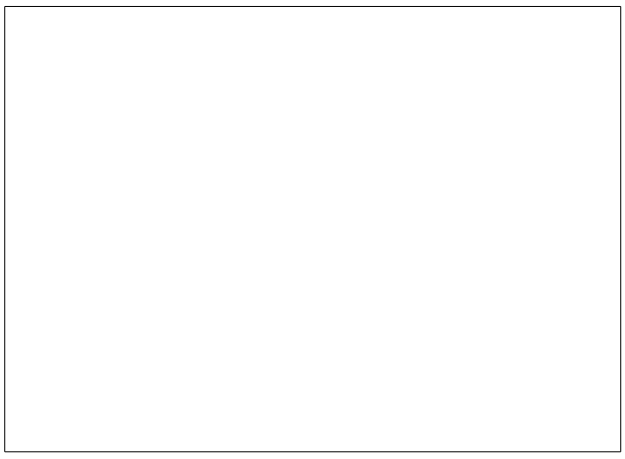




a. Experimental diet – High inclusion SPC

b. “Montes de Oro” diet





c. “Otohime” diet

d. Containers for experimental feeds

FIGURE 5 – FREQUENCY OF DAILY FEEDING DURING THE 75 DAY SPOTTED ROSE SNAPPER *Lutjanus guttatus* FEEDING DEMONSTRATION CONDUCTED BY MARTEC IN 2013.

FIGURE 6 – GROWTH CURVES FOR THE THREE TEST FEEDS EVALUATED IN THE 2013 MARTEC FEEDING DEMONSTRATION WITH SPOTTED ROSE SNAPPER *Lutjanus guttatus*.