

Performance of fishmeal-free rabbitfish (*Siganus lineatus*) feed in Palau¹

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Abstract— Two 56-day trials were conducted to evaluate the effects on the performance and feed utilization efficiency of four experimental diets formulated with different plant-based meals to completely replace fish meal for juvenile golden-lined rabbitfish (*Siganus lineatus*). A commercial and currently employed feed was used as control, while the experimental diets were formulated and manufactured with a combination of duckweed (Lentein), fermented soybean (MrFeed Pro50s), soy protein concentrate (Profine), and soybean meal. Each trial had a total of 120 fish (trial 1: 9.29 ± 0.14 g/fish; trial 2: 7.74 ± 0.25 g/fish) stocked in groups of 10 in a total of 12 floating cages (47.7 L) within two 3-ton flow-through tanks (Koror, Palau). Control and experimental diets were fed to quadruplet cages by hand to visual satiation for one hour four times a day to a maximum of 5% body weight in trial 1, and two times per day to a maximum of 3% body weight in trial 2. Trial 1 tested diet 1 (42% Lentein, 15% MrPro50s) and diet 2 (42% Lentein, 15% soybean meal) with no significant differences ($p > 0.05$) in terms of growth performances (final weight, weight gain and specific growth rate) and feed utilization (feed conversion ratio and protein efficiency ratio), while feed intake was significantly different ($p < 0.05$). Trial 2 tested diet 3 (23% soy protein concentrate, 20% soybean meal) and diet 4 (27% soy protein concentrate, 10% soybean meal) with significant differences in terms of growth performances but no differences in terms of feed utilization and feed intake. Survival was unaffected by the treatments ($p > 0.05$).

Introduction

Aquaculture is an industry that has witnessed steady global growth; since 2010 it has grown by 5.8% and the production of 2016 was 4 million tons more than the previous year (FAO 2018). In 2015, aquaculture produced more than 45% of the global seafood demand in the form of finfish, crustaceans, mollusks and other aquatic organisms (FAO 2017). With a projected continual growth of aquaculture, there will also be an increase in the demand of feed and its related ingredients. In most finfish production systems where a fishmeal-based feed is employed, nearly 70% of production costs are strictly for feeds (Wilson 2002). Due to the relatively static supply of wild forage fishmeal coupled with its increase in demand, the price of aqua feeds has been rising (Henry et al. 2015).

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Because of this scenario, alternative sources of protein intended to replace fishmeal have been researched and developed for commercial applications (Jia & Hing 2017). There exists a large spectrum of plant-based fishmeal replacements for aquafeeds such as soy protein, corn gluten, pea meal and wheat gluten for example, but when presented with these options it appears that soybean meal is regarded as the preferred source of protein for animals and humans (Lock et al. 2015, Baker 2000).

Just as the global aquaculture industry, seafood consumption has witnessed a steady increase as well. In 2015 the global per capita consumption was 18.5 kg, in 2016 it was 20.3 kg, and by 2027 it is projected to reach 21.3 kg (OECD/FAO 2018). Small island developing states (SIDS) like Palau in Micronesia rely on the oceans to provide 50-90% of their animal protein (FAO 2017). These islands face complex challenges in terms of food security and nutrition to become more independent and less reliant on costly imports. Local and foreign governments coupled with non-profit organizations have the capacity and tools to assist SIDS in developing their own food production systems. This is true for Palau as it has been assisted by its own Bureau of Marine Resources, the Taiwanese government and The Nature Conservancy to develop its aquaculture industry since the 1970s with giant clams, crustaceans (mangrove crab, giant freshwater prawns and marine shrimp) and finfish (milkfish, rabbitfish and grouper) (Bueno 2014). Private sector-operated feed supply in Palau is non-existent, meaning that all commercially- used feed is imported from Taiwan which increases costs on the farmers.

Currently, two species of rabbitfish (*Siganus lineatus* and *Siganus fuscenscens*) are being farmed in Palau with year-round local seed production to provide farmers with fingerlings. Rabbitfish are part of the genus *Siganus* of the family Siganidae, an herbivorous teleost that is found in brackish and marine environments across the Indo-West Pacific (Woodland 1990). *Siganus* fry congregate in large schools, and during certain seasons they can be harvested from coastal waters with gill nets, beach seines, small mesh nets and cast nets. This genus is euryhaline which allows them to inhabit shallow reefs, mangroves, estuaries and lagoons which exhibit daily and seasonal water quality fluctuations (Babikian et al. 2017). The main issue with Palauan rabbitfish farming is that the commercially-used feed is intended for milkfish (*Chanos chanos*) with a species-specific nutritional profile and requirement that differs from what rabbitfish might require.

The purpose of this research is to test diets in which fishmeal has been completely replaced with plant-based meals while at the same time increasing growth and feed efficiency. As fishmeal demand is expected to increase while its supply to remain relatively constant, novel plant-based protein ingredients are projected to become more readily available and affordable. This project was intended for the development of practical alternative diets rather than identifying which specific ingredients and at what inclusions levels might have improved growth and efficiency. The funding party of this research was Future of Fish Feed, a non-profit environmental group that focuses on researching and developing alternative feeds for aquaculture and to provide those feed formulae to the public as open source.

Methods

EXPERIMENTAL DIETS

The experimental diets (Table 1) were formulated and produced by Prairie Aquatech, Brookings, South Dakota, USA, using a cooking extruder. The control diet, meant for milkfish (*Chanos chanos*), is currently being used by local farmers in Palau as the conventional feed. Four experimental diets containing different plant-based proteins were formulated. Diet 1 contained 42% Lentein and 15% MrFeed Pro50S, diet 2 contained 42% Lentein and 15% soybean meal, diet 3 contained 23% soy protein concentrate and 20% soybean meal, and diet 4 contained 27% soy protein concentrate and 10% soybean meal. Feeds were stored in a cold room at 4 °C. These diets were not formulated to test each specific plant-based ingredient, but rather to test the effect of the overall

combination of ingredients on growth and feed efficiency. Proximate analyses of diets followed the standard Association of Official Analytical Chemists method (AOAC 2002) and are provided in Table 1. Fiber analysis for diet 2, 3 and 4 was carried out with Filter Bag Technology (ANKOM Technology, Macedon, NY) (AOAC 2002).

EXPERIMENTAL DESIGN

Two trials were conducted lasting each 56 days. Trial 1 began on June 14th and concluded on August 8th, while trial 2 began on August 15th and finished on October 9th. The same number of fish, cages and tanks were used for both trials. Each trial had 120 fish randomly selected from a larger cohort and placed in 12 floating cages of 47.7 L each which were evenly and randomly divided into two 3-ton flow-through tanks. Trial 1 was stocked with 9.29 ± 0.14 g/fish and diets 1 and 2 were tested, while trial 2 was stocked with 7.74 ± 0.25 g/fish and diets 3 and 4 were tested (Table 1). Flow rates were adjusted daily to 0.8 L/5s.

Temperature (°C) and oxygen (mg/l) were recorded daily at 07:00 hr and 16:30 hr using Hanna Instruments® Portable Galvanic Dissolved Oxygen Meter (HI9147). pH was recorded daily at 07:00 hr using Hanna Instruments® Waterproof Pocket pH Tester (HI98107). Alkalinity (ppm) was tested three times per week at 07:00 hr using Hanna Instruments® Seawater/Marine Alkalinity (dKH) Colorimeter (HI772). Salinity (ppt) was tested daily at 07:00 hr with Hanna Instruments® Marine Salinity Tester (HI98319).

FEEDING PROTOCOL

Trial 1 received four 1-hour feedings per day to visual satiation set to a maximum 5% body weight for each treatment. Trial 2 received two 1-hour feedings per day to visual satiation set to a maximum 3% body weight for each treatment.

SAMPLING

For both trials individual fish were weighed at the start (day 0) and at the end of the trial (day 56). During trial 1, group and individual weights were taken. Group weights were carried out on day 7, 21, 35 and 49, while individual weights on day 14, 28 and 42. During trial 2 only group weights were taken on day 14, 28 and 42. Whether group or individual weights, all fish and cages in each trial were weighed. The procedure for group weights was to temporarily move the fish from each cage to a small holding tank, they were subsequently removed from the tank with a dip net and excess water was removed with a cloth and placed in a container with water on a scale which was tared. Once weight was acquired, the fish were returned to their respective cage. The procedure for individual weights followed the same protocol as that of group weights but one fish at a time.

CALCULATION AND STATISTICAL ANALYSIS

Fish performance and feed utilization were evaluated according the following indices (Table 2):

1. Live weight gain (WG, g) = final live weight (Wf, g) – initial live weight (Wi, g)
2. Specific growth rate (SGR, %/day) = $[\text{Ln}(Wf) - \text{Ln}(Wi)]/\text{days} * 100$
3. Food conversion ratio (FCR) = feed distributed (g DM)/WG
4. Daily feeding rate (% biomass/day) = $[\text{feed distributed (g DM)/number of cages}/\text{biomass (g)}] * 100$
5. Protein efficiency ratio (PER) = WG/total protein fed (g DM)
6. Survival rate (%) = $[(\text{fish stocked initially} - \text{mortality})/\text{fish stocked initially}] * 100$

Statistical analysis was carried out using StatGraphics Centurion software (version 16.1). Data was subjected to one-way ANOVA followed by a Levene test for homogeneity of variance. Correlation between the treatments and the performance results was analyzed using Tukey's HSD post hoc test. A significance of $p = 0.05$ was considered for all analysis performed.

Table 1. Ingredient and proximate composition (%) of the control and four test diets

	Experimental Diets				
	Control	Trial 1		Trial 2	
		1	2	3	4
<i>Ingredient composition (%)</i>					
Soy Protein Concentrate, Profine	-	-	23	27	
Lentein	42	42	-	-	
Wheat, whole ground	18.72	17.59	19.24	19.09	
Soybean meal, solvent extracted	-	15	20	10	
MRFeed Pro50S	15	-	-	-	
Corn gluten meal	10.2	10.2	18.2	23	
Fish oil	5.5	6.9	11.7	12.65	
Mono-Dical Phosphate	2.8	2.8	2.8	2.8	
Lysine-HCL	2.58	2.56	2.11	2.43	
Vitamin Premix ARS 702	1	1	1	1	
DL- Methionine	0.7	0.65	0.65	0.73	
Threonine	0.7	0.5	0.5	0.5	
Choline CL	0.6	0.6	0.6	0.6	
Trace Min premix ARS 1520	0.1	0.1	0.1	0.1	
Stay-C	0.1	0.1	0.1	0.1	
<i>Proximate composition (%)</i>					
Dry Matter	-	92.2	90.62	96.37	95.22
Protein	30.88	34.9	33.1	45.5	46
Crude Fiber	3.6	16.5	14.9	8.9	6.9
Ash	5.49	6.65	5.99	6.63	5.76
Energy (kcal/kg)	3,416	980.72	1,674.62	3,815.73	3,952.98
Moisture	8.3	7.8	9.38	3.63	4.78
Fat	10.5	8.25	9.35	13.7	11.7

Table 2. Growth performance and feed utilization indices determined for juvenile rabbitfish fed control and experimental diets for two 56-day trials

	Dietary treatments							
	Trial 1 diets				Trial 2 diets			
	Control	1	2	<i>p</i> -value	Control	3	4	<i>p</i> -value
Initial live weight (g/fish)	9.26 ± 0.04	9.31 ± 0.07	9.30 ± 0.03		7.79 ± 0.10	7.70 ± 0.11	7.73 ± 0.04	
Final live weight (g/fish)	21.82 ± 3.07 ^a	20.05 ± 1.19 ^a	21.28 ± 1.75 ^a	0.5158	20.30 ± 0.35 ^a	30.70 ± 0.43 ^b	27.77 ± 0.08 ^c	0.0000
Live weight gain (g/fish)	12.56 ± 3.04 ^a	10.74 ± 1.18 ^a	11.98 ± 1.74 ^a	0.4947	12.51 ± 0.36 ^a	23.00 ± 0.40 ^b	20.04 ± 0.12 ^b	0.0000
SGR (% per day)	1.52 ± 0.25 ^a	1.37 ± 0.10 ^a	1.47 ± 0.14 ^a	0.4923	1.71 ± 0.04 ^a	2.47 ± 0.03 ^b	2.28 ± 0.01 ^c	0.0000
Total feed distributed (g/cage)	246.97 ± 25.84 ^a	210.30 ± 11.12 ^b	223.53 ± 14.16 ^{ab}	0.0461	190.98 ± 0.02 ^a	228.35 ± 0.00 ^a	221.66 ± 0.00 ^a	0.3883
FCR	2.02 ± 0.30 ^a	1.97 ± 0.12 ^a	1.88 ± 0.17 ^a	0.6391	1.53 ± 0.04 ^a	0.99 ± 0.02 ^a	1.11 ± 0.01 ^a	0.4353
PER	1.62 ± 0.23 ^a	1.35 ± 0.08 ^a	1.45 ± 0.13 ^a	0.1035	1.74 ± 0.05 ^a	2.67 ± 0.05 ^a	2.40 ± 0.01 ^a	0.1785
Feeding rate (% biomass/day)	3.75 ± 0.7	3.37 ± 0.53	3.41 ± 0.68		2.97 ± 0.00	2.97 ± 0.00	2.97 ± 0.00	
Survival rate (%)	100	100	100		100	100	100	

1= diet with 42% Lenten and 15% MrFeed Pro50S; 2: diet with 42% Lenten and 15% soybean meal, solvent extracted; 3: diet with 23% soy protein concentrate and 20% soybean meal, solvent extracted; 4: diet with 27% soy protein concentrate and 10% soybean meal, solven extracted. Values are means and standard deviation. Values with different superscripts letters are significantly different, $P \leq 0.05$ (one-way ANOVA).

Results

Water quality for both trials and for both tanks varied slightly during the course of the experiment. Mean temperature and dissolved oxygen for trial 1 was 29.64 ± 0.69 °C and 3.60 ± 0.67 mg/l respectively, and for trial 2 the parameters were 29.41 ± 0.76 °C and 3.52 ± 1.06 mg/l respectively. pH for trial 1 and trial 2 was 8.14 ± 0.18 and 8.09 ± 0.11 , respectively. Alkalinity for trial 1 and trial 2 was 119.42 ± 3.99 ppm and 112.86 ± 3.19 ppm, respectively. Salinity for trial 1 and trial 2 was 33.63 ± 0.36 ppt and 33.12 ± 0.16 ppt, respectively.

Trial 1 showed no significant difference ($p > 0.05$) in terms of growth (final weight, live weight gain and specific growth rate) and feed utilization (feed conversion ratio and protein efficiency ratio). There was a significant difference ($p < 0.05$) in feed consumption between the experimental diets and the control diet. Trial 2 showed significant difference ($p < 0.05$) in terms of growth (final weight, live weight gain and specific growth rate) and no significant difference ($p > 0.05$) in terms of feed utilization (feed conversion ratio and protein efficiency ratio) and consumption. Survival was 100% across all diets and trials (Table 2).

When looking at SGR and FCR of the four experimental diets, diets with high protein, lipid, and energy, and low fiber had better performance. Diets 3 and 4 with 45.5-46% protein, 11.7-13.7% lipid, 3,815-3,952 kcal/kg of energy, and 6.9-8.9% fiber retained better SGR and FCR than diets 1 and 2 which had 33.1-34.9% protein, 8.25-9.35% lipid, 980-1,674 kcal/kg of energy and 14.9-16.5% fiber.

Discussion

The genus of Siganidae, including *S. canaliculatus*, *S. lineatus*, *S. guttatus* and *S. fuscenscens*, is one of the few groups of herbivorous marine teleost that are cultured along with milkfish (*Chanos chanos*) and a variety of sea chubs (family Kyphosidae) (Xie et al. 2018). The interest in developing aquaculture of these species is mainly because herbivorous marine fish might require less to no fishmeal to be included in practical commercial diets as they naturally graze on algae (Monzer et al. 2017).

Rabbitfish inhabit the coastal areas of the Pacific Ocean, and naturally feed on a variety of macrophytes such as seagrass, rhodophyte, phaeophyte and chlorophyte (Anastasiades et al. 2011). Even though they feed on macroalgae in their natural environment, they have been found to readily accept artificial feeds once introduced in captive environments and begin behaving as omnivores (Abou-Daoud et al. 2014, Barakat et al. 2011). Pillans et al. (2004) looked at macrophyte choice by *Siganus fuscenscens* and found that when presented with a variety of macroalgae, the fish preferred *Acanthophora spicifera*. This choice was attributed to the fact that *A. spicifera* had the shortest gut transit time and had the highest amounts of assimilable biomass, energy, carbon, and nitrogen when compared to the other algae. Furthermore, the authors state that algal cell undergoes lysis by the highly acidic stomach environment of these herbivorous fish to allow access to the cell's contents. Additionally, selection of *A. spicifera* was also attributed to the fact that the cell walls of red algae are more easily degraded by *Siganus fuscenscens* than brown or seagrass algae (Horn 1989, Zemke-White et al. 2000, Lobel 1981).

Plant-based meals are being used more widely as fishmeal replacements to provide the necessary protein, but by utilizing these land-sourced ingredients there is the presence of abundant non-starch polysaccharides (NSP) which are indigestible for fish due to the lack of specific enzymes (Kuz'mina 1996). Studies by Refstie et al. (1999), Storebakken et al. (1999) and Allan et al. (2000) suggest that NSPs have a negative effect on digestion and absorption of energy and nutrients in Atlantic salmon (*Salmo salar*) and Australian silver perch (*Bidyanus bidyanus*). The addition of exogenous NSP-degrading enzymes in feeds formulated with plant-based ingredients has shown to improve nutrient utilization in Japanese seabass (*Lateolabrax japonicus*) and rohu (*Labeo rohita*) by

Ai *et al.* (2007) and Kumar *et al.* (2006). This is believed to be achieved by the successful degradation of the cell walls, reducing digesta viscosity, and/or stimulating gastrointestinal microbiota (Sinha *et al.* 2011). In a publication by Zhou *et al.* (2013), the authors found that algae *Enteromorpha prolifera* can be included up to 5% in diets of *Siganus canaliculatus* without negative effects, but if included at 10-15%, NSP enzymes become necessary to prevent adverse effects on growth, a theme that is in line with Ai *et al.* (2007) and Kumar *et al.* (2006). Similarly, as Zhou *et al.* (2013) states, Mountfort *et al.* (2002) hypothesize that herbivorous marine fish may have adapted to digest complex algal diets by elevated consumption and gut throughput rates, optimizing essential digestive enzymes, and reliance on microbial gut processes. Furthermore, Horn's (1989) hypothesis that herbivorous fish are able to gain energy and nutrients by lysing the algae cells in a highly acidic stomach, grinding the digested food in a muscular stomach or pharyngeal mill, or harboring symbiotic microbes that ferment the algae in a hindgut caecum is in line with what Pillans *et al.* (2003) conclude about *Siganus fuscuscens* feeding on *Acanthophora spicifera*.

It is imperative though to understand the differences in terrestrial and marine plants. Terrestrial animals utilize symbiotic organisms to break down plant cellulose and hemicellulose into simple compounds such as short-chain fatty acids which are assimilated by the host for energy generation and biosynthesis (Stevens & Hume 1995). On the other hand, algae are supported by water and they encompass a lower proportion of structural elements to cell contents (Choat *et al.* 1998). That being said, algae are significantly different in terms of chemical composition than terrestrial plants, and studies on herbivorous marine fish digestion seems to be deficient on accounting for the major differences in alimentary structure, the biochemical composition of the diet, and the inorganic composition of the gut contents (Choat *et al.* 1998, Painter, 1983).

Land-based meals such as soybean and copra cake are more readily available and cost-effective than their marine-based meal counterparts. Shiau *et al.* (1988) looked at replacing fish meal with soybean meal in milkfish (*Chanos chanos*) and found that diets with either 30 or 40% protein containing 100% soybean meal had significant negative effects on weight gain, feed conversion ratio and protein efficiency ratio than the control diet which contained fish meal ($p < 0.05$). Our study demonstrates that golden-lined rabbitfish (*Siganus lineatus*) fed on a diet with 42% protein containing 23% soy protein concentrate and 20% soybean meal to totally replace fish meal (Diet 3, Table 1) had a significant increase in weight gain ($p < 0.05$) with a noticeable yet not significant improvement in feed conversion ratio and protein efficiency ratio ($p > 0.05$). Monzer *et al.* (2017), when testing soybean meal replacement for marbled rabbitfish (*Siganus rivulatus*), found a negative correlation of survival, growth, feed, and protein efficiency with the incremental replacement of fish meal. The study being presented here shows that Diet 3 (42% protein with 23% soy protein concentrate and 20% soybean meal) and diet 4 (43.6% protein with 27% soy protein concentrate and 10% soybean meal), where fishmeal had been completely replaced, had a significant increase in weight gain ($p < 0.05$) with a noticeable yet not significant improvement in feed conversion ratio and protein efficiency ratio ($p > 0.05$) when compared to commercial control diet.

In trial 1 there was a significant difference in feed consumption between the control diet and the experimental diets 1 and 2 ($p < 0.05$). The reasoning behind this occurrence might be due to the daily feed rate being set to a maximum of 5% body weight per treatment, allowing the fish to satiate themselves. Fish fed the control diet (commercial milkfish feed) consumed $3.75 \pm 0.7\%$, while fish fed Diets 1 and 2 consumed 3.37 ± 0.53 and $3.41 \pm 0.68\%$ respectively. The higher consumption of the control diet might be due to fishmeal being present in the diet which has been found to have certain growth and attractant factors (FAO 2002).

Zhu *et al.* (2014) carried out a comprehensive experiment to determine optimum levels of protein and lipid for *S. guttatus*. The results indicated that a protein and lipid level of 42.49 and 6.33%, respectively, incurred the best growth. These results are partially in line with the results gathered from this study. The diets with the best performance had a protein level of 45.5-46%, and a

lipid level of 11.7-13.7%. The protein levels between our diets and those of Zhu et al. (2014) are somewhat similar, but the lipid levels are significantly different.

Meanwhile, our results are in complete contradiction to those by Wang et al. (2009) who investigated dietary protein and lipid in diets of *S. canaliculatus*. Wang et al. (2009) conclude that diets with 40% protein retained the worst performance (WG, PER, and FCR) when compared to the best performing diet with 32% protein. Additionally, diets with 3-9% lipid had a better WG and PER than the dietary groups with 12% lipid. The author finalizes that the optimum dietary protein and lipid levels for *S. canaliculatus* are 29.01-34.37% and 6-9%, respectively. The results from our study indicate that dietary protein and lipid levels of 45.5-46 and 11.7-13.7%, respectively, have better performance than diets with 33.1-34.9 and 8.25-9.35%, respectively.

Ghanawi et al. (2011) investigated the effects of dietary lipid levels on growth of *S. rivulatus* fed a diet containing either 2.5, 4.5, 6.5, 8.5, 10.5 or 12.5% lipid. The authors found that the fish fed the diet with the lowest lipid level had the lowest growth performance. There was an obvious trend of increased viscerosomatic index with increased dietary lipid. Additionally, they state that the suggested minimum dietary requirement for suitable growth of *S. rivulatus* juveniles is 40% protein when digestible energy of the diet is 3,821-4,299kcal/kg which is in close accordance with Diet 3 and 4 that had the best FCR and SGR. Results of our study are comparable to what Ghanawi et al. (2011) concluded in that the performance of the diets improved as the dietary lipid levels increased from 8.25 to 13.7%.

Results from our study may vary from others, but comparisons among species from various studies is difficult as the effect of dietary protein and lipid levels on fish growth performance vary considerably with species, size, age, diet formulation and composition, range of parameters being tested, and rearing conditions. It is possible that the differences in species, initial size and experimental conditions caused the large differences in results between these studies with different rabbitfish species.

CONCLUSION

This study was carried out to test practical diets for golden-lined rabbitfish (*Siganus lineatus*). The main purpose was not to test individual alternative ingredients to replace fish meal, but rather to test complete formulated feeds against the commercially used feed in Palau. A concise conclusion cannot be made for the performance of the individual protein ingredients of Letein, MrPro50s, soy protein concentrate or soybean meal. The resulting performance indices from this study might be more related to the combination of the ingredients of each feed rather than the individual protein component. Further studies with isocaloric, isolipidic and isoenergetic feeds are required to better understand the effects of the individual protein ingredients used in this study. Additionally, rabbitfish are herbivores that constantly graze on algae, so carrying out future feeding trials set in a local growout environment of floating ocean net pens might be a more commercially realistic scenario as the fish will graze on algae growing on the nets. The obtained results originated from an indoor, controlled and experimental setting where no available algae was present, meaning that growth and feed efficiency might be different than in the ocean net pens.

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References

- Abou-Daoud, Y., J. Ghanawi, M. Farran, D.A. Davis & I.P. Saoud. 2014. Effect of dietary protein level on growth performance and blood parameters of marbled spinefoot *Siganus rivulatus*. *Journal of Applied Aquaculture* 26: 103–118. doi.org/10.1080/10454438.2014.901077.
- Ai, Q.H., K.S. Mai, W.B. Zhang, W. Xu, B.P. Tan, C.X. Zhang & H. Li. T. 2007. Effects of exogenous enzymes (phytase, non- starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, *Lateolabrax japonicus*. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* 147: 502-508. <https://doi.org/10.1016/j.cbpa.2007.01.026>
- Allan, G.L., S. Parkinson, M.A. Booth, D.A.J. Stone, S.J. Rowland, J. Frances & R. Warner-Smith. 2000. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: Digestibility of alternative ingredients. *Aquaculture* 186: 293-310. [https://doi.org/10.1016/S0044-8486\(99\)00380-4](https://doi.org/10.1016/S0044-8486(99)00380-4)
- Anastasiades, G. 2011. Biodiversity in eastern Mediterranean marine aquaculture: An approach to new species. PhD dissertation, Univ. of Insubria, Varese, Italy.
- AOAC, 2002. Official methods of analysis, 13th Ed, Association of Official Analytical Chemists, Washington DC, USA
- Babikian, J., N. Nasser & I.P. Saoud. 2017. Effects of salinity on standard metabolic rate of juvenile marbled spinefoot (*Siganus rivulatus*). *Aquaculture Research* 48: 2561–2566. <https://doi.org/10.1111/are.13092>
- Baker, D.H. 2000. Nutritional constraints to use of soy products by animals. In J.K. Drackley (eds), *Soy in Animal Nutrition*, pp. 1-12. Federation of Animal Societies, Savoy, IL.
- Barakat, A., R. Roumieh, N.E. Abdel Meguid, J. Ghanawi & I.P. Saoud. 2011. Feed regimen affects growth, condition index, proximate analysis and myocyte ultrastructure of juvenile spinefoot rabbitfish *Siganus rivulatus*. *Aquaculture Nutrition* 17: 773–780. <https://doi.org/10.1111/j.1365-2095.2010.00847.x>
- Bueno, P.B. 2014. Sub-regional office for the Pacific Islands – Lessons from past and current aquaculture initiative in selected Pacific Island countries. FAO. <http://www.fao.org/3/a-i4139e.pdf>
- Choat, J.H. & K.D. Clements. 1998. Vertebrate herbivores in marine and terrestrial environments: a nutritional ecology perspective. *Annual Reviews Ecology and Systematics* 29: 375–403. <https://doi.org/10.1146/annurev.ecolsys.29.1.375>
- FAO. 2002. Use of fishmeal and fish oil in aquafeeds: further thoughts on the fishmeal trap. FAO Fisheries Circular No. 975. Rome. 61 pp.
- FAO. 2017. World Aquaculture 2015: A Brief Overview. FAO Fisheries and Aquaculture Circular No. 1140. Rome: FAO. <http://www.fao.org/3/a-i7546e.pdf>
- FAO. 2018. The State of World Fisheries and Aquaculture 2018: Meeting the Sustainable Development Goals. Rome. License: CC BY-NC-SA 3.0 IGO. <http://www.fao.org/3/i9540en/i9540en.pdf>
- Ghanawi, J., L. Roy, D.A. Davis & I.P. Saoud. 2011. Effects of dietary lipid levels on growth performance of marbled spinefoot rabbit fish *Siganus rivulatus*. *Aquaculture*, 310: 395–400. <https://doi.org/10.1016/j.aquaculture.2010.11.012>
- Henry, M., L. Gasco, G. Piccolo & E. Fountoulaki. 2015. Review on the use of insects in the diet of farmed fish: Past and future. *Animal Feed Science Technology* 203: 1-22. <https://doi.org/10.1016/j.anifeedsci.2015.03.001>

- Horn, M.H. 1989. Biology of marine herbivorous fishes. Oceanography and Marine Biology Annual Review. Aberdeen University Press/Allen & Unwin: London. ISSN 0078-3218; e-ISSN 2154-9125 27, 167–272.
- Jia, F.T., & W.N. Hing. 2017. Fish feed formulation using black soldier fly pre-pupae from fruit waste. *Journal of Engineering and Applied Sciences* 12: 6393-6397. <https://doi.org/10.3923/jeasci.2017.6393.6397>
- Kumar, S., N.P. Sahu, A.K. Pal, D. Choudhury & S.C. Mukherjee. 2006. Studies on digestibility and digestive enzyme activities in *Labeo rohita* (Hamilton) juveniles: effect of microbial α -amylase supplementation in non-gelatinized or gelatinized corn-based diet at two protein levels. *Fish Physiology and Biochemistry* 32: 209-220. <https://doi.org/10.1007/s10695-006-9002-z>
- Kuz'mina, V.V. 1996. Influence of age on digestive enzyme activity in some freshwater teleosts. *Aquaculture* 148: 25-37. [https://doi.org/10.1016/S0044-8486\(96\)01370-1](https://doi.org/10.1016/S0044-8486(96)01370-1)
- Lobel, P.S. 1981. Trophic biology of herbivorous reef fishes: alimentary pH and digestive capabilities. *Journal of Fish Biology* 19: 365–397. <https://doi.org/10.1111/j.1095-8649.1981.tb05842.x>
- Lock, E.R., T. Arsiwalla & R. Waagbø. 2015. Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt. *Aquaculture Nutrition* 22: 1202–1213. <https://doi.org/10.1111/anu.12343>
- Monzer, S., N. Nasser, J. Babikian & I.P. Saoud. 2017. Substitution of fish meal by soybean meal in diets for juvenile marbled spinefoot, *Siganus rivulatus*. *Journal of Applied Aquaculture* 29: 101–116. <https://doi.org/10.1080/10454438.2016.1272031>
- Mountfort, D.O., J. Campbell, D. Kendall & K.D. Clements. 2002. Hindgut Fermentation in Three Species of Marine Herbivorous Fish. *Applied and Environmental Microbiology* 68: 1374–1380. <https://doi.org/10.1128/AEM.68.3.1374>
- OECD & FAO. 2018. OECD-FAO Agricultural Outlook. https://doi.org/10.1787/agr_outlook-2018-en
- Painter, T.J. 1983. Algal polysaccharides. In G.O. Aspinall (eds), *The Polysaccharides*, pp 196-285. Academic Press, London, United Kingdom.
- Pillans, R. D., C.E. Franklin & I.R. Tibbetts. 2004. Food choice in *Siganus fuscescens*: Influence of macrophyte nutrient content and availability. *Journal of Fish Biology* 64: 297–309. <https://doi.org/10.1111/j.0022-1112.2004.00261.x>
- Refstie S., B. Svihus, K.D. Shearer & T. Storebakken. 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soybean products. *Animal Feed Science Technology* 79: 331-345.
- Shiau, S.Y, B.S, Pan, S. Chen, H.L. Yu, & S.L Lin. 1988. Successful Use of Soybean Meal with a Methionine Supplement to Replace Fish Meal in Diets Fed to Milkfish *Chanos chanos* Forskal. *Journal of the World Aquaculture Society* 19: 14–19. <https://doi.org/10.1111/j.1749-7345.1988.tb00448.x>
- Sinha A.K., V. Kumar, H.P.S. Makkar, G. De Boeck & K. Becker. 2011. Non-starch polysaccharides and their role in fish nutrition: A review. *Critical Review in Food Science and Nutrition* 127: 1409-1426. <https://doi.org/10.1016/j.foodchem.2011.02.042>
- Stevens, C. E. & I.D. Hume. 1995. *Comparative physiology of the vertebrate digestive system*. 2nd ed, Cambridge University Press, Cambridge, United Kingdom
- Storebakken T, I.S. Kvien, K.D. Shearer, B. Grisdale-Helland & S.J. Helland. 1999. Estimation of gastrointestinal evacuation rate in Atlantic salmon (*Salmo salar*) using inert markers and collection of faeces by sieving: evacuation of diets with fish meal, soybean meal or bacterial meal. *Aquaculture* 172: 291-299. [https://doi.org/10.1016/S0044-8486\(98\)00501-8](https://doi.org/10.1016/S0044-8486(98)00501-8)
- Wilson, R.P. 2002. *Protein and Amino Acids*. 3rd ed, Elsevier, Amsterdam, Netherlands

- Woodland, D.J. 1990. Revision of the fish family Siganidae with descriptions of two new species and comments on distribution and biology. Bernice Pauahi Bishop Museum, Honolulu, Hawaii. ISBN: 0646116657
- Xie, D., S. Xu, Q. Wu, F. Chen, S. Wang, C. You & Y. Li. 2018. Changes of visceral properties and digestive enzymes in the herbivorous marine teleost *Siganus canaliculatus* fed on different diets. *Acta Oceanologica Sinica* 37: 85–93. <https://doi.org/10.1007/s13131-018-1165-9>
- Zemke-White, W.L., K.D. Clements & P.J. Harris. 2000. Acid lysis of macroalgae by marine herbivorous fishes: effects of acid pH on cell wall porosity. *Journal of Experimental Marine Biology and Ecology* 245: 57–68. [https://doi.org/10.1016/S0022-0981\(99\)00151-3](https://doi.org/10.1016/S0022-0981(99)00151-3)
- Zhou, S., C. You, S. Wang & Y. Li. 2013. Effects of dietary seaweed *Enteromorpha prolifera* on growth performance, physiological and biochemical characteristics of rabbitfish *Siganus canaliculatus*. *Journal of Fishery Sciences of China* 20: 1257–1265. <https://doi.org/10.3724/sp.j.1118.2013.01257>

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