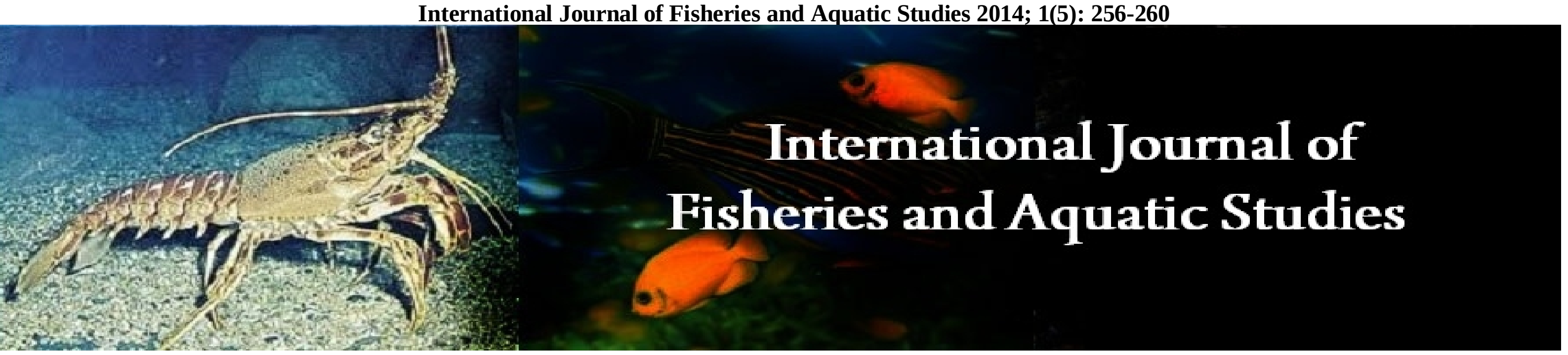

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Replacement of fish meal in cobia *(Rachycentron canadum)* diet with squid waste and squid waste silage

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Abstract
The effects of squid waste and squid waste silage feeds on the growth and feed utilization in the juveniles of cobia, *Rachycentron canadum* were investigated. There was significant decrease in protein and increase in lipid and ash ($P<0.05$) in the squid waste silage than that of squid waste. Five isonitrogenous (45% protein) and isolipidic (15% lipid) experimental diets were formulated incorporating squid waste (SW) and squid waste silage (SWS) at 50 and 100% dietary protein (SW 50, SW 100, SWS 50 and SWS 100) respectively. A control feed without the incorporation of squid waste and squid waste silage was also prepared. Each diet was fed to three replicate groups of fish with an initial weight 10.71 – 11.23 g for 45 days. The fishes fed all diets had similar ($P>0.05$) mean weight gain (53.44-60.28g) and SGR (3.87 – 4.14%). The fishes fed control and SW 50 had similar and best FCR (1.98-1.99), PER (1.12) and mean feed intake (106.50 – 119.65 g) which differed significantly ($P<0.05$) from SW 100, SWS 50 and SWS 100 diets. The HSI was similar (2.00 – 2.20) in fishes fed all squid waste and squid waste silage based diets which was significantly ($P<0.05$) higher than the fish meal based diet (control). There was no significant difference observed in survival between treatments. The whole body composition of cobia fed the squid waste and squid waste silage diets did not show any variations in moisture, protein, lipid and ash. The present study indicated that fish meal can be replaced up to 100% by squid waste and squid waste silage based diets without any adverse effect on the growth, feed utilization and body composition of cobia.

Keywords: *Rachycentron canadum*, squid waste.

1. Introduction

The Cobia, *Rachycentron canadum* (Linnaeus, 1766) is a marine pelagic carnivorous fish distributed in tropical, subtropical and warm temperate seas .Cobia is the only species in the family *Rachycentridae* and commonly called as black kingfish, black salmon, ling, lemonfish and crab eater. They are delicious food fish highly preferred by Asian consumers, especially in Taiwan and Japan. The belly is especially highly valued, having high lipid content, and is often served in restaurants as raw fish [1]. It can grow to a maximum size of 2 m length and 68 kg weight and has the life span of 15 years [2] .Cobia, *Rachycentron canadum* is emerging globally as a potential species for mariculture. Positive culture aspects include capacity to spawn both naturally and through artificial induction in captivity, rapid growth rates in excess of 6 kg per year, high disease resistance, adaptability to a variety of culture and tank conditions, adaptability to commercially available extruded diets and high quality fillets suitable for sashimi as well as white table cloth restaurant markets[3] . Globally, the culture of cobia began in earnest in Taiwan during 1993. At present, in addition to trash fish, commercial feeds based on formulas similar to Asian sea bass or grouper feeds are also used which results in acceptable growth in cobia net cage culture with feed conversion ratios ranging from 1.5 to 1.8 [4]. However, cost-effective feeds to maximize growth and health of this species are yet to be developed. The fish meal replacement studies carried out in cobia is very much encouraging for developing less cost feed. The weight gain, feed conversion ratio, protein efficiency ratio, net protein utilization were good when the replacement of fishmeal with soybean meal was at 40% [4]. It was reported that up to 50% of the fish meal can be replaced with an organic protein source without detrimental impacts on cobia weight gain or feed efficiency [5].

Yeast-based organically certified protein was used as a replacement for fish meal in cobia diet at the levels of 25%, 50%, 75%, and 100% [6]. The replacement of 25% fish meal with yeast-based protein source resulted in similar weight gain and feed efficiency ratio obtained in fish meal diet. Complete replacement of fish meal in the grow out diet of cobia is possible with plant protein. Sources if taurine is added [7]. So far, no work has been carried out utilizing squid waste silage as an alternative feed protein ingredient for cultivable fishes. Therefore, this research was undertaken to evaluate squid waste silage as feed ingredient in cobia.

2. Materials and Methods

2.1. Preparation of squid waste powder and squid waste silage by acid

Squid waste comprised of head, body cuttings and skin were purchased from J.A. Dryfish Company, Tharuvaikulam, Thoothukudi district. They were sundried, grounded well in laboratory pulverizer, seived through a 0.3 mm mesh and used as ingredient in feed formulation for cobia. Squid waste silage was prepared by ensiling squid waste (head, cuttings and skin) with 3% formic acid (Merck, Mumbai). Then the mixtures were stored in 10 litre plastic drums at room temperature (30±2 ºC). The daily pH was measured using pH meter (Cyperscan pH 1100, EUTECH-Instruments, Singapore). The initial pH was 6.2 and then on fourth day, the pH has come down to 3.8. Squid waste silage were sun dried, grounded well in laboratory pulverizer and seived through a 0.3 mm mesh. The proximate composition of the squid waste silage powder was analyzed using standard analytical methods [8].

2.2. Preparation of experimental feeds for cobia

The proximate composition of squid waste and squid waste silage are presented in Table 1. Ingredients and proximate composition of experimental feeds are presented in Table 2 and 3. Five isonitrogenous (45% protein) and isolipidic (15% lipid) experimental diets were formulated. A feed without incorporation of squid waste and squid waste silage was used as the control feed (C). The other four feeds were formulated incorporating squid waste and squid waste silage at 50% and 100% of dietary protein respectively. The squid waste incorporated diets are denoted as SW50 and SW100 and squid waste silage incorporated diets are denoted as SWS50 and SWS100.The feed ingredients were seived through 350 µm die, homogeneously mixed and then made into dough with the addition of water. The dough was cooked in a steam cooker for 20 min. and extruded in a noodle making machine through 2 mm die. The resulted pellets were dried at 60 ºC for 12 hrs and stored in air-tight container.

Table 1: Proximate composition of squid waste and squid waste silage (%dry weight)

Ingredients	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Fibre (%)	NFE (%)	Gross Energy (Kcal/g)
Squid waste	12.03	65.11	6.72	8.55	1.00	6.59	4617
Squid waste silage	10.23	58.81	6.79	12.50	1.84	9.83	4431

Table 2: Ingredients composition of squid waste and squid waste silage incorporated feeds for cobia, *Rachycentron canadum*

Ingredients (%)	Control	SW 50	SW 100	SWS 50	SWS 100
Fish meal (Sardine)	70.0	35	-	35	-
Squid waste	-	28.6	57.10	-	-
Squid waste silage	-	-	-	31.6	63.2
Soybean meal	17.65	16.47	15.57	17.2	16.75
Corn meal	0.19	6.65	12.65	2.9	5.61
Cod liver oil	8.16	9.28	10.68	9.3	10.44
Vitamin mineral mix	2.0	2.0	2.0	2.0	2.0
Carboxy methyl cellulose	2.0	2.0	2.0	2.0	2.0

Table 3: Proximate composition of feeds used for cobia, *Rachycentron canadum*

Parameters (%)	Control	SW 50	SW 100	SWS 50	SWS 100
Moisture	5.92	7.6	9.27	7.81	9.69
Protein	44.98	44.95	44.96	44.98	44.98
Lipid	14.98	14.99	14.99	14.99	14.98
Ash	18.71	12.21	5.95	13.7	8.94
Fibre	1.23	1.2	1.19	1.46	1.69
NFE	29.16	19.05	23.64	17.06	19.72
Gross Energy (K cal/g)	3589.58	3780.81	3752.22	3693.62	3797.59

2.3. Growth experiment for cobia

Cobia seeds purchased from Rajiv Gandhi Centre for Aquaculture (RGCA) Pozhiyur, Kerala were maintained in 5000 liter capacity FRP tank at Maritech Research and Extension Centre, Tharuvaikulam, Thoothukudi district. After 1 month, the fishes were transferred to the experimental system and acclimated for two weeks before start of the experiment. The growth experiment for cobia was conducted at Maritech Research and Extension Centre, Tharuvaikulam. Five groups of 10 cobia juveniles (mean initial weight of 10.71-11.23 g) were stocked in each 300 liter capacity FRP circular tanks filled with 250 litre seawater and adapted to a new condition for two weeks fed with grouper feed. Growth experiment was conducted with five diets viz., squid waste 50% (SW50),squid waste 100% (SW100),squid waste silage 50% (SWS50),squid waste silage 100% (SWS100) along with control. Three replicate group of fishes were maintained for each treatment. The cobia juveniles were fed twice daily at 10.00 am and 5.00 pm at a rate of 5 % of body weight per day for 45 days. The fishes were weighed every fifteenth day and the amount of feed adjusted accordingly. The faecal matter was removed every day. The uneaten feed was collected daily by siphoning 2 hr after each feeding, dried in a hot air oven at 60 ºC and weighed for evaluating growth parameters.

2.4. Proximate analyses
The moisture, crude protein, lipid, ash, fiber, nitrogen free extract (NFE) and gross energy in the squid waste, squid waste silage, squid waste incorporated diets, squid waste silage incorporated diets and whole body of cobia was analyzed according to standard procedure [8]. Moisture was determined by oven drying at 105 ºC-110 ºC for 6 hrs and protein by Micro kjeldhal method after acid digestion. Lipid was determined by Soxhlet's method by extracting in ether which is continuously volatilized at 60-80 ºC.Crude fiber was estimated by dried fat free residues after digestion with dilute acid (0.255N) and alkali (0.313N).Ash was determined by ignition at 600 ºC for 6 hrs in a muffle furnace. The gross energy (GE) was estimated using Digital Bomb Calorimeter (Model. No. RSB, Rajdhani Scientific Inst. Co. New Delhi, India).The nitrogen free extract (NFE) was calculated using the following formula.

NFE= [100-(crude protein+crude lipid+ash+crude fiber+moisture)]

2.5. Water quality analyses
The samples for water quality parameters were taken at fortnight intervals prior to water exchange between 10.00 to 10.30 am. The temperature, dissolved oxygen, hardness, alkalinity, ammonia, nitrite and nitrate, were recorded [9]. The pH and salinity were measured by pH meter (1100, EUTECH-Instruments, Singapore) and Refractometer (HANNA, Taiwan,).

2.6. Statistical analyses
One way analysis of variance (ANOVA) was carried out to find out whether there is any significant difference among the growth related parameters in growth experiment [10].

3. Results
3.1. Proximate composition of squid waste silage
The lipid, fibre, ash and nitrogen free extract of the squid waste silage were significantly increased than that of squid waste. There was significant increase in ash level of (8.55% to 12.50%), nitrogen free extract from (6.59% to 9.83%) and fibre level from (1.00% to 1.84).There was significant decrease in protein level of squid waste silage than that of squid waste. The protein content was significantly decreased from 65.11 to 58.81%. The gross energy also decreased in squid waste silage (4431 kcal/g) while compared to squid waste (4617 Kcal/g). Significant difference ($P<0.05$) in proximate composition between the cobia fed on squid waste and squid waste silage was observed.

3.2. Effect of squid waste and squid waste silage incorporated diets for cobia
The result for the growth related parameters of *Rachycentron canadum* fed the diets supplemented with squid waste and squid waste silage for duration of 45 days is presented in Table 4. Cobia fed on SW50 diet attained maximum weight gain of 60.28 g followed by SWS100 (56.77 g).

Table 4: Growth performance and feed utilization of juvenile cobia, *Rachycentron canadum* fed with experimental diets for 45 days

Treatment	Mean initial weight (g)	Mean final weight (g)	Mean weight gain (g)	SGR	PER	FCR	Mean feed intake(g)	HSI	Survival (%)
Control	10.78±0.0989	64.55±1.1849	53.77±1.3859	3.97±0.0283	1.12a±0.0240	1.98a±0.0424	106.50a±5.0275	1.89a±0.0919	100.00
SW50	11.04±0.1626	71.31±1.6829	60.28±1.8455	4.14±0.0848	1.12a±0.0042	1.99a±0.0070	119.65a±4.0870	2.00b±0.1414	100.00
SW100	11.08±0.2213	67.84±1.5697	56.77±1.7889	4.03±0.0919	0.87b±0.0091	2.55b±0.0282	140.91b±2.4465	2.15b±0.0707	95.00±7.0710
SWS50	11.20±0.0494	64.64±6.3427	53.44±6.2932	3.87±0.1838	0.86b±0.0070	2.56b±0.0212	134.68b±9.6378	2.18b±0.0353	95.00±7.0710
SWS100	10.95±0.0353	65.82±6.0881	54.87±6.1235	3.98±0.2121	0.85b±0.0502	2.61b±0.1555	140.36b±29.2176	2.20b±0.1414	95.00±7.0710

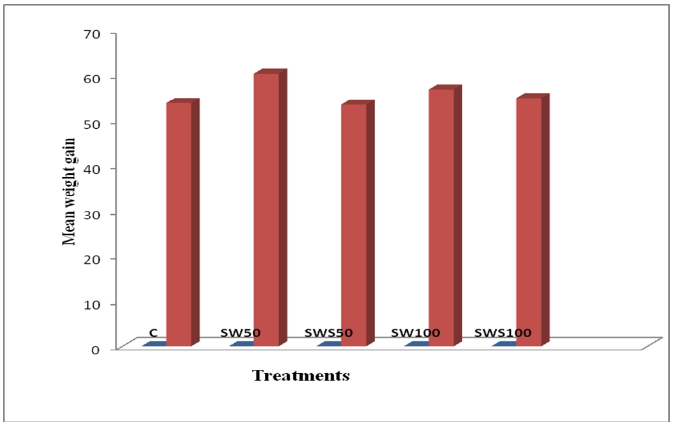

Fig 1: Mean weight gain of cobia fed with squid waste and squid waste silage based diets for 45 days

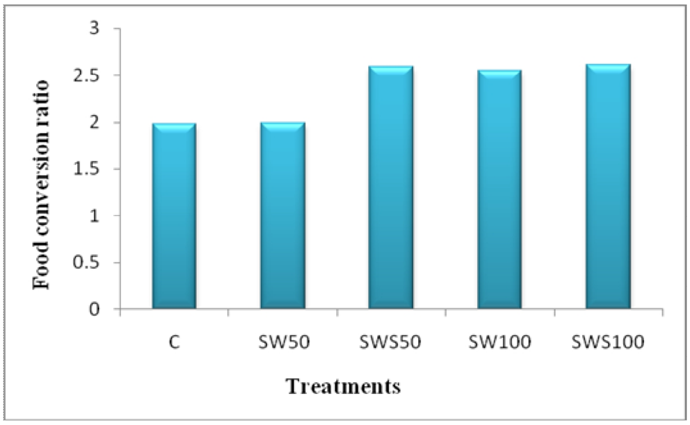

Fig 2: Food conversion ratio of cobia fed with squid waste and squid waste silage based diets for 45 days

The minimum weight gain was observed in cobia fed with SWS50 diet (Fig.1). However no significant difference ($P>0.05$) in mean weight gain was recorded between the cobia fed on squid waste and squid waste incorporated diets.
The highest specific growth rate (SGR) of 4.14 was observed in cobia fed on SW50 diet and the lowest SGR (3.87) was recorded in cobia fed on SWS 50 diet. No Significant difference ($P>0.05$) in specific growth rate was observed between the cobia fed with squid waste and squid waste incorporated diets. The mean feed intake was maximum in cobia fed SW100 diet (140.91 g) and SWS100 (140.36 g). Minimum mean feed intake was observed in cobia fed control diet. Significant difference ($P<0.05$) in mean weight gain was observed between the cobia fed with squid waste and squid waste incorporated diets. The cobia fed on SWS50 and control diets showed good food conversion ratio (FCR) of 1.99 and 1.98.The cobia fed on SWS100 showed poor FCR. Significant difference ($P<0.05$) in food conversion ratio was observed between the cobia fed with squid waste and squid waste incorporated diets (Fig. 2).

3.3. Effect of squid waste and squid waste silage on the body composition (% wet weight) of cobia

The moisture content of cobia fed on SW50 diet showed higher value of 74.18%.The protein content was higher (21.80%) in the cobia fed with SWS50 diet. The lipid content was higher (2.19) in cobia fed with control diet. The ash content was higher (1.92) in cobia fed with SW100 diet. No significant difference ($P>0.05$) in moisture, protein, lipid and ash was observed between squid waste and squid waste silage incorporated diets.

4. Discussion

4.1. Proximate composition of squid waste silage

The ash content of squid waste was 8.55%. There was significance increase in ash content of squid waste silage (12.50%). Similarly significant increase in crude fibre from 1.0 to 1.8 in squid waste silage was observed. In acid fish silage an increase in ash content when compared to raw fish waste was reported [11]. In the present study, the protein contents of the squid waste silage showed significant decrease from 65.11 to 58.81%. Silage protein was lower than the protein content of the raw materials comparable with other reports [12, 13]. Acid preserved Atlantic salmon residue showed diminished protein levels while compared to fresh residue [14]. During storage, the moisture level of silage reduced and ash and lipid content was stable in condition [15].

4.2. Growth parameters in cobia

Various researchers have reported that when feeding carnivorous fish, fish feed can be substituted with other protein sources and this produced different results with regard to the growth of the fish and the feed utilization [16, 17, 18, 19]. In the present investigation the mean weight gain and specific growth rate were similar and there was no significant difference between the squid waste, squid waste silage and control diets. This study was consistent with other studies reported in Rainbow trout (95-135g) [20] and Atlantic Salmon (320-1400g) [21, 22, 23] where fish fed 400-500 kg^{-1} silage based diets showed no differences in weight gain compared to fish fed fish meal or raw fish based diets. It was reported that there was no significant difference among the raw fish and fish silage based diets in weight gain, feed efficiency and protein efficiency ratio [24]. In a study carried out on dry pellet feed and silage, the authors reported that the best growth was in the group fed with commercial feed and 50% silage [22]. Young eels fed with fish silage obtained from sardines showed best growth in the group fed with feed containing 15 to 20% silage [16]. On the contrary, it was reported that lower weight gain and feed efficiency were achieved by the cobia groups fed silage based diets; particularly the fish fed the crab silage and mixed fish/crab silage diets [25]. The growth rate was higher in cobia fed the diet without added silage than in fish fed the diet with 130 gkg^{-1} added silage, while no significant differences in PER and FCR between them were observed[15].The FCR and PER were best in fishes fed control and SW50 diets. They were significantly different from SW100, SWS50 and SWS100 diets. The feed intake was higher in fishes fed SW100, SWS50 and SWS100 diets. SW100 contained higher level of squid waste and SWS50 and SWS100 contained squid waste silage. The non-performance of squid waste silage diets indicates that utilization of the feed ingredients must be poorer. This suggests that protein from the silage based diets could be of higher digestibility but possess lower utilization for growth which is consistent with [23, 24] studies. The HSI values of the present study indicated that the squid waste and squid waste silage fed fishes did not show significant difference among them. The study on silage based diets showed that lower levels of HSI in silage based diets and it was suggested it might be that formic acid was probably responsible for the liver stress ultimately caused reduced appetite, digestive efficiency and depression in weight gain [24]. In the present study, squid waste silage was used and since the silage product is completely different, formic acid might not have stressed the liver of cobia.

4.3. Muscle composition of cobia

There was no significant difference in the protein, lipid and ash levels of whole body composition of cobia among the squid waste and squid waste silage based feeds. This is in agreement with the study conducted by Mach *et al.* (2010) [25] in cobia using raw fish / crab and fish/crab silage diets. The protein and ash content of the whole body of rainbow trout did not change [16, 26]. However the lipid content increased in proportion to the percentage of silage used in rainbow trout [26].
The cobia juveniles fed the fish meal based diet and diets completely replaced with both squid waste meal and squid waste resulted in similar growth and did not show significant variations. There was no adverse effect observed in feed utilization and body composition of cobia. Therefore, it is concluded that squid waste and squid waste silage could be used as complete fish meal replacer in the diets of cobia juveniles. The cobia growers can take the advantage of this research where sea food processing industries are more.

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