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Artificial feed development through fishmeal replacement with non conventional feed stuff for mud crab (*Scylla serrata*) fattening

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Abstract
A feeding trial was set to investigate the potential of selected plant and animal-offal based ingredients to reduce the fishmeal content in grow out diets formulated for mud crab fattening. Ten isonitrogenous feeds, five for each of animal protein (AP) and plant protein (PP) based diets were prepared while tilAPia was used as control. Feeding trial was conducted for 120 days and feeds were APPlied at the rate of 5% body weight. Significantly highest (p<0.05) net benefit was gained with AP based feed of AP_2 followed by AP_3 and AP_1 at Tk. 63.14, 56.06 and 52.28 respectively. Survival rate of female was much higher in the control (100%), compared to AP (96.30%) and PP (83.1%) based feeds. The body protein contents of crabs under feeding trial were higher than wild crabs although differences were insignificant. The result might be useful to reduce the feed cost and dependency on trash fish in fattening process.

Keywords: Artificial feed, fishmeal, non conventional feed stuff, mud crab, fattening

1. Introduction
Mud crab (*Scylla serrata*), belonging to the family Portunidae and class crustacea under the phylum arthropoda [1], is one of the important fishery commodities in the coastal region of Bangladesh. Although, shrimp is still the top listed aquatic product of this country but disease problem especially WSSV (White Spot Syndrome Virus) in shrimp aquaculture has posed a serious threat to the public livelihood and growth of brackish water aquaculture which promotes people seeking for crab culture as an alternative livelihood option because of its larger size, capacity to live out of water for a long period, compatibility with other species like shrimp, mullets and milk fish, high meat quality and nutritive value [2, 3, 4]. To increase the market value of female mud crab, fattening (female crabs are raised for a certain time for gonadal development) of wild caught crablets are still popular in Bangladesh. During these processes crabs are fed with different types of feed like trash fish, snail, wheat, boiled rice, commercial prawn and shrimp feed etc in excessive amounts which ultimately contaminate the rearing water and lead to high mortality rates [5, 6]. Therefore, the possible expansion of crab farming in Bangladesh would greatly depend on the development of artificial feed that can be produced from selected available raw materials. Moreover, the available commercial feeds are mostly based on fish meal that are getting scarce day by day resulting in price hike of the crab feed [7]. Therefore, searching alternate protein source has become so crucial. Regarding this, plant-based and animal-based proteins have been used in many aquafeeds. Chen (1994) [8] and Luo (2011) [9] found that 33-40% fish meal protein could be replaced by soybean cake and rapeseed meal mixture (1:1 ratio) for crab without reducing growth. As because,these plant protein sources are high in anti-nutritional and antigenic factors including protease inhibitors, oligosaccharides (e.g., stachyose, raffinose), sAPonins, isoflavones, phytate, and tannins [10]. Similarly, poultry offal as animal protein source can subsidize fish meal in crab feed as it contains high protein [11]. Bhaskar (2014) [12] found that the protein and lipid contents were 60.67% and 12.05% respectively in poultry viscera which can be used as an alternative source of fish meal. However, the degradability and digestibility of poultry offalis lower than soybean meal but higher than that of meat and bone meal [13, 14]. Considering the nutritional value of soy bean meal (SBM) and poultry offal (PO) the current study was conducted to develop low cost alternative feed for mud crab using plant and

animal based protein as an alternate of fish meal and evaluate the growth performance through fattening process in cages.

2. Materials and methods

2.1 Experimental design

The study was conducted at Rampal upazila of Bagerhat district, Bangladesh. The area is under the influence of Sundarban along the coastal region where climate induced natural disaster hamper the agricultural activities very frequently. Ten experimental feeds under two major groups namely animal protein (AP) and plant protein (PP) based feeds were prepared and trials conducted for female (F) crab with 3 replications each along with one conventional trial using trash fish as control (Table 1).The trials were carried out in separate experimental cages set into the farmer's pond at Rampal Upazila, Bagerhat district.

Table 1: Number of cages under different treatments (feed types) for female crab (F)

Feed-PP_1	Feed-PP_2	Feed-PP_3	Feed-PP_4	Feed-PP_5	Feed-AP_1	Feed-AP_2	Feed-AP_3	Feed-AP_4	Feed-AP_5	Control (Trash fish)
F	F	F	F	F	F	F	F	F	F	F
3	3	3	3	3	3	3	3	3	3	3
Total	33 cages									

2.2 Feed formulation

Feeds were formulated from locally available feed ingredients following Pearson's technique. Proximate analysis of the ingredients and formulated feed was done in the Fish nutrition laboratory, Faculty of Fisheries, Bangladesh Agricultural University.

2.2.1 Animal protein based (AP) and Plant protein based (PP) feed formulation

AP feed was formulated through progressive replacement of fishmeal (FM) with poultry offal (PO) as a substitute of animal protein along with other ingredients. The poultry viscera was collected, processed and dried and used along with the other feed ingredients (Table 2). In contrast, the PP feed was formulated in the same way where fishmeal was replaced with soya bean meal (SBM) instead of PO as a substitute of plant protein along with other ingredients (Table 3). An extruder pellet machine with 3mm dies was used to formulate pellet feed. The pellets were dried at 50°C in a drying oven, overnight to remove the excess moisture. The prepared feed was tested with crab placed in cages set in the farmer's pond and put individual crab in each chamber to avoid fighting for the feed and cannibalism.

2.3 fattening of crab

Experimental cages were set in the selected farmers' ponds where the cages were provided with shade of palm leaves, bamboo and wooden poles to prevent direct sun exposure. Immature healthy crabs (100-150g or more) were procured locally when available and stocked with one female crab per plastic cage (19.5cm x 28cm x 22cm). The carapace length, width and weight were recorded before release. Then the crabs were assigned randomly into ten groups and housed individually in the cages until the female become gravid. The crabs were fed experimental diets twice daily (at 9:00 am and 5:00 pm) at a feeding rate of 5% body weight (BW) per day which was subsequently adjusted according their demand. Growth performance in terms of carapace length and width, weight gain, mortality were recorded at weekly interval whereas with the water quality parameters such as temperature, dissolved oxygen (DO), pH, salinity, nitrite and nitrate was measured fortnightly using hand held oxygen and pH meter and salinity refract meter. Other parameters like nitrite, nitrate and ammonia were measured using water testing reagent in the laboratory. The crab was harvested and sold in the local market immediately after maturation. Carapace length and width, weight and days required were recorded prior to sale the crab.

2.4 Data analysis

All the collected data entered into the Microsoft Excel and Xlstat (version 2013) analytical tools was used to perform statistical analysis like ANOVA, average and significance level was determined at $p>0.05$. Duncan Multiple Range Test (DMRT) of the differences between the categories with a confidence interval of 95% was carried out for plant protein and animal protein based feed and water quality to see the significant level with the data obtained.

Table 2: Dietary Inclusion rate in animal protein based (AP) feed, where 100, 75, 50, 25 and 0% of poultry offal were replaced with 0, 25, 50, 75 and 100% fish meal respectively

Ingredients	100% poultry offal and 0% fishmeal (AP1)	75% poultry offal and 25% fishmeal (AP2)	50% poultry offal and 50% fishmeal (AP3)	25% poultry offal and 75% fishmeal (AP4)	0% poultry offal and 100% fishmeal (AP5)
	Amount (g)	Amount (g)	Amount (g)	Amount (g)	Amount (g)
Poultry offal	39	29.25	19.50	9.75	0
Fish meal	0	10	20	30	40
Wheat bran	12	11.75	3.5	4	9
Mustard oil cake	20	21	22	23	24
Rice bran	24	23	30	28.25	22
Starch	3	3	3	3	3
Binder	1	1	1	1	1
Mineral	0.5	0.5	0.5	0.5	0.5
Vitamin	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Table 3: Dietary Inclusion rate in plant protein based (PP) feed, where 100, 75, 50, 25 and 0% of fish meal were replaced with 0, 25, 50, 75 and 100% soya meal respectively

Ingredients	100% fish meal and 0% soy meal (PP1)	75% fish meal and 25% soy meal (PP2)	50% fish meal and 50% soy meal (PP3)	25% fish meal and 75% soy meal (PP4)	0% fish meal and 100% soy meal (PP5)
	Amount (g)	Amount (g)	Amount (g)	Amount (g)	Amount (g)
Fish meal	52	26.25	17.5	8.75	0
Soybean	0	15	30	45	60
Mustard oil cake	13	42	36	30	24
Wheat bran	16	6	6	6	6
Rice bran	14	5.75	5.5	5.25	5
Starch	3	3	3	3	3
Binder	1	1	1	1	1
Mineral	0.5	0.5	0.5	0.5	0.5
Vitamin	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Table 4: Proximate composition of formulated feed

Name of items	Moisture (%)	Crude Lipid (%)	Crude Protein (%)	Ash (%)	Crude Fiber (%)	Carbohydrate (%)
AP1	10.15	8.1	28	12.08	5.1	36.57
AP2	8.44	7.85	29.4	12.79	4.9	36.62
AP3	7.17	9.46	31.5	15.24	4.4	31.83
AP4	8.47	8.94	31.5	16.57	4.4	30.12
AP5	8.2	8.2	32.2	17.78	4.5	29.12
PP1	9.63	8.56	32.9	16.08	5.2	27.63
PP2	6.79	9.4	37.8	12.35	3.8	29.86
PP3	6.88	9.1	35	11.19	3.9	33.93
PP4	8.73	8.88	35.7	10.11	4.7	31.88
PP5	8.41	9.35	35	8.93	4.6	33.71

3. Results and discussion

3.1 Performance of female crabs fed plant and animal protein based feed

While feeding animal protein (AP) and plant protein (PP) based feed to the crab under fattening, higher weight gain and gonad development were observed with the AP than the PP based feeds. The highest average weight gain was obtained with AP_3 in 23.0 days among the ten feeds along with the control, followed by AP_4 in 23.33 days. On the other hand, fewer days (16.33) were required with the control to weight gain of 8.56 g. However, while feeding plant protein based feed to the female the highest weight (8.33g) was obtained with PP_5 and PP_4 in 23.63 days and 24.00 days respectively. The highest feed cost was BDT (Bangladeshi Taka) 54.79 Per kg found in PP_1 (100% fishmeal with 0% soya bean meal) followed by AP_5 (BDT 49.24) where 100% fishmeal and 0% poultry offal was used. Moreover, the lowest crab weight gain (6.50g) was with PP_2 followed by AP_2 (7.0g) in 26.88 and 23.0 days respectively. On the other hand, significantly highest (p<0.05) net benefit was gained with AP based feed in particular AP_2 followed by AP_3 and AP_1 of BDT 63.14, 56.06 and 52.28 respectively. In contrast, significantly higher feed cost per cycle of crab production required with PP_1 followed by PP_2, PP_4, PP_3 and PP_5 of BDT 76.54, 68.25, 64.01, 61.91 and 58.61 respectively. The lowest net benefit was also attained with PP based feed of PP_5 followed by PP_2 and PP_3 of BDT 28.85, 31.96 and 34.13 respectively. Table 5 showed the weight gain, per kg feed cost, net benefit and per g crab production cost for female crab rearing in cages with plant and animal offal based feed. Moreover, per g female crab production cost was significantly lower with AP_1 followed by AP_3 and AP_2 of BDT 2.28, 3.26 and 3.96 respectively. In addition, per g crab production cost was significantly higher with PP_2 than the control and most of the AP based feed followed by AP_5 and PP_1 of BDT 7.71, 7.46 and 7.13respectively (Table 5). Cost effective, nutritionally balanced and good quality commercial feed development for any organism is crucial to reduce feed cost as it required nutritional requirement data for that organism first [15]. Chin (1992) [16] first reported the crude protein requirement in >600 g size mud crabs was 35-40%, whereas, Unnikrishnan (2010) [17] mentioned 45% crude protein requirement in 0.25 g size juvenile mud crabs. Whereas, Sheen (1999) [18] noted lipid requirement was ranging from 5.3 to 13.8%. Moreover, Catacutan (2002) [19] in a study found dietary protein and lipid requirement of mud crab was ranging from 32 to 40% and 6-12% for best growth performances. Wilson (2005) [20] managed to reduce mud crab feed cost of 21% than the trash fish fed group. All the above findings more or less match with the present findings. On the other hand, Primavera (2009) [21] gained 38.5% return on investment fed trash fish and formulated diet which is much lower than the present study.

Table 5: Cost benefit analysis of crab fattening with AP and PP based feeds

Feed Type	Average weight gain	Days required	Feed cost Tk/ Kg	Total cost	Net benefit	Production cost/g crab (Tk.)
C1	8.56(±2.13)^{ab}	16.33(±3.50)^{b}	40.00(±1.5)^{de}	49.87(±1.99)^{c}	45.80(±2.96)^{bcd}	4.67(±0.77)^{cd}
AP1	8.38(±2.62)^{ab}	20.75(±1.04)^{ab}	18.79(±1.31)^{g}	40.43(±0.8)^{a}	52.96(±3.12)^{bcd}	2.24(±0.13)^{a}
AP2	7.00(±2.74)^{b}	23.00(±8.26)^{ab}	26.40 (±2.85)^{f}	50.16(±1.83)^{c}	64.13(±4.52)^{d}	3.77(±0.76)^{bc}
AP3	10.67(±4.39)^{a}	23.00(±8.26)^{ab}	34.02(±1.74)^{e}	38.39(±1.14)^{c}	56.67(±2.81)^{cd}	3.19(±0.30)^{ab}
AP4	8.56(±4.85)^{ab}	23.33(±7.81)^{ab}	41.63(±2.11)^{cd}	43.7(±2.12)^{b}	51.57(±3.33)^{bcd}	4.86(±0.65)^{d}
AP5	6.50(±1.93)^{b}	23.63(±5.50)^{ab}	49.24(±0.89)^{a}	51.98(±2.77)^{c}	36.97(±3.09)^{bc}	7.58(±0.47)^{e}
PP1	7.83(±1.17)^{b}	27.00(±1.10)^{a}	54.79(±2.21)^{a}	76.54(±2.54)^{g}	38.59(±1.82)^{bc}	7.00(±0.60)^{e}
PP2	6.25(±2.55)^{b}	26.88(±3.44)^{a}	47.44(±2.34)^{bc}	68.25(±2.15)^{f}	32.76(±3.65)^{b}	7.59(±0.59)^{e}
PP3	7.89(±1.96)^{b}	24.33(±3.04)^{ab}	45.04(±1.84)^{bc}	61.91(±2.59)^{de}	34.55(±1.92)^{b}	5.71(±0.58)^{d}
PP4	8.33(±1.86)^{ab}	24.00(±0.00)^{ab}	42.64(±2.12)^{cd}	64.01(±2.42)^{e}	44.49(±3.96)^{bcd}	5.12(±0.13)^{d}
PP5	8.33(±2.78)^{ab}	23.63(±3.00)^{ab}	40.24(±2.59)^{d}	58.61(±2.94)^{d}	28.85(±3.85)^{a}	4.83(±0.83)^{d}

The values in the same column having similar letter(s) do not differ significantly otherwise differ significantly (p<0.05) as per Duncan Multiple Range Test (DMRT).

3.2 Survival rate of crab fed with experimental feeds

The average survival rate of female crabs was much higher with AP (96.30%) than PP based feed (83.1%) (Fig. 2). The difference in performance of the two types of feeds was might be due to the difference in the physical properties of the feeds. Survival of mud crab in cages with trash fish and gastropod meat was obtained 53.2 by David (2009) [22], which is significantly low for female than the present study.

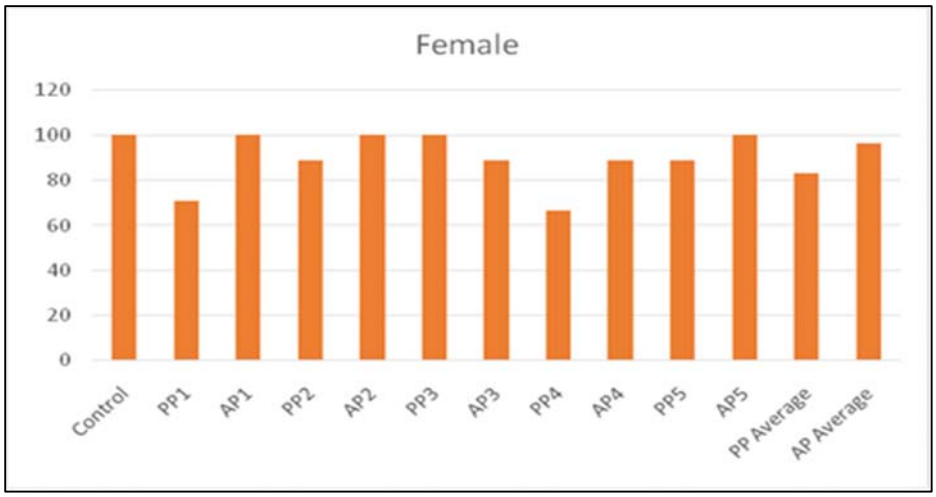

Fig 1: Survival rate of female crab fed experimental diets

3.4 Proximate composition of crabs

The proximate composition of crabs caught from nature, fed with formulated PP and AP based feeds reflected better body protein percentage (Table 6) than wild crabs. However, no significant differences were found in the proximate composition of crabs fed with different formulated feeds. Crude protein and lipid were significantly higher in fattened crab than the naturally caught crab. Sarower (2013) [23] reported similar result in their comparative study of biochemical and composition of naturally caught and fattened mud crab from the coastal area of Munshigonj, Satkhira districts of Bangladesh. They have mentioned that the small crab had less lipid percentage (0.20-7.51%) compared to the large fattened female crab. Musaiger (2005) [24] also reported that *Portunus pelagicus* crab consumed in Bahrain have a high level of protein (17.5-18.8%) and very low amount of fat (0.6-1.4%) both of the study signify the result of the current study.

Table 6: Proximate composition of wild and fattened female crab fed PP and AP feed

		Moisture %	Protein %	Lipid %	Ash %	Carbohydrate %
Wild crab	Female	80.66(±0.57)ab	11.51(±1.26)c	0.37(±0.03)c	6.77(±0.03)a	0.68(±0.03)c
Fattend PP_1	Female	81.07(±0.72)a	15.16(±0.86)b	0.42(±0.08)bc	2.16(±0.41)c	1.19(±0.1)a
Fattend PP_2	Female	80.57(±1.58)ab	15.5(±0.6)b	0.44(±0.08)abc	2.25(±0.26)c	1.24(±0.06)a
Fattend PP_3	Female	77.8(±1.42)c	18.3(±0.8)a	0.5(±0.04)ab	2.51(±0.39)c	0.89(±0.07)abc
Fattend PP_4	Female	77.5(±1.27)c	18.51(±0.51)a	0.65(±0.03)a	2.32(±0.19)c	1.02(±0.05)ab
Fattend PP_5	Female	78.87(±1.85)bc	15.51(±0.71)b	0.52(±0.04)ab	4.23(±0.22)b	0.86(±0.06)bc
Fattend AP_1	Female	80.57(±0.03)ab	15.5(±0.03)b	0.44(±0.03)abc	2.25(±0.03)c	1.24(±0.03)a
Fattend AP_2	Female	77.5(±0.03)c	18.51(±0.03)a	0.65(±0.03)a	2.32(±0.03)c	1.02(±0.03)ab
Fattend AP_3	Female	77.8(±0.03)c	18.3(±0.03)a	0.5(±0.03)ab	2.51(±0.03)c	0.89(±0.03)abc
Fattend AP_4	Female	81.07(±0.03)a	15.16(±0.03)b	0.42(±0.03)bc	2.16(±0.03)c	1.18(±0.03)a
Fattend AP_5	Female	78.87(±0.03)bc	15.51(±0.03)b	0.52(±0.03)ab	4.23(±0.03)b	0.86(±0.03)bc

The values in the same column having similar letter (s) or without letters do not differ significantly otherwise differ significantly (p<0.05) as per DMRT.

Moisture and ash content was significantly higher in naturally caught crab than the fattened crab. The range of ash content was in between 1.98-6.77%, where higher percentage of ash was found in naturally caught crab than the fattened crab regardless of sexes. Sarower (2013) [23] reported similar amount of ash content (2.02-7.65%) from the natural and fattened mud crab. In contrast, carbohydrate content did not follow any definite trend, although significantly low amount of carbohydrate were recorded in naturally caught crab than the fattened crab with both AP and PP based feeds (Table 6) that conforms with Musaiger (2005) [24].

3.5 Water quality parameters over the study period

The parameters recorded over the period were: temperature (27.7-30.93°C); salinity (7.25-14.50 ppt); dissolved (DO) oxygen (3.68-5.43 ppm); pH (7.7-8.25); turbidity (15.50-62.63); nitrite (0.0-0.25); nitrate (0.0-0.86); and ammonia (0.0-0.25). The values were within the acceptable range reported by Triño (1999, 2001) [25, 26]. However, salinity was bit lower in the ghers which is the general phenomena of the area where the experiment was carried out but the ranges are within the suitable limit of crab culture as the crab has ability to withstand adverse situation than the shrimp and prawn (Shelley 2011) [4]. Southeast Asian Fisheries Development Center (SEAFDEC 1997) [27] reported that mud crabs are highly euryhaline and can tolerate extremely high salinity range of 10 to 34 ppt, pH, 8.0 to 8.5, temperature, 23 to 30 ºC and dissolved oxygen content more than 3 ppm.

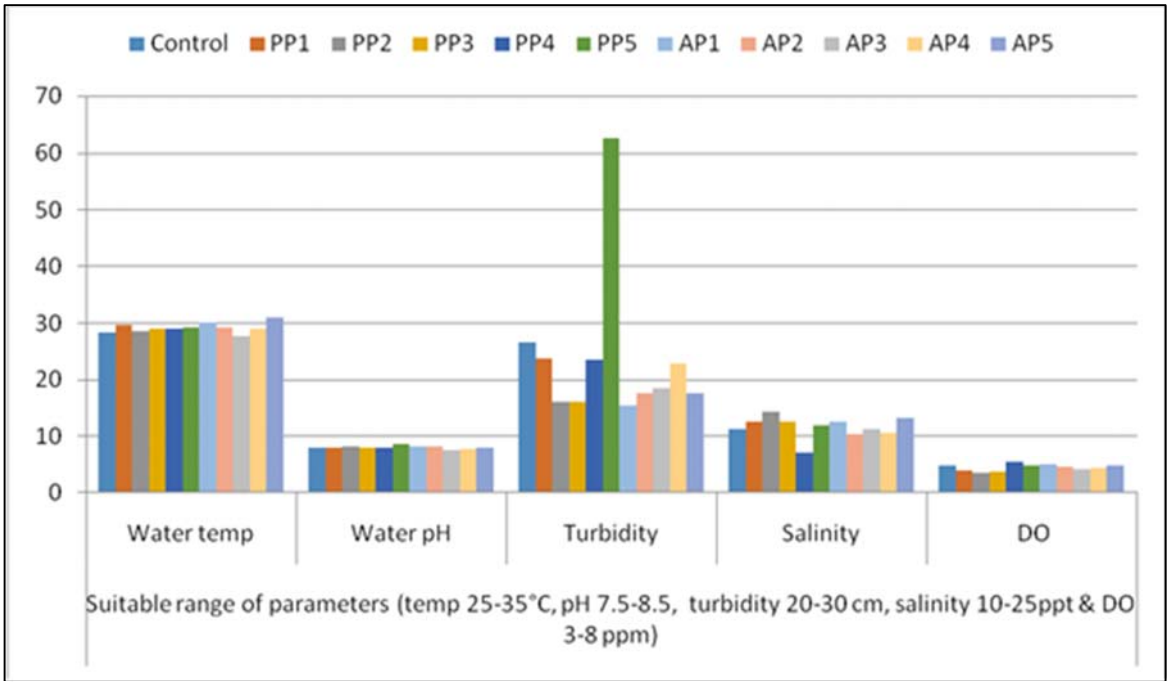

Fig 2: Water quality parameters

4. Conclusion

It is evident from the present study that the female crab accepted the formulated feeds. The performance of both the animal protein based(AP) and plant protein based (PP) feed were satisfactory in comparison to control feed, suggesting that the result might be useful to reduce the feed cost and dependency on trash fish in fattening process, save the farmers time and reduce health hazard as well as address environmental pollution. In the long run, this study is expected to bring about a revolutionary change in crab economy along the coastal region.

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