Fish meal replacement with chicken waste meal in Asian seabass (*Lates calcarifer*) feeds

S. NANDAKUMAR, K. AMBASANKAR, J. SYAMA DAYAL
C. RAMAN AND SYED RAFFIC ALI
*Central Institute of Brackishwater Aquaculture, 75, Santhome High Road, Raja Annamalai Puram
Chennai - 600 028, Tamil Nadu, India
e-mail: ambasankar@ciba.res.in*

ABSTRACT

An eight week growth trial was conducted to study the effect of replacement of fish meal with processed chicken waste meal (CWM) in the diet of Asian seabass, *Lates calcarifer*. Analysis of CWM showed that it has 53% crude protein and 32% crude lipid. Effect of replacement of fish meal (FM) with CWM was carried out by including CWM at 0, 5, 10, 15 and 20% (W/W) levels in an isonitrogenous (40%) and isolipidic (10%) diet. The experiment was carried out in a completely randomised design with three replicates for each treatment and each replicate containing ten animals (average initial body weight of 3.09±0.04 g). The results showed that there was no significant ($p>0.05$) difference in the final body weight (FBW), weight gain %, feed intake, feed conversion ratio (FCR), condition factor (CF) and average daily gain (ADG) in the fish, fed with diets containing CWM upto 10%. Inclusion of CWM at more than 10% level showed a significant ($p<0.05$) reduction in the growth parameters, compared to control diet. There was no significant difference in survival rate among the fish fed with different experimental diets. Similarly, hepatosomatic index (HSI) and viscerosomatic index (VSI) showed no significant difference ($p>0.05$) among the treatment groups. The results from this study indicate that the CWM is a potential ingredient in the diet of Asian seabass *L. calcarifer* and it can be included up to 5-10% levels, replacing fish meal. However further studies are needed to optimise the level of CWM in the diet of seabass.

Keywords: Asian seabass, Chicken waste meal, Fish meal replacement, Growth

Introduction

Asian seabass *Lates calcarifer*, popularly known as bhetki or barramundi, is an economically important species and it is being widely cultured in South-east Asia and Australia under extensive or intensive system in fresh, brackish and marine water resources (Harpaz *et al*., 2005; Glencross, 2006). In India, it is being considered as a potential candidate species for coastal aquaculture. The carnivorous feeding habit of the fish necessitates going for fish meal as the major source of dietary protein (Williams *et al*., 2003; Glencross *et al*., 2011). The high demand and the limited supply of fish meal make this dietary protein source as an unsustainable one in the long run. This situation resulted in the search for alternate protein sources. Protein sources derived from plant and terrestrial animal origin are considered as a suitable alternative to fish meal, due to their availability at low cost compared to fish meal. The inclusion of plant protein (partially or totally replacing fish meal) as an alternate protein source for fish meal has been evaluated by many investigators in Asian seabass (Boonyaratpalin *et al*., 1998; Tantikitti *et al*., 2005). Rendered protein sources from terrestrial animals have been considered as promising alternatives. Chicken waste meal, for its high protein and lipid content, is considered to be a potential novel ingredient to replace fish meal in the diet of fish. Chicken waste meal (CWM) is the dry, ground, rendered clean part of chicken carcass, with some meat with trimmed fat. These meals vary in nutritional value and composition, depending on the processing applied and the materials that are included in the meal. Chicken waste meal (poultry byproduct meal) has been tested in diets for various species, such as rainbow trout (Serwata, 2007), cobia (Saadiah *et al*., 2011), catfish (Giri *et al*., 2010) and Florida pompano (Waldemar *et al*., 2012). The utility of chicken waste meal in the diet of seabass has not been explored so far. and the present study was undertaken to assess the utility of chicken waste meal as a replacement for fish meal in the diet of *L. calcarifer*

Materials and methods

Preparation of experimental diets

Chicken waste meal for the study was collected from the chicken meat processing industry, Suguna Foods at Coimbatore in Tamil Nadu during June 2011. The cost of the CWM at the time of collection was ₹ 37 per kg. The effect of replacement of fish meal with chicken waste meal was studied, by including CWM at 0, 5, 10, 15 and 20% (W/W) levels in an isonitrogenous (40%) and isolipidic (10%) diet. The ingredients and proximate composition of the experimental diets are given in Table 1 and 2. Dry feed ingredients were powdered in an electrical grinder, passed through a 0.5 mm sieve, mixed with additives and then homogenised thoroughly in an electrical blender. The diet mix was made into a soft dough by adding water at about 40%, steam cooked (at atmospheric pressure) for 5 min, cooled and then pelletised in a hand pelletiser using a 2 mm die. The diet was dried in a hot air oven at 70 °C to moisture content of less than 9% and stored in a dessicator until use.

The proximate composition of the ingredients and experimental diets was analysed following standard procedures (AOAC, 1990). Moisture content was estimated by gravimetric analysis after oven drying at 105 °C for 12 h. Crude protein (CP) was determined by Kjeldahl method (N x 6.25) after acid hydrolysis (Kjeltec 2100, FOSS, Tecator, Sweden). Crude lipid (CL) was calculated gravimetrically after extraction with petroleum ether in a soxhlet system (SOCS, Pelican, India). Total ash was determined gravimetrically by ignition at 600 °C for 6 h in muffle furnace. Crude fibre was estimated gravimetrically after acid and alkali digestion and loss in mass by combustion at 600 °C for 3 h. Nitrogen free extract (NFE) was calculated by difference.

Fish rearing and experimental design

Asian seabass juveniles were collected from the wild and were acclimatised to the laboratory conditions for two weeks before the start of the experiment. During the acclimatisation period, the animals were fed with pellet feed developed by Central Institute of Brackiswater Aquaculture (CIBA), Chennai. The experiment was conducted in a completely randomised design with three replicates for each treatment and each replicate containing 10 animals (average initial body weight of 3.09±0.04 g). The weight and total length of the fish were measured individually at the beginning of the experiment. Fish were hand fed to satiation

Table 1. Ingredient composition of experimental diets, containing varying levels of CWM

Diets	CWM0	CWM5	CWM10	CWM15	CWM20
Ingredients (%)					
Fish meal	35	30	25	20	15
Shrimp meal (*Acetes* spp.)	10	10	10	10	10
Chicken waste meal	0	5	10	15	20
Soyabean meal	21	21	21	21	21
Groundnut oil cake	8	8	8	8	8
Wheat flour	5	6	7	8	9
Rice flour	6	6	6	6	6
Maize	6	6	6	6	6
Fish oil	5.5	4.5	3.5	2.5	1.5
Lecithin	0.5	0.5	0.5	0.5	0.5
Binder [¶]	1	1	1	1	1
Vitamin and mineral mixture[*]	1.8	1.8	1.8	1.8	1.8
Vitamin C [#]	0.1	0.1	0.1	0.1	0.1
Choline chloride	0.1	0.1	0.1	0.1	0.1

[¶] - Poly Methylol carbamide
[*]- Commercially sourced premix and each kg contains Vitamin A - 2000000IU, Vitamin D - 400000 IU, Vitamin E – 300 U, Vitamin K - 450 mg, Riboflavin - 800 mg, Panthothenic acid - 1 g, Nicotinamide - 4 g, Vitamin B12 - 2.4 mg, Choline chloride - 60 g, Ca - 300 g, Mg – 11 g, I - 400 mg, Fe - 3 g, Zn - 6 g, Cu - 800 mg, Co - 180 mg
[#] - Rovimix Stay - C 35

Table 2. Proximate composition (% dry matter basis) of experimental diets containing varying levels of CWM

Proximate composition/ Diets	CWM0	CWM5	CWM10	CWM15	CWM20
Moisture	7.39	7.2	7.68	7.32	7.54
Crude protein	41.08	40.83	40.58	40.33	40.08
Ether extract	9.66	10.05	10.38	10.64	10.95
Total ash	13.38	11.02	10.57	10.08	8.56
Crude fibre	2.12	2.02	2.14	2.42	2.52
NFE	26.37	28.88	28.65	29.21	30.35

twice daily; morning (10.00 hrs) and evening (16.00 hrs). The unconsumed feed pellet was siphoned out from the tanks to determine the feed intake. Fish were held in 1000 l fibre reinforced plastic (FRP) tanks and total water was replaced twice a day throughout the experimental period of 8 weeks. Continuous aeration was provided with air stones. Animals were group weighed at fortnightly intervals for adjusting the feed ration.

Sampling and data collection

On termination of the experiment, the total length and weight of the fish were measured individually. Three fish from each experimental group were randomly selected to measure the biological indices, such as condition factor (CF), hepatosomatic index (HSI) and viscerosomatic index (VSI). The whole body composition of fish was analysed as per AOAC (1990).The growth parameters were calculated using the following formulae:

Weight gain (%)	=	(FBW – IBW/ IBW) x 100
Daily growth coefficient, DGC (% d^{-1})	=	$[(FBW^{1/3}-IBW^{1/3})/ D]$ x 100
Specific growth rate, SGR (% d^{-1})	=	[(Ln FBW – Ln IBW)/ D] x 100
Average daily gain, ADG (mg day^{-1})	=	[(FBW – IBW)/D] x 1000
Condition factor, CF	=	[(Live weight, g) / (Length, cm)^3] x 100
Hepatosomatic index, HSI	=	(Liver weight/body weight) x 100
Viscerosomatic index, VSI	=	(Visceral weight/body weight) x 100
Survival (%)	=	N_2 /N_1 x 100

where, IBW = Initial body weight (g); FBW = Final body weight (g) and D = duration of experiment in days; N_1 = Number of fish at the start of the experiment; N_2 = Number of fish on termination of the experiment.

During the experimental period, the water quality parameters in the rearing tanks *viz*., pH, temperature, salinity, dissolved oxygen, nitrate and ammonia were measured following standard methods (APHA, 1998).

Statistical analyses

Data were analysed using one-way ANOVA to compare significant differences between treatments, whereas Duncan's multiple range test was used to compare the means of the treatments. Data in percentage values (SGR, DGC and survival) were subjected for arcsine transformation prior to analysis of variance. All the data were analysed using SPSS version 16.0 software.

Results

The CWM used in the study had 8.29% moisture, 53.54% crude protein, 31.37% crude lipid, 1.72% crude fibre and 5.55% ash. The water quality parameters recorded in the experimental tanks were : salinity : 29-31 ppt, pH : 7.4-8.1, temperature : 27-29 ºC, dissolved oxygen; 6.1-7.3 mg l^{-1}, nitrate : 0.4-0.-0.7 mg l^{-1} and ammonia: 0.08-0.11 mg l^{-1}.

Growth performance and feed utilisation

The proximate composition of the diet is presented in Table 2 and the results showed that there is no significant difference in crude protein and lipid content of the experimental diets. However, the ash content decreased linearly as the level of inclusion of CWM increased from 0 to 20%. The effect of replacement of fishmeal with CWM on growth performance, and body indices of juvenile seabass fed with CWM based diets are shown in Table 3. The survival rate obtained in this experiment ranged from 80 – 93% and it was not significantly different among the experimental diets. The control diet containing CWM0 recorded the highest final body weight (12.59 g) and this

Table 3. Growth performance and feed utilisation of seabass fed experimental diets with varying levels of CWM (8 weeks).

Parameters	CWM 0	CWM 5	CWM 10	CWM 15	CWM 20
Initial weight (g)	3.07 ^{a} ±0.09	3.08 ^{a} ±0.05	3.12 ^{a} ±0.11	3.04 ^{a} ±0.12	3.12 ^{a} ±0.09
Final weight (g)	12.59 ^{a} ±0.24	12.37 ^{a} ±0.49	11.78 ^{ab} ±0.34	10.79 ^{b} ±0.32	8.15 ^{c} ±0.12
Weight gain %	309.9 ^{a} ±3.7	300.9 ^{a} ±12.8	278.2 ^{ab}±16.1	255.5 ^{b} ±5.5	162.1 ^{c} ±8.0
ADG (mg d^{-1})	158.6 ^{a} ±2.6	154.7 ^{a} ±7.6	144.3 ^{ab} ±5.7	129.2 ^{b}±3.7	84.0 ^{c} ±2.3
Survival %	93.33±3.33	90.0±5.77	90.0±0.01	80.0±5.77	83.3±8.82
FI (g $fish^{-1}$)	15.85 ^{a}±0.06	16.09 ^{a}±0.53	15.30 ^{ab}±0.62	14.20 ^{b}±0.18	9.93 ^{c}±0.44
FCR	1.67 ^{a}±0.03	1.74 ^{ab}±0.03	1.77 ^{ab}±0.02	1.83 ^{b}±0.03	1.97 ^{c}±0.06
SGR (% d^{-1})	2.35 ^{a}±0.02	2.31 ^{ab}±0.05	2.21 ^{bc}±0.07	2.11 ^{c}±0.03	1.60 ^{d} ±0.05
DGC	1.45 ^{a} ±0.01	1.42 ^{ab} ±0.04	1.35 ^{bc} ±0.04	1.27 ^{c} ±0.01	0.92 ^{d} ±0.03

All values are means ± SE of three observations
Means bearing same superscript in a row do not differ significantly ($p > 0.05$)

was not significantly different with CWM5 and CWM10 diets. A similar trend of no significant difference in weight gain %, average daily gain (ADG) and feed intake was observed in groups fed with CWM0, CWM5 and CWM10 diets. The feed conversion ratio (FCR) showed that the fish fed the CWM0, CWM5 and CWM10 diet showed no significant difference, while CWM20 diet showed significantly ($p<0.05$) poor FCR among the experimental diets. The specific growth rate (SGR) and daily growth coefficient (DGC) obtained for fish fed CWM0 diet (2.35% and 1.45% respectively) showed no significant difference with that of CWM5 (2.31% and 1.42%) diet.

Whole body composition and biological indices

Whole body composition of seabass fed with the experimental diets is presented in Table 4. Analysis of moisture, crude protein, crude lipid, and ash contents of seabass fed with experimental diets showed significant differences ($p<0.05$) among the experimental diets. Significantly ($p<0.05$) high moisture content was observed in fish fed with CWM0 diet while the CWM20 fed group showed significantly ($p<0.05$) low moisture content. Fish fed with CWM0 diet showed significantly higher ($p<0.05$) crude protein. Significantly ($p<0.05$) low lipid content was observed in fish fed with CWM0 diet while the CWM20 fed group showed significantly ($p<0.05$) high lipid content. The total ash content showed a significantly ($p<0.05$) higher value (4.11) in the CWM0 diet. The condition factor (CF) for the fish fed with CWM5 and CWM10 diets showed no significant ($p>0.05$) difference with that of fish fed with control diet containing CWM0. The HSI and VSI values showed that there was no significant difference among the experimental diets (Table 5).

Discussion

The results of the present study demonstrated that fish meal can be replaced with a considerable amount of CWM in the diet of Asian seabass, *L. calcarifer* without adverse effects on the growth performance and on body indices. In India, modern methods of poultry processing result in the availability of large quantities of byproducts like feathers, viscera, head, feet and blood. These byproducts have been successfully converted to digestible meals by steam pressure cooking. At present, poultry byproducts like feather meal, poultry offal meal and blood meal are used as commercial feed stuffs in many countries. Recycling of wastes from poultry slaughter houses is of importance economically, biologically and environmentally (Sahraei *et al.,* 2012). The nutritional content of CWM showed that it is high in protein and lipid with a potential for inclusion in carnivorous fish feeds. The high protein and lipid content in the CWM indicates that it has the nutritional potenial for use in the feeds of seabass, as it requires high protein and high lipid diet.

CWM is a cheap source of protein compared to fish meal and is available in large quantities in the poultry processing plants in India. In India CWM is presently used in the pet food and poultry feed industry. The CWM is used at very limited levels in the feed of aquatic animals, especially in certain carnivorous fish feeds. The protein (53.57%) content of CWM is marginally lower than the fish meal (60%) while the lipid (31.37%) content is very high compared to the fish meal (6-8%). The high lipid content and the type of lipid present in the CWM are to be considered while formulating feed with CWM for aquatic animals. The fatty acid content, especially the beneficial highly unsatutuarted fatty acids (EPA and DHA), in the

Table 4. Whole body composition (% wet weight) of seabass fed experimental diets with varying levels CWM

Diets	Moisture	Crude protein	Crude lipid	Total ash
CWM0	$69.09^{a}\pm0.12$	$16.75^{a}\pm0.25$	$6.06^{d}\pm0.05$	$4.11^{a}\pm0.02$
CWM5	$68.07^{b}\pm0.02$	$16.30^{b}\pm0.03$	$7.08^{c}\pm0.18$	$3.53^{b}\pm0.04$
CWM10	$67.92^{b}\pm0.11$	$16.39^{b}\pm0.02$	$7.62^{b}\pm0.01$	$3.60^{b}\pm0.07$
CWM15	$67.64^{c}\pm0.09$	$16.25^{b}\pm0.07$	$7.67^{b}\pm0.07$	$3.54^{b}\pm0.06$
CWM20	$67.48^{c}\pm0.13$	$16.33^{b}\pm0.10$	$8.41^{a}\pm0.16$	$3.13^{c}\pm0.01$

All values are means ±SE of three observations
Means bearing same superscript in a column do not differ significantly ($p > 0.05$)

Table 5. Biological indices of seabass fed experimental diets with varying levels of CWM for 8 weeks

Parameters (%)	CWM0	CWM5	CWM10	CWM15	CWM20
CF	$1.32^{a}\pm0.07$	$1.30^{a}\pm0.05$	$1.21^{ab}\pm0.02$	$1.16^{b}\pm0.03$	$1.08^{b}\pm0.01$
HSI*	2.40±0.06	2.43±0.08	2.46±0.04	2.44±0.10	2.45±0.11
VSI*	9.18±0.31	9.25±0.41	9.66±0.64	9.91±0.54	9.97±0.08

* -Non significant ($p > 0.05$)
All values are means ±SE of three observations
Means bearing same superscript in a row do not differ significantly ($p > 0.05$)

feed needs to be taken care as these fatty acids are indispensable in most of the marine and brackishwater fish and shrimp. The cost of CWM is ₹ 37 per kg against ₹ 52 per kg for the fish meal used in this study. Therefore, feed cost can be reduced substantially by including CWM in the diet of seabass and possibly in other carnivorous cultured marine fish species. Poultry or chicken waste meal has been used in the diet of various fish species to evaluate the nutritional significance of replacing fish meal. Saadiah *et al.* (2011) reported that fish meal can be replaced with poultry byproduct meal (PBM) up to 60% in the diet of cobia (*Rachycentron canadum*) without any effect on growth performance. Shapawi *et al.* (2007) reported that 75% of pet grade as well as feed grade PBM can replace FM in the diets for the humpback grouper (*Cromileptes altivelis*) without any effect on growth and feed utilisation as compared to that of fish meal based control diet. Kureshy *et al.* (2000) reported that PBM can substitute FM up to 66.7% in the diets for juvenile red drum (*Sciaenops ocellatus*). PBM can replace FM in the range of 35 – 70% in the diets for hybrid striped bass (Rawles *et al.,* 2006). It is pertinent to note that Waldemar *et al.* (2012) reported that the inclusion of PBM can be increased from 10% to 15% with methionine and taurine supplementation in the diet of Florida pompano (*Trachinotus carolinus*). Hence, there is further scope to improve the inclusion level of CWM by supplementing with the limiting amino acids.

Growth performances of seabass fed with CWM containing diets indicated that CWM can be included up to a significant level in the diet. Fish fed with the diets CWM5 and CWM10 showed good growth rate, feed intake, FCR and ADG. However, SGR and DGC showed no significant variation between CWM0 and CWM5**.** Poor growth performance of fish fed with CWM20 in this study can be attributed to the reduced feed intake observed in that group. Similar results were observed by Yigit *et al.* (2006) in black sea turbot (*Psetta maeotica*) when fish meal was replaced by CWM. Similarly, Nengas *et al.* (1999) reported poor growth performance in gilthead seabream (*Sparus aurata*) fed diet containing a similar grade PBM (CP 53% and CL 30%) at 30% (w/w) inclusion level. On the contrary, Glencross *et al.* (2011) reported good growth performance in Asian seabass (*L. calcarifer*) at 30% (w/w) inclusion of poultry offal meal (POM), replacing fish meal. Che Utama *et al.* (1999) reported that POM can be included in Asian seabass diet up to 50% (w/w) without any negative effect on the growth performance, compared to that of control diet. CWM varies in nutritional value and its composition depends on the processing method and the materials that are included in the meal**.** Further, it is pertinent to note that chicken waste meal is deficient in certain essential amino acids and n-3 fatty acids (Nengas *et al.,* 1999; Rawles *et al.*, 2006; Yigit *et al.,* 2006; Shapawi *et al.,* 2007; Pine *et al.,* 2008). The processing condition would affect the chemical nature of the material, such as amino acid availability, lipid oxidation and reduced digestibility, which resulted in poor performance of the fish when it included in the diet at higher level. Thus, the acceptability of CWM, when it is used in the diet, varied with the fish species.

The whole body composition of seabass fed with experimental diets containing varying levels of CWM showed significant variations. Significantly highest moisture content was observed in fish fed with CWM0 diet (69.09%) and the groups fed with CWM supplemented diets showed significantly lower moisture content than the control diet CWM0. The crude lipid content showed lowest value in CWM0 diet and it was significantly different from the CWM supplemented diets. Further, the crude lipid content showed a linear increase in value as the level of CWM increased from 0 to 20%. This observation was similar to the findings of Nengas *et al.* (1999) and Giri *et al.* (2010) that inclusion of chicken byproduct meal results in higher lipid content. This may be due to the high lipid content in CWM and comparatively higher lipid content in fish fed coupled with its higher digestible energy in CWM supplemented diets.

The condition factor was highest in the group fed with CWM0 (1.32%) diet and it showed a linear decreasing trend as the level of inclusion of CWM increased from 0 to 20%. However, the difference was not significant among CWM0, CWM5 and CWM10 fed groups. Shapawi *et al.* (2007) opined that there was a tendency for lower CF as the level of poultry byproducts increased in the diet of humpback grouper. Condition factor is used to compare the 'condition', 'fatness' or 'well being' of fish and are based on the hypothesis that heavier fish of a given length are in better condition. The HSI ranged from a lower value of 2.40% in CWM0 diet to a higher value of 2.46% in CWM10 diet and the differences are not significant among the experimental diets. Similarly insignificant differences in HSI were observed by Rawles *et al.* (2006) in hybrid striped bass fed diets containing PBM at 7.73% and 17.20%. VSI also showed no significant variation among the experimental diets and it ranged from 9.18% in the CWM0 diet to 9.97% in CWM20 diet, similar to the results of HIS. This was in close agreement with the report of Shapawi *et al.* (2007).

Results from this study showed that chicken waste meal can be included in the range of 5 to 10% (w/w basis) levels in seabass diet without affecting the growth performance. However further studies on digestibility of amino acids and fatty acids are needed to optimise the inclusion level of CWM and its nutritional potency in Asian seabass diet.

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