CHAPTER 3

Materials and methods

3.1 Materials

3.1.1 Feed ingredients

Fish Protein Hydrolysates dry powder was supplied by Chonburi aquatic animal feed technology research and development center, Department of Fisheries, Ministry of Agriculture and Cooperative, Thailand. It mainly consists of head, skin, trimmings, fins, frames, viscera, and roes of Slender rainbow sardine (*Dussumieria elopsoides)* hydrolyzed by *Lactobacillus plantarum* 541 for 16 hours and then spray dried at 105°C. The chemical composition is indicated with 41%, 7.79 %, 16.22%, and 5.11% for crude protein, moisture, fat, and ash respectively. Essential and non-essential amino acids of this FPH are specified in Table 3

Table 3 Essential and non-essential amino acids of fish protein hydrolysates from Chonburi aquatic animal feed technology research and development center

Essential amino acid	g/100g	Non-essential amino acid	g/100g
Histidine	1.04	Taurine	0.72
Isoleucine	1.72	Aspartic acid	3.58
Leucine	3.05	Threonine	1.84
Lysine	3.94	Serine	1.41
Methionine	1.28	Glutamic acid	5.76
Phenylalanine	1.70	Proline	1.90
Threonine	1.84	Glycine	3.00
Valine	2.09	Alanine	2.86
		Cystine	0.35
		Tyrosine	0.71

Figure 3.1 Sample of Fish Protein Hydrolysates powder tested

The other feed ingredients were bought from RPM farm and feed company, ChiangMai, Thailand. Vitamin and mineral premix were bought from Premer feed company,. The nutritional content of this feed ingredients are indicated by the manufacturer as lined out in Table 4.

Table 4 Nutrition content of the feed ingredients used in the experiment diets (%)

Raw material	Moisture	Protein	Fat	Fiber	Ash
Fish meal	6.46	60.1	12.09	0.72	21.13
Soybean meal	8.35	44.4	6.71	3.35	1.4
Corn meal	8.63	9.5	0.77	6.67	7.19
Rice bran	14.6	8.41	2.2	9.9	2
Wheat flour	8.9	14.6	13.52	13.26	13.32

. **3.1.2 Experimental fish**

The red-tail catfish fry for the experiment were acquired from Thongchai Fish Farm, ChiangMai, Thailand. This farm revealed the red-tail catfish bloodstock from the Phisanulok Inland Research and Development Center. This institution has during the past years successfully developed brood-stock of red-tail catfish to supply the aquaculture industry with stocking material. Juvenile red tail catfishes were immediately stocked in cycle fiber tank upon arrived, raring until two week for adaptation before experimental started

3.2 Methods

3.2.1 Experimental design

The experiment was designed as Completely Randomized Comparison (CRD) with 4 treatments and 3 replications each. The treatments refer to the 4 experimental diets with increased replacement of fishmeal by protein hydrolysate (FPH): 0 (FPH0), 50 g or 16.67 % (FPH50), 100 g or 33.33 % (FPH100) and 150 g or 50 % (FPH150). Each treatment diet was standardized at 32% crude protein by adding different levels of FPH (Table 5).

Figure 3.2 Mixing experimental diets

Table 5 Test feed composition with different replacement levels of fishmeal by FPH for rearing red-tail catfish (g/kg)*

	Replacement levels			
	(FPH0)	(FPH50)	(FPH100)	(FPH150)
Ingredients	0%	16.67%	33.33%	50%
Fish meal	300	250	200	150
FPH	0	50	100	150
Soybean meal	252	273	295	315
Corn meal	100	100	100	100
Rice bran	185	164	142	122
Wheat flour	100	100	100	100
Soy bean oil	20	20	20	20
Vitamin C	3	3	3	3
Vitamin premix	20	20	20	20
Mineral premix	20	20	20	20
Total (g)	1000	1000	1000	1000
Nutritional content by proximate composition analysis (%)**				
Dry Matter	81.24	81.77	83.44	84.88
Crude Protein	32.15	32.55	32.12	32.13
Crude lipid	8.43	8.32	8.18	8.01
Ash	9.71	9.13	8.48	7.94
Crude Fiber	4.22	3.48	5.26	4.34
NFE	45.49	46.52	45.96	47.58

* calculated on manufacturer specifications.

** calculated by proximate analysis (AOAC, 1990)

3.2.2 Preparation and evaluation of experimental diets

The diets were prepared by mixing of the dry ingredients with soybean oil and water by mixer and then putting the wet dough through a grinder, extruded to pass through a 2 mm mesh sieve. Finally the moist pellets were dried at 50 °C for 24 hours, and then stored in the fridge at 5°C for further use. Samples of the pelleted experimental diets were analyzed regarding their feeding value (dry matter, crude protein, fat, ash, crude fiber and NFE) in the laboratory of Animal and Aquatic Science, the CMU-Agricultural Faculty according AOAC standards from 1990.

Figure 3.3 Pelleting machine

Figure 3.4 Pelleted experimental diet

3.2.3 Experimental Setup

The experiment was carried out in a closed recirculation unit with 12 circle fiber tanks of 750 L at the Mae Hia Agricultural Research, Demonstration and Training Center, Faculty of Agriculture, Chiang Mai University. The acquired fish fry were conditioned and reared in 4 circle fiber tanks up to fingering size. A total of 720 juvenile red-tail catfish with an average initial body weight of 5.45±0.05 g by stocking density of 60 fishes per tank (600 L) entered the experiment. The experiment lasted for 14 weeks. Sinking feed were daily hand-fed to apparent satiation twice (08:00 and 20:00) daily for 14 weeks. The tank water at a flow rate was 5 L/min was circulated through biological and mechanical filters utilizing high-density polyester screens to

remove particulate material and provide substrate in trickling filter for nitrifying bacteria. Water temperature was maintained at 26±1°C by using a heating rod. All rearing tanks were provided with continuous aeration. Any uneaten feed was collected 1 h after every feeding.

Figure 3.5 Fish were grown in circle fiber tanks run in a closed water recirculation system

3.2.4 Parameters of growth performance

Weight and length of the fishes sampled every 2 weeks (20 fish /tank) in order to calculate the weight gain (WG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate data.

WG (g) = [Actual weight (Wt) – Initial weight (Wi)/Wi] x 100
SGR (%/day) = (Ln final weight - Ln initial weight)/days x100
FCR = Total feed intake (g)/total wet weight gained (g)
ADG (g) = (Initial weight – final weight)/period of experiment
TL (cm) = Total length/ no. of fish
Survival (%) = 100 x (final fish number)/(initial fish number)
PER (%) = (g weight gained/g protein intake) x 100

Figure 3.6 Weight recording

Figure 3.7 Length measurement

3.2.5 Water quality

Water samples were collected 15 cm below water surface from each replicated tank and they were used for the determinations level of dissolved oxygen (DO) contents, pH, total hardness and ammonia-nitrogen (total NH_3) content. For analysis titrate tube kits were employed following Winkler method (Norwegian Standard, 1988) rovided by Union Science. All parameters were measured at a two-week interval throughout the experimental period.

3.2.6 Carcass and meat evaluation

At the end of the feeding trial, 3 fishes (5%) from each tank were randomly slaughtered for flesh quality assessment. Slaughtering procedure followed the method of Huidobro *et al*, (2001). Fish were immobilized in a box with iced water for 5 min before slaughtering .Liver, intestine, kidney were removed and the fillets were prepared by separating head, skin, trimmings, fins, frames, viscera, roes and bones. Fillets were kept in an ice box before transporting to laboratory for chemical analysis.

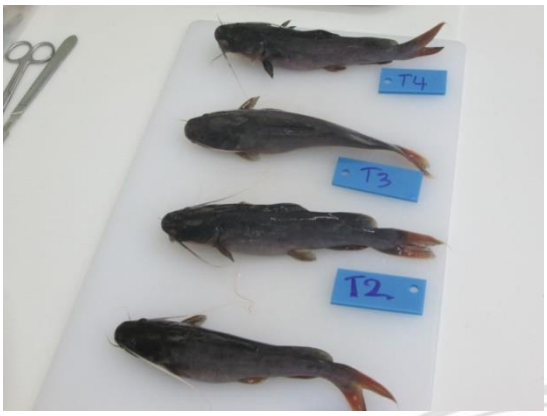

Figure 3.8 Appearance at end of experiment

Figure 3.9 Preparing of fillets

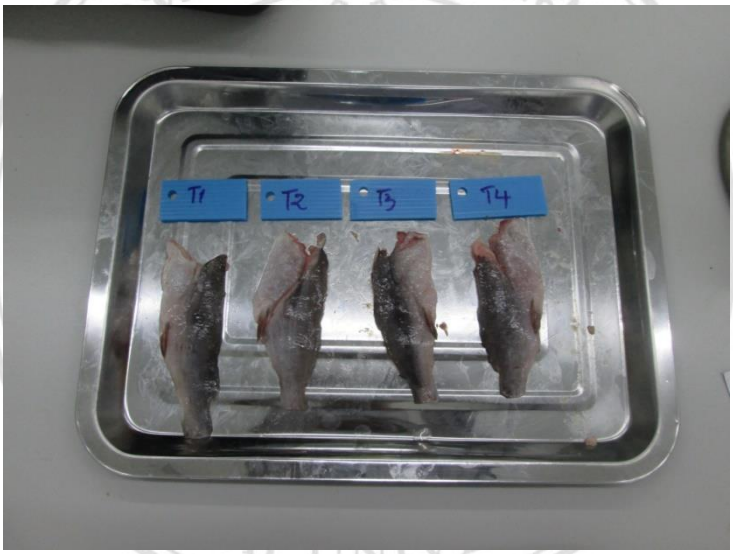

Figure 3.10 Carcass appearance

Proximate analysis of muscle composition. The moisture content was determined by drying samples at a temperature of 105◦C to constant weight (AOAC, 1990); the total protein was determined by Kjeldahl's method applying a multiplier of 6.25; and the lipid content was determined by Soxhlet's method, with petroleum ether as solvent. The ash amount was determined by the mineralization of the samples at a temperature of 550–600◦C (AOAC, 1990). The pH was measured at 25◦C using a pH meter (Testo205 instrument, Germany) applied directly on flesh.

Measurement of carcass quality fillet yields with skin (FY) were calculated for carcass without head and viscera, viscera weight (VW), head weight (HW), liver weight

(LW) were recorded seperately. All yield rates were calculated in relation to total weight.

Measurement of flesh color The color measurements of the fish samples were carried out using the Konica Minolta Chromometer (CR 400 Osaka, Japan). Following calibration with the white reference tile, it was applied directly to the fish slices and fish skin samples. The measurements of lightness (L∗ value) and the color (a∗: red/green, b∗: blue/yellow) were taken threefold. The results are presented as the mean ± *SD* for the triplicate samples.

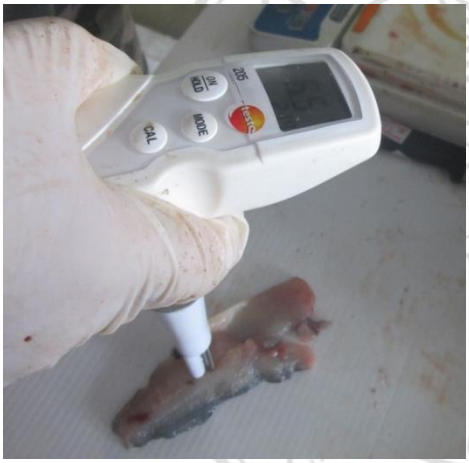

Figure 3.11 pH measurement

Figure 3.12 Color measurement

3.2.7 Lysozyme analysis

After 14 weeks, blood samples were collected by puncture of vena caudalis from 3 fish per tank by using a 120 1 ml syringe at the end of the feeding trial. The samples were immediately withdrawn into the Eppendorf 1.5 ml tubes without anticoagulant, allowed to clot (1 h at room temperature and 4 hours at 4^{o}C) and centrifuged at 1500 × g, 5 minutes, and 4^{o} C. The serum was finally collected and stored at -20^{o} C until assay. Serum lysozyme activity was determined according to the method suggested by (Parry *et al.*, 1965). The equivalent unit of the activity of the sample (compared with the standard) was determined and expressed in µg ml^{-1} serum.

Figure 3.13 Blood collected by puncture of vena caudalis

3.3 Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple range test. The statistic model applied was:

$$Y_{ijk} = \mu + \tau_i + r_{ij} + \varepsilon_{ijk}$$

where: Y_{ijk} is the k^{th} observation of the i^{th} treatment

μ is the population mean

τ_i is the treatment effect of the i^{th} treatment

r_{ij} is the replication effect of the j^{th} replication of the i^{th} treatment

ε_{ij} is the random error

The level of significance was set at $P<0.01$ or $P<0.05$