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***Full Paper***

# Optimisation of dietary protein level for rearing juvenile spotted scat (*Scatophagus argus* Linnaeus, 1766)

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**Abstract:** An 8-week feeding trial was conducted to determine the optimum dietary protein level for growth, feed utilisation, chemical composition and amino acid profile in juvenile spotted scat (*Scatophagus argus*). Five isoenergetic diets containing 31.70, 35.39, 40.71, 45.88 and 51.48% crude protein (T1–T5 respectively) were formulated and fed to triplicate groups of *S. argus* (4.92 ± 0.06 g, n = 15 for each group). The percent weight gain (WG), specific growth rate (SGR) and average daily gain (ADG) showed significant quadratic response to dietary protein level with the highest values observed in the T4 group. The feed conversion ratio (FCR) and feed intake (FI) showed significant quadratic response to the dietary protein level, with the highest efficiency in the T3 and T4 groups. Crude protein and most amino acid levels increased with increasing dietary protein. The optimum dietary protein level for tested parameters of this species amounted to 41-45%. However, WG, SGR, ADG, FCR and FI showed subtle and/or insignificant differences between the dietary protein levels of 35.39 and 45.88% (T2–T4 groups). These results suggest that diets containing ~35% protein can be a practical and cost-effective option for rearing juvenile spotted scat.

**Keywords:** spotted scat, dietary protein requirement, growth performance, feed utilisation

## INTRODUCTION

Spotted scat (*Scatophagus argus* Linnaeus, 1766), also known as tiger scat, argus fish, spotted butter fish and kitang, is a popular food fish species that is also attracting attention as an ornamental fish because of its beautiful body colouration. Spotted scat is an omnivorous fish species that predominantly feeds on benthic animals, zooplankton and detritus with high flexibility in

changing their feeding habits based on the food availability in their habitat [1-3]. Furthermore, this species has a high ability to cope with environmental fluctuations as represented by its wide salinity tolerance [3]. There have been extensive studies on different aspects of the species, such as morphology, food and feeding habits and reproductive biology [2-7], and the knowledge is expected to improve the aquaculture of this species. Global production of spotted scat has not yet been reported, but fishery production of this species in Thailand recorded in Songkhla Lagoon was approximately 12-26 metric tons/year during 2013 and 2019 [8].

In aquaculture information regarding nutritional requirements is critical because feed constitutes 40-50% of the total investment [9, 10]. In particular, the level of protein, the major nutrient of fish, substantially affects fish growth and feed cost [10, 11]. Insufficient protein intake results in growth retardation or stagnation [12], whereas fish are unable to utilise excess dietary protein [13]. Ammonia excretion increases with the increase in protein consumption, which may cause a negative effect on water quality [14]. Normally, the optimum dietary protein level for fish depends on the feeding habit of the species in the following order: carnivorous fish > omnivorous fish > herbivorous fish. The optimum dietary protein levels have been reported for many aquaculture species at the juvenile stage: 40% for salmonid fishes, 30-35% for channel catfish (*Ictalurus punctatus* Rafinesque, 1818), 37-42% for common carp (*Cyprinus carpio* Linnaeus, 1758) [15], 30% for cachama (*Colossoma macropomum* Cuvier, 1816) [11] and 61.4% for Leopard mandarin fish (*Siniperca scherzeri* Steindachner, 1892) [16]. However, there have been no studies on the optimum protein level for spotted scat. Several dietary replacement studies have been conducted employing diets with protein levels of 33-34% [17] and approximately 39% [18] using wild fingerling (3.31-3.39 g) and hatchery fish (0.62-0.67 g) respectively, but in principle the precise determination of the optimum dietary protein level should precede these interventions.

The objective of this study is to determine the optimum dietary protein level for the juvenile spotted scat using several culture parameters including growth, protein retention efficiency, body composition and amino acid profile. The information from this study will be useful in developing protein-balanced diets for growth promotion and the efficient culture of this promising aquaculture species.

## MATERIALS AND METHODS

### Experimental Diets

Five isoenergetic diets were formulated with different protein levels by the incorporation of different levels of fish meal (30, 35, 40, 45 and 50 g/100 g of diet) and soybean meal, which corresponded to proximate crude protein contents of 31.70 (T1), 35.39 (T2), 40.71 (T3), 45.88 (T4) and 51.48% (T5) respectively (Table 1). All ingredients were finely ground, mixed and pelleted through a 1.98-mm sieve of a pellet granulator machine (Hobart HL 200, Troy, USA). The dietary pellets were dried in a hot-air oven at 70°C and subsequently stored at 4°C.

### Experimental Fish and Trial Conditions

The feeding trial was conducted at the marine fish hatchery at the Coastal Aquaculture Research and Development Regional Center 6 (Songkhla), Thailand. The fish meal was bought from JFK Feed, 11 Na Wang Road, Mueang district, Nakhon Pathom province, Thailand. Prior to the feeding trial, juvenile spotted scat specimens raised in the institution were acclimatised to laboratory conditions with a commercial diet for 2 weeks in two 1,000-L tanks at a density of

approximately 200 fish/tank. After the acclimation, 15 groups (5 protein levels with 3 replicates), each consisting of 15 fish, were established and randomly distributed to 15 cylindrical fibreglass tanks with continuous aeration. The initial mean body weight was 4.92±0.06 g. Each tank was filled with 200 L of water prepared from sea water (salinity = 30-33 ppt), which was kept in a sedimentation tank for a week and then sterilised with 30 ppm sodium hypochlorite in an outdoor tank for 4-5 days. The sterilised sea water was pumped through a filter bag (5 µm) to an indoor tank, diluted with freshwater to the salinity of approximately 15 ppt, and pumped through a filter bag into experiment tanks.

**Table 1.** Formulation and proximate composition of experimental diets

| **Ingredient** | **T1** | **T2** | **T3** | **T4** | **T5** |
|---|---|---|---|---|---|
| **(g/100 g)** | (31.70% protein) | (35.39%) | (40.71%) | (45.88%) | (51.48%) |
| Fish meal (protein 66%) | 30 | 35 | 40 | 45 | 50 |
| Soybean meal (protein 55%) | 4 | 10 | 16 | 22 | 28 |
| Broken rice | 21 | 20 | 15 | 14 | 12 |
| Wheat flour | 36 | 26 | 20 | 10 | 1 |
| Sardine liver oil | 8 | 8 | 8 | 8 | 8 |
| Mineral mix [a] | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Vitamin mix [b] | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Vitamin C | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| BHT [c] | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **Proximate analysis** | | | | | |
| Crude protein (%) | 31.70 | 35.39 | 40.71 | 45.88 | 51.48 |
| Crude fat (%) | 9.12 | 10.09 | 9.80 | 9.89 | 10.24 |
| Crude fibre (%) | 14.07 | 15.14 | 15.55 | 13.67 | 11.47 |
| Ash (%) | 17.26 | 17.43 | 17.31 | 17.50 | 18.34 |
| Moisture (%) | 1.19 | 1.26 | 1.65 | 1.61 | 0.86 |
| Gross energy (kcal/100 g) | 403.4 | 403.4 | 398.4 | 397.5 | 405.2 |

[a] Per kg of mineral mixture: iron 12,000 mg; copper 12,000 mg; zinc 15,000 mg; manganese 6,000 mg; iodine 200 mg; selenium 25 mg; magnesium 50,000 mg; calcium 100,000 mg; phosphorus 80,000 mg

[b] Per kg of vitamin mixture: vitamin A 600,000 IU; vitamin D3 200,000 IU; vitamin E 6,000 IU; vitamin K 1,200 mg; vitamin B1 5,000 mg; vitamin B2 6,000 IU; vitamin B6 5,000 mg; vitamin B12 6 mg; niacin 20,000 mg; pantothenic acid 16,000 mg; folic acid 1,000 mg; biotin 200 mg; Endox Dry 20,000 mg

[c] butylated hydroxytoluene

The fish in each tank were fed to satiation twice a day at 08:30 am and 4:00 pm. During the trial, the weight of diets fed to each tank was recorded daily. Before feeding, faeces and pellet residues were removed every day by siphoning and half the water in each tank was replenished twice a day (morning and evening). The fish were weighed at the beginning of the experiment and then biweekly during the 8-week feeding trial period. Before weighing, fish were starved for 24 hr to allow the gut to empty. The proximate composition was determined for fish (five specimens) before the feeding trial to calculate the protein productive value (PPV). At the end of the feeding trial, five fish from each tank were anaesthetised, weighed and sacrificed for the determination of the whole-body chemical composition and hepatosomatic index.

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Animal experiments were conducted in 2015. All fish were maintained with care throughout the feeding trial and handled following the guidelines of the Animals for Scientific Purpose Act 2015 and the National Research Council of Thailand. A licence for using animals for scientific experiments was also acquired (licence nos. U1-04670-2559 and U1-05152-2559).

**Analytical Methods**

Whole-body samples of 5 individuals per tank were pooled and dried at 70°C for 72 hr before analysis. The proximate composition was determined according to the standard methods [19]. The moisture content was determined by oven-drying of samples at 100±1°C for 24 hr. Crude protein content was determined by the micro-Kjeldahl method. Crude lipid content was determined by petroleum ether extraction at 40-60 °C in a Soxhlet apparatus (ST 243 Soxtec™ Foss, Sweden). Crude fibre content was determined by extraction with 0.5 M $H_2SO_4$. Ash content was determined by using a muffle furnace at 600°C for 15 hr. Gross energy was measured by the ballistic bomb calorimetric method [20]. Amino acid content was determined by an HPLC system [Waters Alliance 2695 with heater, Jasco FP2020 fluorescence detector (EX: 250 and EM: 395 nm)] with a Hypersil gold column C18 (4.6×150 mm, 3 µm) at 35°C at the Central Instrument Facility, Faculty of Science, Mahidol University, Bangkok.

**Growth Performance and Feed Utilisation**

Growth performance and feed utilisation were estimated in terms of mean final body weight (FBW), per cent weight gain (WG), specific growth rate (SGR), average daily gain (ADG), feed conversion ratio (FCR), food conversion efficiency (FCE), feed intake (FI), protein efficiency ratio (PER), PPV and survival rate (SR). These indices were calculated as follows:

FBW (g) = fish biomass in tank (g)/no. of fish in tank

WG (%) = [final body weight (g) – initial body weight (g)] × 100

SGR (%/d) = [ln(final body weight) – ln(initial body weight)]/no. of feeding days × 100

ADG (g/d) = [final body weight (g) – initial body weight (g)]/no. of feeding days

FCR = feed intake (g)/weight gain (g)

FCE = [weight gain (g)/feed intake (g)] × 100

FI = feed intake (g) × 100/[(initial body weight (g) + final body weight (g))/2 × (initial fish quantity+ final fish quantity)/2 × experimental period)]

PER = body weight gain (g)/protein intake (g)

PPV = protein gain (g)/protein intake (g)

SR (%) = (final no. of fish/initial no. of fish) × 100

**Water Quality Analysis and Measurement**

During the experimental period, water quality was measured before feeding. The salinity, dissolved oxygen and water temperature were measured daily, and the ammonia-nitrogen ($NH_3$-N) content was measured biweekly. Dissolved oxygen was measured by a portable DO meter (WTW Multiline P4, Germany), whereas ammonia and nitrite contents were measured using standard test kits (V-unique, Better Syndicate Co., USA).

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### Statistical Analysis

Data are expressed as means ± SD and differences among the dietary groups were tested using one-way analysis of variance (ANOVA) followed by Duncan's test. Orthogonal polynomial contrasts were determined to test whether there were quadratic effects of dietary protein levels. We subsequently conducted the second-degree polynomial regression analysis to determine the optimum dietary protein level for the culture parameters [21]. These analyses were conducted using SPSS (version 22, Chicago, USA). The Akaike information criterion (AIC) was calculated by R version 4.0.0 and used to determine the degree of polynomial fitting with a range of 0 to 2. Differences were considered significant at $P < 0.05$.

## RESULTS

### Growth Performance

Among the growth parameters tested, the dietary protein level has significant quadratic effects on WG, SGR and ADG, but not on FBW (Table 2), although the association is close to statistical significance ($P = 0.052$). No significant effects are detected by ANOVA for these parameters ($P > 0.05$) and thus post-hoc group comparisons were not conducted.

The WG is quadratically ($P = 0.035$) affected by the dietary protein level and the highest WG is recorded in the T4 group. The SGR and ADG also show significant quadratic correlations with the dietary protein level ($P = 0.029$ and $P = 0.043$ respectively). The SGR and ADG values are also highest in the T4 group, followed by T3. Overall, the T3 or T4 dietary treatment results in the highest growth performance. Treatment T1 appears to be the least appropriate since it records the lowest values for FBW (8.64 g), WG (76.74%), SGR (1.01%/d) and ADG (0.067 g/d), although some values do not show significant differences from those of the other treatments.

**Table 2.** Effects of dietary protein level on growth performance and feed utilisation of spotted scat

| | T1 | T2 | T3 | T4 | T5 | Pooled SD | *P* value | |
|---|---|---|---|---|---|---|---|---|
| | 31.70% protein | 35.39% | 40.71% | 45.88% | 51.48% | | ANOVA | Quadratic |
| IBW (g) | 4.89 ± 0.08 | 4.88 ± 0.08 | 4.92 ± 0.06 | 4.85 ± 0.02 | 4.90 ± 0.05 | | | |
| FBW (g) | 8.64 ± 0.90 | 9.20 ± 0.65 | 9.74 ± 0.08 | 10.12 ± 0.94 | 9.19 ± 0.20 | 0.78 | 0.138 | 0.052 |
| WG (%) | 76.74± 16.44 | 88.35± 10.72 | 97.99 ± 2.33 | 108.35 ± 18.52 | 87.68 ± 4.23 | 14.48 | 0.080 | **0.035** |
| SGR (%/d) | 1.01 ± 0.16 | 1.13 ± 0.10 | 1.22 ± 0.02 | 1.31 ± 0.16 | 1.12 ± 0.04 | 0.13 | 0.070 | **0.029** |
| ADG (g/d) | 0.07 ± 0.02 | 0.08 ± 0.01 | 0.09 ± 0.00 | 0.09 ± 0.02 | 0.08 ± 0.00 | 0.01 | 0.105 | **0.043** |
| FCR | $1.39 \pm 0.29^{c}$ | $1.16 \pm 0.22^{bc}$ | $0.87 \pm 0.05^{ab}$ | $0.82 \pm 0.09^{a}$ | $1.03 \pm 0.08^{ab}$ | 0.20 | **0.013** | **0.014** |
| FI | $1.34 \pm 0.17^{c}$ | $1.26 \pm 0.14^{bc}$ | $1.02 \pm 0.01^{a}$ | $1.01 \pm 0.01^{a}$ | $1.12 \pm 0.05^{ab}$ | 0.13 | **0.012** | **0.027** |
| PER | 2.35 ± 0.55 | 2.49 ± 0.47 | 2.83 ± 0.20 | 2.70 ± 0.32 | 1.90 ± 0.13 | 0.43 | 0.079 | **0.014** |
| PPV | $0.58 \pm 0.06^{d}$ | $0.52 \pm 0.03^{cd}$ | $0.47 \pm 0.05^{c}$ | $0.39 \pm 0.04^{b}$ | $0.30 \pm 0.01^{a}$ | 0.05 | **< 0.001** | 0.335 |

Note: IBW = initial body weight, FBW = final body weight, WG = % weight gain, SGR = specific growth rate, ADG = average daily gain, FCR = feed conversion ratio, FI = feed intake, PER = protein efficiency ratio, PPV = productive protein value. Values are mean of three replications. Means within each row not sharing a common superscript are significantly different ($P < 0.05$). P values of ANOVA and quadratic regression lower than 0.05 are shown in bold.

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**Feed Utilisation, Hepatosomatic Index and Survival Rate**

The FCR and FI are both significantly affected by the dietary protein level with significant quadratic effects (Table 2). Fish in the T4 group exhibit the best FCR (0.82), although the FCR in the T4 group does not significantly differ from those of T3 and T5 groups in the post-hoc test ($P > 0.05$). The FIs in the T4 (1.01), T3 (1.02) and T5 (1.12) groups are very similar with no significant differences ($P > 0.05$), but these values are significantly lower than those of the T1 group. The PER and PPV are significantly affected by the dietary protein level with negative quadratic effects ($P < 0.001$). There are no significant differences in the SR values (98.33-100%) among the groups ($P > 0.05$).

**Determination of Optimum Protein Level**

The optimum protein levels were determined for WG, SGR, ADG, FCR, FI and PER, on which the dietary protein level has significant quadratic effects. FBW is included in this analysis since the quadratic effect is close to statistical significance (P = 0.052). The AIC supports the use of the quadratic model in this regression for these parameters. The optimum dietary protein levels for FBW, WG, SGR and ADG are calculated to be 44%, 43%, 44% and 42% respectively (Figure 1). Similarly, the optimum dietary protein levels for FCR, FI and PER are 44%, 45% and 41% respectively (Figure 2).

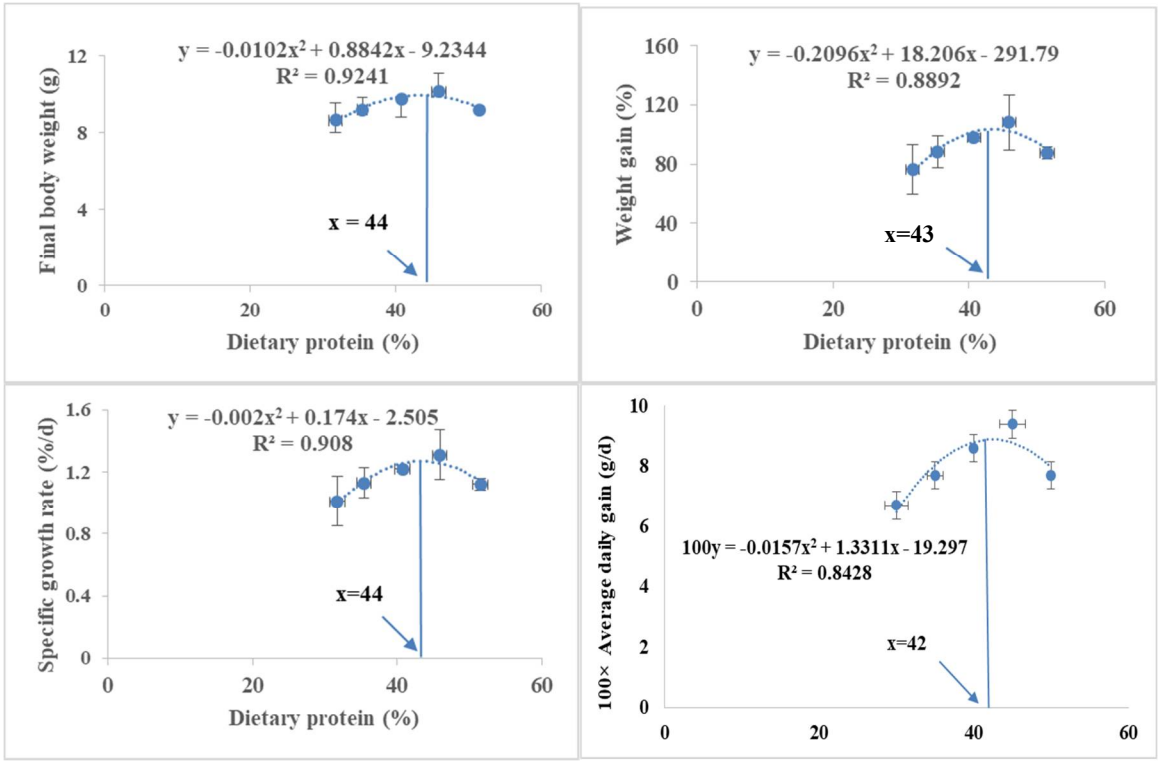


**Figure 1**. Second-order polynomial relationship of final body weight (FBW), weight gain (WG), specific growth rate (SGR) and average daily gain (ADG) to dietary protein levels for spotted scat. Note that ADE values are multiplied 100 times to obtain accurate coefficients.

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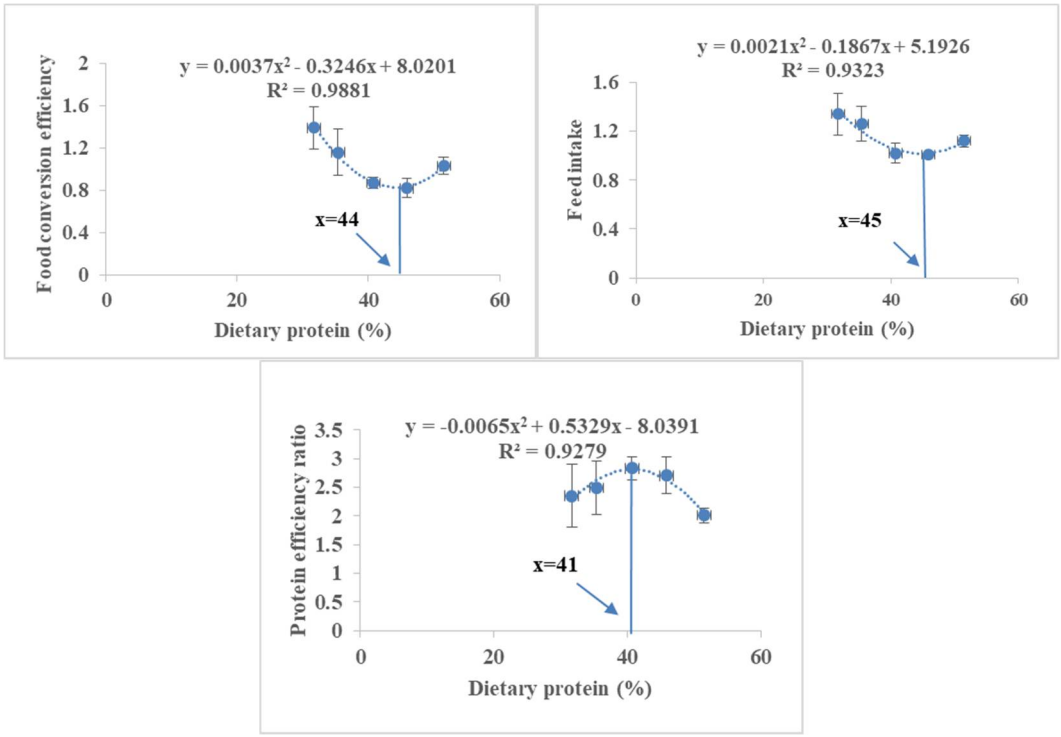


**Figure 2**. Second-order polynomial relationship of food conversion efficiency (FCE), feed intake (FI) and protein efficiency ratio (PRR) to dietary protein levels for spotted scat

## Body Composition

The dietary protein level has significant effects on the moisture, crude protein and crude lipid contents but not on the ash content (Table 3, ANOVA). There are no significant quadratic effects of the dietary lipid content on these parameters ($P > 0.05$). The crude protein content tends to increase with increasing level of dietary protein, which is the opposite of the crude lipid content. The highest crude protein level (20.53%) is recorded in the T4 group, but the crude protein content in the T4 group does not significantly differ from those of the T3 and T5 groups (19.98 and 19.46% respectively). Similarly, the crude lipid content is the lowest in the T5 group (6.49%) but this value does not significantly differ from those of the T3 and T4 groups.

**Table 3.** Effects of dietary protein level on whole body composition of spotted scat

| | T1 | T2 | T3 | T4 | T5 | Pooled SD | *P* value | |
|---|---|---|---|---|---|---|---|---|
| | 31.70% protein | 35.39% | 40.71% | 45.88% | 51.48% | | ANOVA | Quadratic |
| Crude protein | 19.20$^{a}$ (58.55 ± 0.31) | 20.34$^{b}$ (61.14 ± 1.73) | 19.98$^{bc}$ (61.91 ± 0.51) | 20.53$^{c}$ (63.61 ± 1.14) | 19.46$^{c}$ (63.63 ± 0.88) | 1.15 | **0.001** | 0.185 |
| Crude lipid | 8.63$^{a}$ (26.31 ± 2.30) | 8.72$^{a}$ (26.20 ± 1.71) | 7.78$^{a}$ (24.10 ± 0.96) | 7.13$^{a}$ (22.10 ± 1.70) | 6.49$^{a}$ (21.20 ± 1.54) | 1.85 | **0.019** | 0.548 |
| Ash | 5.57 (16.99 ± 0.40) | 5.92 (17.78 ± 1.77) | 5.78 (17.90 ± 1.76) | 5.78 (16.76 ± 1.43) | 5.47 (17.90 ± 1.42) | 1.55 | 0.853 | 0.711 |
| Moisture | 67.20 ± 0.86$^{a}$ | 66.73 ± 1.40$^{a}$ | 67.73 ± 0.16$^{ab}$ | 68.40 ± 1.17$^{b}$ | 69.42 ± 0.82$^{b}$ | 1.08 | **0.047** | 0.149 |

Note: Means within each row not sharing common superscript are significantly different ($P < 0.05$). Values in parentheses represent % dry matter. P values of ANOVA and quadratic regression lower than 0.05 are shown in bold.

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**Amino Acid Profile**

The whole-body total amino acid content is significantly affected by the dietary protein level (Table 4, ANOVA, $P < 0.001$). The total amino acid content tends to increase with increasing level of dietary protein up to 35% (T1 and T2 groups) and declines thereafter (T3, T4 and T5 groups). The maximum level of total amino acid content (48.36 mg/100 mg) is found in the fish in the T2 group. Significant quadratic effects of dietary protein level on the total amino acid content are found ($P < 0.001$).

The levels of essential and non-essential amino acids (EAAs and non-EAAs respectively) in the bodies of the fish fed different levels of dietary protein are also determined (Table 4). The amounts of EAAs are in the order of histidine < isoleucine < phenylalanine < threonine < valine < leucine < arginine < lysine, while those of non-EAAs are glutamic acid > glycine > aspartic acid > alanine > proline > serine > tyrosine. ANOVA and the trend estimation using orthogonal polynomial contrasts detects significant effects of dietary protein levels on the content of most amino acids except for histidine, lysine, glutamic acid, glycine and aspartic acid ($P < 0.05$). The levels of tyrosine, isoleucine, threonine, valine, arginine, leucine, phenylalanine, alanine, proline and serine tend to follow the trend of total amino acids — the levels of both EAAs and non-EAAs are high in T1–T3 groups and low in T4 and T5 groups. The highest levels are recorded in the T2 group for most EAAs (isoleucine, phenylalanine, threonine, valine, leucine and arginine) and non-EAAs (glycine, alanine, proline, serine and tyrosine).

**Table 4.** Effects of dietary protein level on amino acid profiles of spotted scat

| Amino acid (mg/100 mg dry diet) | T1 31.70% protein | T2 35.39% | T3 40.71% | T4 45.88% | T5 51.48% | Pooled SD | *P* value ANOVA | *P* value Quadratic |
|---|---|---|---|---|---|---|---|---|
| **EAA** | | | | | | | | |
| Histidine | 0.83 ± 0.06 | 0.91 ± 0.04 | 0.87 ± 0.05 | 0.83 ± 0.05 | 0.85 ± 0.00 | 0.05 | 0.129 | 0.147 |
| Isoleucine | 1.77 ± 0.06$^{ab}$ | 1.90 ± 0.04$^{c}$ | 1.84 ± 0.10$^{bc}$ | 1.74 ± 0.04$^{ab}$ | 1.68 ± 0.06$^{a}$ | 0.07 | **0.016** | **0.014** |
| Phenylalanine | 1.91 ± 0.03$^{bc}$ | 2.04 ± 0.03$^{d}$ | 1.91 ± 0.07$^{c}$ | 1.86 ± 0.06$^{ab}$ | 1.75 ± 0.02$^{a}$ | 0.06 | **<0.001** | **0.003** |
| Threonine | 2.18 ± 0.03$^{b}$ | 2.30 ± 0.04$^{c}$ | 2.23 ± 0.06$^{b}$ | 2.16 ± 0.02$^{b}$ | 2.05 ± 0.02$^{a}$ | 0.05 | **<0.001** | **<0.001** |
| Valine | 2.20 ± 0.05$^{ab}$ | 2.34 ± 0.05$^{c}$ | 2.27 ± 0.10$^{bc}$ | 2.17 ± 0.05$^{ab}$ | 2.14 ± 0.06$^{a}$ | 0.07 | **0.020** | **0.025** |
| Leucine | 3.28 ± 0.08$^{b}$ | 3.49 ± 0.09$^{c}$ | 3.36 ± 0.16$^{b}$ | 3.21 ± 0.04$^{b}$ | 3.13 ± 0.06$^{a}$ | 0.11 | **0.008** | **0.014** |
| Arginine | 3.48 ± 0.09$^{c}$ | 3.60 ± 0.07$^{d}$ | 3.43 ± 0.04$^{bc}$ | 3.31 ± 0.08$^{b}$ | 3.08 ± 0.05$^{a}$ | 0.08 | **<0.001** | **<0.001** |
| Lysine | 3.49 ± 0.16$^{a}$ | 3.63 ± 0.29 | 3.74 ± 0.14 | 3.62 ± 0.17 | 3.59 ± 0.11 | 0.21 | 0.620 | 0.190 |
| **non-EAA** | | | | | | | | |
| Glutamic acid | 6.97 ± 0.19 | 7.25 ± 0.49 | 7.20 ± 0.12 | 6.77 ± 0.27 | 7.11 ± 0.14 | 0.32 | 0.287 | 0.679 |
| Glycine | 5.20 ± 0.20 | 5.33 ± 0.32 | 5.10 ± 0.11 | 4.99 ± 0.26 | 4.85 ± 0.10 | 0.25 | 0.146 | 0.404 |
| Aspartic acid | 4.66 ± 0.10 | 4.82 ± 0.38 | 4.83 ± 0.10 | 4.50 ± 0.24 | 4.67 ± 0.14 | 0.26 | 0.406 | 0.531 |
| Alanine | 3.48 ± 0.09$^{bc}$ | 3.60 ± 0.07$^{c}$ | 3.43 ± 0.04$^{bc}$ | 3.31 ± 0.08$^{a}$ | 3.08 ± 0.05$^{ab}$ | 0.06 | **0.005** | 0.139 |
| Proline | 3.05 ± 0.05$^{b}$ | 3.12 ± 0.16$^{b}$ | 3.03 ± 0.04$^{b}$ | 2.99 ± 0.13$^{b}$ | 2.78 ± 0.05$^{a}$ | 0.12 | **0.023** | 0.050 |
| Serine | 2.21 ± 0.10$^{abc}$ | 2.32 ± 0.08$^{c}$ | 2.24 ± 0.06$^{bc}$ | 2.15 ± 0.02$^{ab}$ | 2.08 ± 0.03$^{a}$ | 0.08 | **0.012** | **0.022** |
| Tyrosine | 1.30 ± 0.04$^{ab}$ | 1.42 ± 0.03$^{c}$ | 1.35 ± 0.06$^{b}$ | 1.33 ± 0.04$^{ab}$ | 1.28 ± 0.00$^{a}$ | 0.04 | **0.007** | **0.003** |
| Total | 46.3 ± 0.20$^{a}$ | 48.4 ± 0.95$^{a}$ | 47.2± 0.80$^{ab}$ | 45.3± 0.09$^{ab}$ | 44.8 ± 0.08$^{b}$ | 0.67 | **<0.001** | **<0.001** |

Note: Values are mean of three replications. Means within each row not sharing a common superscript are significantly different ($P < 0.05$). P values of ANOVA and quadratic regression lower than 0.05 are shown in bold.

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### Water Quality

The temperature, salinity, dissolved oxygen and ammonia-nitrogen concentration of water are shown in Table 5. Dietary protein levels have positive quadratic ($P < 0.05$) effects on ammonia nitrogen. The ammonia level fluctuates; it is low in the treatments T1, T2 and T3 (0.59, 0.21 and 0.07 mg/l respectively), high in T4 (0.93 mg/l) and low in T5 (0.34 mg/l). The minimum level of ammonia is found in the water used in fish culture in treatment T3. Dietary protein level does not have significant effects on other water quality parameters.

**Table 5.** Effects of dietary protein level on water quality for culturing spotted scat fed the experimental diets

| | T1 | T2 | T3 | T4 | T5 | Pooled SD | *P* value | |
|---|---|---|---|---|---|---|---|---|
| | 31.70% protein | 35.39% | 40.71% | 45.88% | 51.48% | | ANOVA | Quadratic |
| Temperate (˚C) | 27.4 ± 0.1 | 27.6 ± 0.5 | 27.6 ± 0.0 | 27.6 ± 0.1 | 27.7 ± 0.1 | 0.48 | 0.986 | 0.826 |
| Salinity (ppt) | 15.2 ± 0.1 | 15.1 ± 0.1 | 15.2 ± 0.1 | 15.2 ± 0.1 | 15.1 ± 0.1 | 0.12 | 0.926 | 0.674 |
| Dissolved oxygen (mg/l) | 5.52 ± 0.17 | 5.29 ± 0.12 | 5.15 ± 0.07 | 5.21 ± 0.09 | 5.11 ± 0.10 | 0.14 | 0.858 | 0.601 |
| Ammonia-nitrogen (mg/l) | $0.59 \pm 0.09^{d}$ | $0.21 \pm 0.09^{b}$ | $0.07 \pm 0.04^{a}$ | $0.93 \pm 0.02^{e}$ | $0.34 \pm 0.05^{c}$ | 0.08 | **<0.001** | **<0.001** |

Note: Values are mean of three replications. Means within each row not sharing a common superscript are significantly different ($P < 0.05$). P values of ANOVA and quadratic regression lower than 0.05 are shown in bold.

## DISCUSSION

The dietary protein level is of fundamental importance in aquaculture because it influences both fish growth and the rearing cost. In the present study the dietary protein level has a significant quadratic effect on the growth (WG, SGR and ADG), feed utilisation (FCR, FI and PER), and amino acid profiles of juvenile spotted scat. The optimum dietary protein levels for the growth and feed utilisation parameters were determined to be 41-45%, which is generally consistent with those in previous reports on other species, e.g. 55% for the larvae of carnivorous African obscure snakehead (*Parachanna obscura* Günther, 1861) [22] and 43% for the omnivorous North African catfish (*Clarias gariepinus* Burchell, 1822) [23]. Further increase in the dietary protein level decreases the growth and feed utilisation parameters in this study, as also reported in stinging catfish (*Heteropneustes fossilis* Bloch, 1794) [24], cachama [11] and African obscure snakehead [22]. On the other hand, even though the optimum dietary protein level is calculated to be 41-45%, there are no significant differences in growth performance parameters in treatments T2-T4. These results suggest that the omnivorous spotted scat has a certain range of dietary protein requirements, and diets containing ~35% protein would be of economic advantages in rearing juvenile spotted scat.

The FCR, FI and PER are generally used as indices of food and protein utilisation in fish. In the present study the FCR and FI values show a similar trend. When the dietary protein level increases, the fish eat less food (low FI) and the FCR improves (i.e. is reduced), except for treatment T5, where the protein content is higher than the optimum level. It is interesting to note that while increasing levels of dietary protein generally increase protein intake, they lead to a decrease in the PER once the level of dietary protein exceeds the level appropriate for growth, which is in agreement with a previous study [22]. This is probably because protein catabolism

requires higher energy [24] although the activity of digestive enzymes generally increases with the dietary protein level for effective digestion (e.g. [25]). Meanwhile, there are relatively large variations in the FCR and FI in this study. This may be due to fish behaviour; fish are known to stop eating once their energy requirements are met [26]. Potentially due to the large individual variations, PER values of treatments T1-T3 are not significantly different ($P > 0.05$) from those of T4 and T5.

In the present study the whole-body proximate composition of the fish is influenced by the dietary protein level. The protein and moisture contents tend to increase with the dietary protein level while the lipid content has an inverse relationship. The ash content is not significantly affected by the dietary protein level. Previous studies have reported similar results for the body protein and ash contents of the North African catfish [27] and cachama [11]. A number of previous studies have reported the inverse relationship between carcass lipid content and dietary protein level in fish, including Asian redtail catfish (*Mystus nemurus* Valenciennes, 1840), Malabar grouper (*Epinephelus malabaricus* Bloch & Schneider, 1801), silver perch (*Bidyanus bidyanus* Mitchell, 1838), red porgy (*Pagrus pagrus* Linnaeus, 1758), cachama, and topmouth culter (*Culter alburnus* Basilewsky, 1855) [11, 28-32]. However, this trend may be species-specific. Stinging catfish and North African catfish show a positive correlation between dietary protein and carcass lipid level [24, 27] while Sankian et al. [16, 33] reported that the chemical composition of leopard mandarin fish (*Siniperca scherzeri* Steindachner, 1892) is not significantly affected by dietary protein levels of 35-55% and 45-65%.

Amino acids are essential for fish as the basic components of protein biosynthesis and as a major regulator of metabolism. The whole-body content of essential amino acids in banded astyanax (*Astyanax fasciatus* Cuvier, 1819) is in the order of histidine < isoleucine < phenylalanine < threonine < valine < arginine < leucine < lysine [34], which is similar to those in the present study except for arginine and leucine. In the present study the highest values of amino acid content are generally observed in the T2 and T3 groups, whereas the lowest values are observed in the T5 group. Normally, fish fed diets with the optimum protein level show the highest total body amino acids [24]. In the present study, however, the dietary protein level of 35.39% (T2 group), which is lower than the calculated optimum protein levels, results in the highest whole-body amino acid levels. These results suggest that the T2 diet may have provided a balanced amino acid composition for spotted scat. This hypothesis is worth further validation because the amino acid balance has a direct influence on fish growth, at least partly because of the function of individual amino acids. For example, leucine is known to stimulate the synthesis of muscle proteins [35]; histidine is important for growth, tissue repair, nourishment of the myelin sheath of neurons and elimination of metals [36]. Lysine is often the first limiting EAA in fish nutrition and adequate lysine supplementation enhances growth and feed efficiency of fish [37]. The lysine-rich ingredients of fish feeds are fish meal and blood meal, which are important for improving the cost-effectiveness of feeds [34].

The dietary protein level does not significantly affect water salinity, temperature and dissolved oxygen level in the present study. The frequent water changes might also have contributed to the marginal difference in water quality between groups. On the other hand, a higher ammonia concentration is observed in T4 group, which might have reduced fish growth. The ammonia excretion level is usually proportional to the dietary protein level because ammonia is produced from protein catabolism [38], but the relationship between dietary protein level, ammonia excretion and fish metabolism is often not simple. A quadratic relationship between protein content and ammonia excretion has also been reported [14]. Our results in which spotted scat show low

ammonia excretion at dietary protein levels of 40.71% and 35.39% (T2 and T3 groups) may suggest a well-balanced protein metabolism with these levels of dietary protein. If this is the case, feeding fish diets containing 35-40% protein would be an economically feasible option for rearing juvenile spotted scat.

A limitation of the present study is the short experimental period. Spotted scat grew from about 4.9 g to 8.5–10 g during the feeding trial, which corresponds to up to about 100% weight increase. The low weight increase obtained is primarily attributed to the high initial weight of about 4.9 g. In previous growth trials spotted scat juveniles of up to about 1-640 mg were used [17, 18, 39, 40]. This has made our regression analysis conservative, although we were still able to detect significant effects of the protein level on most parameters (Figures 1 and 2). A longer experimental period may identify the optimum protein levels on other parameters. Another limitation is that our feeding trial did not include a digestibility analysis, which may explain the changes in the PER observed. More comprehensive feeding trials using spotted scat of a small initial size will be useful for generalising our results further.

## CONCLUSIONS

The present study has determined for the first time the optimum dietary protein for juvenile spotted scat in terms of growth, feed utilisation, body composition and amino acid profiles. While the regression analysis leads to an optimum dietary protein level of 41-45%, many parameters indicate the advantage of using diets containing ~35% protein, which is considered to be a practical culture setting for reduction of the cost of diet, efficient conversion of protein for growth, and low ammonia excretion to the environment. The breeding of this species has just started at the industrial level and little information is available on the raising conditions. The results of this study may be useful in developing protein-balanced diets for establishing intensive and sustainable cultures of spotted scat.

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