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Evaluation of conventional or hydrolyzed stickwater from food-grade skipjack tuna by-product in diet for hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂)

Yuexing Zhang [1], Lei Zhang [1], Liying Huang [2*], Zhiyong Dong [1,3], Qiang Lu [1,3], Yuan Zou [1,3], Feng Tang [4], Shuaibing Zhao[5], Trond Storebakken[3]

[1] Zhejiang Ocean University, Marine Science and Technology College, National Engineering Research Centre for Marine Aquaculture, Zhoushan, Zhejiang, 316022, China

[2] Zhejiang Marine Fisheries Research Institute, Zhoushan, Zhejiang, 316021, China

[3] Norwegian University of Life Sciences, Faculty of Biosciences, Department of Animal and Aquacultural Sciences, NO-1432 Ås, Norway

[4] Zhejiang Fengyu Marine Organism Products Co., Ltd., R&D Centre, Zhoushan, Zhejiang 316100, China

[5] Ningbo Tech-Bank Feed Industry Co., Ltd., Yuyao, Zhejiang, 315400, China

*Corresponding author. Phone +8615957098788; Email: hlypcd@163.com

Abstract

The aim of this study was to investigate how dietary conventional (CSW) or hydrolyzed (HSW) stickwater from skipjack tuna by-product affected growth and feed utilization of hybrid grouper. Nine isonitrogenous (500-505 g crude protein (CP) (kg DM)$^{-1}$) and iso-energetic (20.3-20.9 MJ gross energy (GE) (kg DM)$^{-1}$) extruded diets were formulated. A control diet (FMC) was produced with 450 g kg^{-1} inclusion of fish meal (FM), but without stickwater. The other eight diets were made with a 1:1:1 mixture (dry matter based) of soy protein concentrate (SPC), corn gluten meal (CGM), and CSW or HSW replacing 10, 20, 40 and 60% of FM from the FMC diet. The diets were fed to triplicate groups of hybrid grouper 4 times a day for 8 weeks. The fish were raised in a recirculated aquaculture system (RAS) with 25 ppt salinity and 30°C average temperature. Feed intake (FI) did not significantly ($P > 0.05$) differ among the dietary treatments. Gradually increased replacement with CSW resulted in linear, negative dose response in weight gain (WG), and a linear positive response in FCR. The hepatosomatic index (HSI) decreased linearly. Whole body CP, lipid and ash, and the apparent digestibility (ADC) of energy and the amino acids Ile and Met decreased in a quadratic manner. Linear, negative responses were seen for the ADC of Lys, Thr, Val, and the sum of essential and total amino acids. Replacing with HSW did not significantly affect WG, FCR, HSI, VSI, whole body dry matter, CP, fat contents, protein- or energy efficiencies. Whole-body ash content showed a negative quadratic response. Factorial ANOVA showed that fish fed HSW grew significantly faster and converted the feed more efficiently than the CSW. There was also a tendency ($P=0.061$) that fish fed the HSW diets ate more than the fish receiving the CSW feeds. In conclusion, both feeds with CSW and HSW supported rapid growth and efficient feed conversion of hybrid grouper. Fish fed diets with HSW grew faster and utilized the nutrients more efficiently than did the fish fed diets with CSW.

Keywords: Hydrolysate, Stickwater, Tuna by-product, Hybrid grouper, Growth performance, Digestibility

1. Introduction

High market price and demand for fish meal (FM) in the fish feed industry and rising ecological concerns about FM use has prompted research to identify alternative protein sources to minimize the use of FM in fish feeds (Shi et al., 2019, Siddik et al., 2019). High quality protein sources in terms of palatability, essential amino acid profiles and high digestibility are needed to satisfy these demands. Plant protein sources can successfully be used as FM replacer in feeds for omnivorous fish species (Egerton et al., 2020). However, replacing fishmeal with plant proteins may affect growth and health of carnivorous fish (Green et al., 2013). Plant protein sources have been associated with indigestible carbohydrates and antinutritional factors which hinders feed intake, growth, digestion, nutrient absorption, and disease resistance in fish (Shi et al., 2019; Richard et al., 2017). Most of plant protein sources are deficient in one or several essential amino acids and low molecular-weight compounds like taurine and choline which are vital for growth and health of carnivorous fish (Faudzi et al., 2017; Hansen et al., 2020). In this regard, other marine protein sources other than FM are increasingly used in fish feeds. This use of marine feed ingredients has been reported to support growth, digestibility, and immunity like that obtained in fish fed FM (Estruch et al., 2020; Kotzamanis et al., 2007).

Hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) is a species firstly created in 2006 in Malaysia (Ch'ng & Senoo, 2008). Commercial aquaculture of this hybrid has a comparative advantage over its parental species, the brown-marbled grouper (*E. fuscoguttatus*) and giant grouper (*E. lanceolatus*) in the term of growth rate (Dennis et al., 2020), disease resistance, and ability to adapt to a wide salinity range (Ye et al., 2020). This has made hybrid grouper a promising marine cultured species in China. As a carnivorous fish, it has a high protein demand in feed. Yong et al. (2019) recommended 50% dietary protein for the juveniles of this species while Jiang et al. (2016) suggested an optimal protein requirement of 53.5%. A few studies had attempted to replace a fraction of FM in diet of hybrid grouper with different kind of ingredients such as hemoglobin powder (Yao et al., 2018), poultry by-product meal and insect meal (Mohamad-zulkifli et al., 2019), soybean meal (Zhou et al., 2020), peanut meal (Ye et al., 2020) and soy protein concentrate (SPC) (Wang et al., 2020).

Currently, fractions from FM processing are partially recovered to be used as fish feed ingredients. Stickwater (SW) consists of water solubles from FM processing. This feed ingredient has been used to produce aquatic species with good performance including yellow catfish *(Pelteobagrus fulvidraco)* (Wu et al., 2018), giant freshwater prawn (*Macrobrachium rosenbergii*) (Wattanakul et al., 2017), and Atlantic salmon (*Salmo salar* L.) (Berge and Storebakken, 1996; Kousoulaki et al., 2009). The good performance is ascribed to water soluble peptides, free amino acids, taurine, lipid, vitamins, and minerals (Mahdabi & Hosseini Shekarabi, 2018; Wu et al., 2018; Bechtel, 2005). SW of high freshness can also be used as feed attractant (Wattanakul et al., 2017). The concentration of water-soluble proteins is high and makes SW a digestible pellet binder. This leads to improved feed physical quality, reducing nutrient leaching and pollution of the environment (del Valle & Aguilera, 1991; Samuelsen et al., 2012). Despite of the potentiality of SW as aquafeed ingredient, little or no information is known about its potential for use in feeds for grouper.

Hydrolysates of fish protein are characterized by a balanced amino acid profile (Chalamaiah et al., 2012) and antioxidant properties which can boost immunity of fish to fight pathogens (Saadaoui et al., 2019; Murray et al., 2003). Gisbert et al. (2018) reported that a diet containing marine protein hydrolysate enhanced non-specific humoral immunity of European sea bass (*Dicentrarchus labrax*), affected by an outbreak of *Vibrio pelagius* infection. Dietary SW and hydrolysates have also been reported to increase appetite, enhance digestible enzyme activities, and improve absorption of nutrients, resulting in boosted fish growth (Aksnes et al., 2006; Kousoulaki et al., 2012; Ospina-Salazar et al., 2016; Khosravi et al., 2015). Protein hydrolysates had also been reported to improve nutrient absorption due to their content of readily digestible free amino acids and small peptides (Olsen & Toppe, 2017). However, excessive use of SW and protein hydrolysates may result in decreased growth performance (Hevrøy et al., 2005).

It is possible to have good growth performance in carnivorous fish like Atlantic salmon fed a diet completely devoid of marine protein, but with marine lipids (Davidson et al., 2016). However, a combination of marine co-products and terrestrial protein sources may ease formulation and processing of high-quality fish feed. Thus, the current study aimed to: 1) to investigate how conventional (CSW) or hydrolyzed (HSW) stick water from skipjack tuna (*Katsuwonus pelamis*) by-product affected growth and feed utilization of hybrid grouper, 2) to compare the effect of the two types of SW as alternative protein of FM to reveal if hydrolyzation would improve the performance on the above responses and 3) to determine the optimal inclusion level of CSW or HSW in hybrid grouper diet. This study also aims at contributing to the development of cost effective and nutrient-balanced diets for hybrid grouper using SW.

2 Materials and methods

2.1 Main ingredients and diets

CSW or HSW were produced from the same batch of filleting by-product (heads, guts, trimmings, and frames) from food-grade skipjack tuna. The CSW was made by vacuum concentrating SW from a fishmeal processing line, while the HSW was subject to enzymatic hydrolysis prior to vacuum concentration. The CSW was an experimental batch produced especially for current experiment. The HSW is commercially available from Zhejiang Fengyu Marine Organism Products Co., Ltd., Zhoushan, Zhejiang, China. The characteristics of the two stickwater qualities is presented in Table 1. Compared to the CSW, the dry matter of the HSW contained more acid soluble protein, peptides, and amino acid nitrogen. Histamine was higher in the CSW. The two hydrolysates had similar concentrations of hydrolyzed amino acids and total free amino acids in their dry matter. Peptides in the CSW had the highest contribution to peptides ranging 500 Dalton (Da) and below, and 5 000 Da and above, both when expressed as a part of dry matter, and as a part of acid soluble protein. The hydrolysate had a lower proportion of peptides sized less than

180 Da than in the range of 500-180 Da. Except from this, the contribution of peptides declined in a polynomial manner with peptides ranging from 500-180 Da (337.0 g (kg soluble protein)$^{-1}$), to more than 10 000 Da (3.3 g (kg soluble protein)$^{-1}$).

The analyzed compositions of the protein-rich ingredients are presented in Table 2. The most notable difference in proximate composition between CSW and HSW was that the CSW contained 47% dry matter (DM), while the DM content of the HSW was 5 percent units higher. There also was a high degree of similarity between the two hydrolysates with respect to the amino acid composition.

Nine isonitrogenous (500-505 g crude protein (CP) (kg DM)$^{-1}$) and iso-energetic (20.3-20.9 MJ gross energy (GE) (kg DM)$^{-1}$) diets were formulated as described in Table 3. The FMC was a control diet with 450 g kg^{-1} high-quality FM, but without CSW or HSW. In the other 8 diets a 1:1:1 (dry matter based) mixture of SPC, corn gluten meal (CGM), and stickwater (CSW or HSW) was used to gradually replace FM from the FMC diet. A 2*4 factorial design was adopted with 2 stickwater types (CSW or HSW) in combination with 4 levels of FM replacement (10, 20, 40 and 60%). The 8 diets in which FM was replaced were named as CSW10 or HSW10, CSW20 or HSW20, CSW40 or HSW40, and CSW60 or HSW60, respectively. Crystalline *L*-lysine and *D, L*-methionine were also supplemented to optimize the dietary EAA profile of the diets with 40 and 60% replacement. The same diets were also supplemented with mono calcium phosphate (MCP). Yttrium oxide (Y_2O_3) was used as inert marker for digestibility measurement in accordance with Austreng et al. (2000).

The diets were extruded at the Feed Technology Laboratory of the Sino-European Aquatic Nutrition and Feed Resources Institute, Zhejiang Ocean University. All dry ingredients were ground through a 0.18-mm screen and mixed. Then 40% of water and relevant SW were sprayed into the mash and mixed for 30 min. The moistened mash was kept at room temperature for 12h to facilitate water penetration. The moistened mash was heated to 95-100 °C for 5 min by microwave preconditioning before it was extruded by a laboratory scale twin-screw extruder (Saibainuo, SYSLG30-IV, Jinan, China) with 5.0 mm die. The extrusion parameters that were controlled included feeding rate, screw speed and 4 individual barrel section temperatures, targeting a slowly sinking pellet with bulk density of the extrudate at around 520g l^{-1}. After forced air drying, the pellets were coated with fish oil using a laboratory scale vacuum coater. The oil had been pre-heated to 50 °C in a water bath. After sieving and removal of damaged pellets, the finished diets were stored at −10 °C until used.

2.2 Fish and feeding

A batch of about 3000 hybrid grouper fingerlings with individual weight around 5g were obtained from a local hatchery (Hongsheng Aquaculture Co., Xiangshan, Zhejiang) and kept for one year in the Fish Laboratory of Sino-European Aquatic Nutrition and Feed Resource Institute, Zhejiang Ocean University, where they were fed a commercial feed with 52% CP and 8% fat. Four weeks prior to the start of feeding trial, 300 groupers with uniform size were anaesthetized with MS-222 (1g l^{-1}) and tagged by injecting a Passive Integrated Transponder (PIT, Smartrac N.V., Amsterdam, Netherland) under the superficial skin close to the dorsal fin.

The feeding trial was conducted in an indoor recirculated aquaculture system (RAS). Before the trial started, the fish were deprived of feed for 48h. Then a total of 540 hybrid grouper with average weight of 0.36 kg were randomly distributed into 27 cylindrical fiberglass tanks with a total volume of 500 l. Ten fish were tagged and the remaining 10 individuals per tank were tagged. Each tank was supplied with seawater at a flow rate of 4-5 l min^{-1} and aeration 24h day^{-1}. A photoperiod of 14L:10D was maintained. The water temperature ranged from 27.5 to 32.0 °C, with an average at 30.0 °C. Dissolved oxygen levels were above 5.0 mg l^{-1} in the outlet water, ammonia less than 0.2 mg l^{-1}, and salinity around 25 ppt, based on daily measurements. Each diet was manually fed to fish in 3 parallel tanks, 4 meals per day (08:00, 11:30, 15:00 and 20:00) with 45 min per meal. After each feeding, all uneaten pellets were siphoned out immediately and counted. Uneaten feed was quantified by the method of Zhang et al. (2012a), except the average pellet weight for each feed was obtained by counting 3×1000 pellets. The daily feeding rate was tentatively set 10% in excess based on the average feed intake over the last 3 day's feeding, and fish received more feed if they showed signs of feeding at the end of each meal. The feeding trial lasted for 8 weeks. The entire study consisted of a 56-day feeding trial and a simultaneous 30-day digestibility trial.

2.3 Sampling

Before the feeding trial started, 3×5 fish from the acclimation tank were depleted of feed for 24 h, killed by an overdose of MS-222, and kept at -20 °C for whole-body analysis. In the beginning (Day 0) and at Days 14, 28, and 42, all fish were depleted of feed for 24 h, then gently netted out and anaesthetized. The PIT tagged groupers were weighed individually while the other fish were weighed in batch after wipe-drying.

At the end of feeding trial (Day 57), 3 PIT tagged fish were randomly sampled from each tank for plasma and liver samples. Fish were weighed individually, blood was drawn from the caudal vein with heparinized vacutainers, kept on ice until centrifugation (3000 G for 10 min). The obtained plasma was aliquoted into two separate Eppendorf tubes, frozen in liquid N_2 and kept at -80 °C until analysis. The same fish were dissected to remove whole viscera. The contents in stomach and intestine, liver, and carcass were weighed separately. Another 3 untagged fish were randomly taken from each tank, measured for weight and body length individually, and then killed by a blow to the head. Fish were dissected to remove the contents in stomach and intestine. The whole viscera, liver and carcass were weighed separately, and then stored at -20 °C for whole body analysis.

Each of the diets was also fed to another triplicate tanks of fish for digestibility assessment, with 22 fish (320g in average) in each tank. The same feeding strategy and husbandry condition as growth trial were adopted. Fecal samples were obtained by careful stripping from the last 5 cm of the distal intestine of fish after anaesthetization on Day 5, Day 15, and Day 30, and then pooled by tank and stored at -20 °C prior to analysis.

2.4 Analyses

The initial and final whole-body samples within same tank were homogenized by a meat grinder, autoclaved (YXQ-LS, Xunbo, Shanghai, China) at 120 °C for 30 min, re-homogenized, oven-dried (Jinghong, Zhejiang, China) at 80 °C, and finely ground into powder prior to analysis. Pooled feces samples were freeze-dried and ground with a pestle and mortar. The processed fish samples, feed and ingredient samples were analyzed for dry matter (105 °C to constant weight), Kjeldahl N (Opsis KD-310, Sweden), crude lipid (ether extraction, Opsis SX-360, Sweden), gross energy (Parr, 1271, USA), and ash (550°C, Muffle furnace). Amino acids (except tryptophan) of all samples were analyzed by amino acid analyzer (Hitachi, L-8900, Japan) with 6 M HCl hydrolysis for 22-24 h at 110°C. The sulphur-containing amino acids were determined based on a process of oxidative hydrolysis with performic acid for 30 min at 55°C. Yttrium concentration in feed and feces were determined by inductively coupled plasma mass spectroscopy (Agilent, ICP-MS 7900, USA) after complete digestion of homogenized and dried sample in HNO_3 after cooking in a microwave oven for 1 h (Zhang et al., 2012b). Chemical compositions and peptide contents of CSW and HSW were analyzed from Analysis and Testing Center of Jiangnan University (Wuxi, China) according to the method of GB/T 22729-2008 and previous study (Wu et al., 2018). Briefly, acid soluble protein and peptide molecular weight distribution were analyzed by Kjeldahl method and the HPLC, respectively.

2.5. Calculations and statistical analysis

Feed intake (FI) was quantified by subtracting uneaten feed from the amount of fed on a dry matter basis. Weight gain (WG, g) was calculated as: WG = FBW − IBW, where FBW and IBW represent final body weight and initial body weight, respectively. Feed conversion ratio (FCR) was calculated as: $FI \times (FBW - IBW)^{-1}$. Apparent digestibility coefficients of individual nutrient and energy were calculated as: $100 \times (1 - (Y_d \times Y_f^{-1} \times N_f \times N_d^{-1}))$, where Y_d and Y_f represent the concentration of yttrium in diet and faeces, N_d and N_f represent the concentration of individual nutrient or energy in diet and faeces, respectively. Nutrient and energy retentions were calculated as: $100 \times (N_1 \times FBW - N_0 \times IBW) \times (N_d \times FI)^{-1}$, where N_0 and N_1 represent the nutrient or energy concentration in the initial and final whole-body samples, respectively. Hepatosomatic index (HSI, %) or viscerosomatic index (VSI, %) was calculated as: $100 \times (\text{weight of organ}) \times (\text{total fish weight})^{-1}$. Condition factor (CF) was calculated as: $100 \times (\text{fish weight}) \times (\text{body length})^{-3}$, where weight is expressed in g and length is in cm.

Statistical analysis was done within each type of SW (with or without hydrolysis), and differences were considered significant for $P < 0.05$. The analyses were linear or quadratic regressions based on which model gave best fit to the data, and one-way ANOVA. Quadratic regressions were only presented when the regression coefficient of the 2_{nd} degree component was statistically significant ($P < 0.05$). Maxima or minima in quadratic regressions were calculated by setting the first derivative of the equation to 0. Significant differences in ANOVA were ranked by the Pdiff routine under LSMEANS, and indicated by different superscript letter a, b, c. A two-way factorial ANOVA with the factors SW type (n=2) and replacement level (n=3) was also conducted. Only the effects of SW are reported since the effects of replacement level effects were analyzed more in detail by regression within SW type. The statistical analyses were conducted using the SAS version 9.4 computer software (SAS Institute Inc., Cary, NC, USA).

3. Results

No mortality occurred, and all the fish had high feed intake and grew well. FI, WG, and FCR of grouper fed diets with different levels of CSW or HSW combinations substituting FM are presented in Table 4. Both FBW and FI did not significantly differ among any of the dietary treatments. WG was linearly decreased in response to CSW combination partly replacing FMC. Only fish fed CSW60 had significantly lower WG than fish fed the FMC diet. FCR was linearly increased in response to the increasing dose of CSW combination. The fish fed CSW40 and CSW60 had significantly higher FCR than fish fed the FMC diet. No significant difference in WG or FCR were found in the fish fed the diets containing HSW. Furthermore, none of the diets containing HSW resulted in WG or FCR being significantly different from the results obtained with the FMC diet. The factorial analysis (Table 5) revealed a trend ($P = 0.061$) indicating higher feed intake in the fish fed the HSW than what was seen with the CSW diets. FBW and WG were significantly higher and FCR was significantly lower in fish fed the HSW than in the fish fed the CSW diets. SW inclusion of 10 and 20% resulted in higher WG than 60%, while 10 and 20% inclusion resulted in lowest FCR.

The only somatic index that came out significant was the HSI of the groupers fed the diets with CSW (Table 6). The regression analysis showed that HSI significantly decreased with increasing dietary concentration of CSW, in a linear manner. The HSI of grouper fed the FMC and CSW10 were significantly higher than that of the fish fed CSW40 and CSW60. VSI and CF did not significantly different among fish fed diets with CSW. Simultaneously, diets containing HSW did not significantly affect HSI, VSI or CF. The factorial ANOVA (Table 7) confirmed that HSI was significantly higher in fish fed HSW than CSW. The significant interaction of SW type and inclusion level is rationalized by HSI of the grouper being affected by dietary level of CSW, but not that of HSW.

Whole-body dry matter, protein retention (PRE), and energy retention (ERE) efficiencies were not significantly affected by gradually replacing FM in FMC diet with CSW or HSW combinations (Table 8). The response in whole-body crude protein to gradually replacing FMC by CSW was best described by a quadratic curve with maximum at 39.1 % replacement. Whole-body fat content responded in a similar manner, with a maximum at 39.3 %. Fish fed CSW40 also contained significantly more lipid than those fed FMC. Crude protein was significantly higher in grouper fed the CSW 20-60 diets than those fed the FMC. Whole body ash was apparently reduced in response both to increased CSW and HSW. However, SPC was added to the diets proportionally to two SW. The factorial analysis (Table 9) furtherly confirmed the significant differences in ash content only caused by inclusion level not the SW type.

Apparent digestibility coefficients (ADC) of crude protein, energy, essential and semi-essential amino acids are shown in Table 10. No significant effects of dietary CSW inclusion on the ADC of N, Arg, Cys, His, Leu, Phe, and Tyr were found. The regression of CSW on the ADC of energy was quadratic, with a maximum at 20.0 %, while quadratic responses of ADC of Ile and Met had minimum at 37.3 % and 36.7 %, respectively. The ADC of Lys, Thr, Val, and the ADC of the sum of EAA, and that of the sum of AA responded to increasing concentration of CSW by linear decline. ADC of Met and Val showed quadratic responses to increasing HSW with respective minima at 45.9 and 11.3 %. The ADC of Arg, Ile, Leu, Lys, Thr, Tyr, the sum of EAA, and that of

AA were linearly negative, while ADC of N, energy, Cys, His, and Phe were not significantly affected by dietary HSW inclusion. The factorial analysis (Table 11) did not reveal significant differences caused by SW type. Inclusion level significantly affected all the analyzed digestibilities except of N and Phe. Generally, ADC values moderately (1-3%) declined when the inclusion level increased from 10 to 60%, with some exceptions (ADC of Cys 4%, Lys 5%, and Thr 5%).

4. Discussion

This study was designed to explore if CSW or HSW from processing of food grade tuna by-product had a potential for partially replacing FM in diets for hybrid grouper. The main criteria used to evaluate these novel feed ingredients were growth rates, feed utilization, body composition and nutrient digestibilities. Throughout the whole experiment period, all groups of hybrid grouper were active and readily accepted the feeds. Feed intake was not significantly influenced by different dietary treatments, but a trend indicates that the HSW was preferred when compared to the CSW. The observation that the palatability of feed was not significantly affected by type of SW, or rate of replacement ranging from 0 to 60% of the dietary FM is contrary to what has been obtained with other ingredients by which feed intake decreased when a high proportion of FM was replaced (Yao et al., 2018; Glencross et al., 2011). One probable explanation to the high feed intake both in grouper fed CSW and HSW, is the high freshness of the food-grade skipjack tuna by-products from which the SW was produced. Histamine concentration is used as an indicator of freshness in fish meal, and 670 mg histidine (kg DM)$^{-1}$ in the CSW was similar to values obtained from high-quality Chilean fish meal by Anderson et al. (1997).

WG linearly decreased with an increase in inclusion levels of CSW whereas WG of fish fed HSW were not significantly affected. The highest WG value was observed in fish fed HSW20. Similar results were observed when yellow catfish fed diets with HSW and FM grew faster than those fed CSW (Wu et al., 2018). Feeding rice field eel (*Monopterus albus*) with low inclusion levels of HSW (10-15%) was reported to increase the WG (Shi et al., 2019). Inclusion of HSW, even in highest level (HSW60) did not have significant negative effect in fish growth, defying the decreased growth performance observed when large portion of FM was replaced by fish hydrolysate (Zheng et al., 2012). In this study, negative effect on the FCR was observed as FM replacement with CSW increased in the diets. Poor feed utilization may be a result of the change in amino acid profiles (Wattanakul et al., 2017).

The lack of significant differences obtained for FCR between fish fed FMC diet and those fed diets with HSW inclusion suggest that HSW can replace a major proportion of FM without negatively affecting growth and feed utilization of hybrid grouper. Likewise, an attempt to replace FM with SPC was done in hybrid grouper but there was a significant negative effect in survival, growth, and feed utilization (Wang et al., 2020). FM was replaced with peanut meal (PNM) up to 50 % with no significant effect on growth performance, but negatively affected immunity and intestinal microbiota of hybrid grouper (Ye et al., 2020). WG of fish groups fed HSW was numerically higher than that of corresponding groups fed CSW at the same level. Also, relatively lower FCRs were observed in fish fed FMC and HSW diets, implying that feed was highly utilized by fish fed those diets. Similar results on growth and feed utilization were obtained when FM was replaced by SW in Nile tilapia (*Oreochromis niloticus*) (Wattanakul et al., 2019). The factorial analysis conducted in this experiment also showed that fish fed diets with HSW combination had significantly higher FBW and WG but lower FCR than that of fish fed diets with CSW combination. Similarly, juvenile brown-marbled grouper (*E. fuscoguttatus*) fed milkfish offal hydrolysate had significantly improved feed intake, growth and feed utilization compared to that fed milkfish offal (Mamauag & Ragaza, 2016). The results of present study also agreed in extent with the studies in which the inclusion of low levels of HSW in diets enhanced feed utilization and growth of rice field eel (Shi et al., 2019). Other marine protein hydrolysates have also brought positive effect on growth of Atlantic salmon (Berge and Storebakken, 1996; Refstie et al., 2004).

Generally, improved growth and feed conversion can be expected due to improved utilization of protein from HSW because the SW being hydrolyzed (Tonheim et al., 2005). Nutrients within the hydrolysates then tend to be rapidly absorbed and assimilate through the intestinal membrane. The reason for rapid absorption of HSW is that the amino acid chains had been hydrolyzed to release shorter peptides and free amino acids (Potier & Tomé, 2008). The factor that contributed most to grouper fed HSW growing faster than those fed the CSW diets seemed to be the tendency (P=0.061) for higher feed intake in the fish fed the diets with HSW.

The lower DM content of CSW than HSW probably reflects higher water binding in conventional than partially hydrolyzed fish proteins (Kristinson & Rasco, 2000). No significant effect was observed on VSI and CF between fish fed FMC diet and diets with CSW. However, fish fed the CSW40 and CSW60 diets had significantly lower HSI than that of fish fed FMC, CSW10 and CSW20 diets. HSI values did not correlate with body lipid content, contrary to previous studies in juvenile silvery-black porgy (*Sparidentex hasta*) (Yaghoubi et al., 2016) and Japanese flounder (*Paralichthys olivaceus*) (Ye et al., 2011). No significant differences were observed on HSI, VSI, and CF between fish fed FMC diet and diets with HSW. Similar results were observed in the same species when FM was replaced to PNM up to 50% (Ye et al., 2020). In contrast, HSI was found to increase when FM was replaced by plant protein (Ye et al., 2019a) and by animal protein blend of poultry by-product meal, spray-dried blood meal and shrimp meal (Ye et al., 2019b).

Two alternative explanations may explain why fish fed CSW and HSW had lower whole-body ash content than the ones fed the FMC diet. One was the characteristics of the FM and the two SW. This contrasts with the results obtained by Kousoulaki et al. (2009) who found that ash content of Atlantic salmon increased with the increase in SW levels replacing FM. The other explanation was that reduced ash content was due to phytic acid in SPC, and which was added to the diets proportionally to two SW. Dietary concentrations of SPC paralleled that of CSW and HSW (Table 3). The regressions of percentwise dietary SPC inclusion on whole-body ash also paralleled the results obtained with CSW (R^2=0.66) and HSW (R^2=0.78). Dietary phytic acid is known to reduce whole-body ash in Atlantic salmon (Denstadli et al, 2006), both due to poor availability of P from phytic acid, and binding cationic elements such as Mg, Zn, and the Ca: P-ratio. These mineral deficiencies cause vertebrae deformities in Atlantic salmon (Helland et al., 2006). It is difficult to solve this challenge in coldwater fish because phytase does not work at low temperatures (Denstadli et al., 2007). In warmwater fish like grouper, phytase can be carefully coated into the feed after extrusion and drying. Hydrolyses of phytic acid will then occur like other digestive processes.

Neither CSW nor HSW inclusion affected the retention of protein and energy. Crude protein in fish whole body was significantly increased with an increase in CSW in fish diets. No significant differences in whole body dry matter, crude protein and crude fat were observed between fish fed FMC and HSW diets. The results agree with those of a previous study in juvenile snakehead (*Ophiocephalus argus*) (Yun et al., 2014).

Determining the digestibility of nutrients is crucial in evaluating the use of novel feed ingredient (de Magalhães et al., 2016; Allan et al., 2000). In this study, the apparent digestibility (ADC) of protein, energy, and individual EAA in diets with SW inclusion ranged from 84.1% to 96.0%, indicating that nutrients were effectively digested by hybrid grouper. The ADC of protein was not affected by HSW or CSW inclusion in diets. Contrary to this study, the ADC of protein decreased with an increase in SPC inclusion in diet for hybrid grouper (Faudzi et al., 2017; Chen et al., 2020). Meanwhile, no significant differences in the ADC of energy were observed between FM and diets with HSW inclusion. CSW60 resulted in significantly lower ADC of energy than CSW10. Apart from amino acid composition, quality of protein source is determined by the bioavailability of essential amino acids which is reflected by digestibility (Wu et al., 2017; Potier & Tomé, 2008). Gut morphology and oxidative stress was reported to be influenced by dietary arginine (Wu et al., 2018) while dietary leucine affected growth hormone (GH) level (Zhou et al., 2019) in hybrid grouper. Therefore, the optimum amount of digested essential amino acids must be examined because of their importance in overall fish performance. Generally, the ADC of amino acids was high in fish fed FMC and diets with low inclusion of SW (10% and 20%). ADC of some amino acids were not affected with FM replacement while there was a correspondingly decrease in ADC of a few amino acids with an increase in SW inclusion levels. Digestibility of the sum of EAA, and that of total AA by fish fed FM, CSW10, HSW10 and HSW20 were significantly higher than other dietary groups. A few studies have touched on the ADC of protein, fat, energy, and phosphorus in fish fed diets with SW inclusion (Kousoulaki et al., 2012; Kousoulaki et al., 2009), but none of them included the ADC of amino acids. Replacing FM with other ingredients has been associated with reduction in digestibility of nutrients, even when the diets meet requirements of specific species (Kousoulaki et al., 2009). However, partial replacement of FM with low levels of SW (10% of FM with SW and up to 20% of FM with HSW) did not generally result in significant negative effects on ADC of nutrients.

5. Conclusion

The current study showed that both CSW and HSW from food-grade skipjack tuna co-product are promising protein sources for hybrid grouper feed. The overall performance of HSW was better in both fish growth and feed utilization than CSW. The HSW combined with SPC and CGM with the mixing ratio of 1:1:1 could effectively decrease the inclusion of high-quality FM from 450 g kg^{-1} to 360 g kg^{-1} in extruded practical diet for hybrid grouper without impairing the feed intake, growth performance, feed utilization, whole-body composition, retentions of protein and energy, nutrient digestibility, and all somatic indices.

6. Acknowledgements

This study was mainly supported by Zhejiang Fengyu Marine Organism Products Co., Ltd., Zhoushan, Zhejiang, China, and partly supported by the National Natural Science Foundation of China (Grant No: 31502182), Key R&D Program of Zhejiang Province (2019C02048), and Scientific Research Startup Foundation of Zhejiang Ocean University (Grant No. Q1402).

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Table 1 Characteristics of conventional (CSW) and hydrolyzed stickwater (HSW)[1].

	CSW	CSW	HSW	HSW
Solids, g (kg wet weight)$^{-1}$	533		580	
Acid soluble protein, g (kg dry matter (DM))$^{-1}$	294		674	
Hydrolyzed amino acids, g (kg DM)$^{-1}$	576		572	
Total free amino acids g (kg DM)$^{-1}$	65.1		65.0	
Peptides, g (kg DM)$^{-1}$	229		609	
Amino acid nitrogen, g (kg DM)$^{-1}$	14.1		15.7	
Histamine, mg (kg DM)$^{-1}$	670		406	
Peptide size ranges, Da	g (kg DM)$^{-1}$	g (kg acid soluble protein)$^{-1}$	g (kg DM)$^{-1}$	g (kg acid soluble protein)$^{-1}$
<180	34.9[2]	118.7[3]	130.0[2]	192.7[3]
500 - 180	43.7	148.4	227.3	337.0
1 000 - 500	8.2	27.9	143.1	212.2
2 000 -1 000	10.2	34.5	98.9	146.7
3 000 -2 000	7.9	27.0	35.5	52.7
5 000 - 3 000	13.2	13.2	26.3	39.0
10 000 - 5 000	31.1	105.7	11.1	16.4
>10 000	145.0	492.8	2.2	3.3

[1] Conventional stickwater (CSW) and hydrolyzed stickwater (HSW) from food-grade tuna by-product, Zhejiang Fengyu Marine Organism Products Co., Ltd., Zhoushan, China.

[2] Calculated by the percentage of the normalized area method x acid soluble protein content (g 100g^{-1} DM).

[3] Percentage of the area normalized method (g 100g^{-1} acid soluble protein)

Table 2 Composition of fish meal (FM), conventional (CSW) or hydrolyzed (HSW) stickwater, soy protein concentrate (SPC), corn gluten meal (CGM), soybean meal (SBM) and wheat gluten used in experiment (on dry matter basis)

Ingredient	FM [1]	CSW	HSW	SPC [2]	CGM[3]	SBM[4]	wheat gluten[5]
Composition, kg^{-1}							
Dry matter (DM), g	933	470	524	928	913	882	926
In DM							
Crude protein, g	750	812	822	683	693	532	815
Crude fat, g	107	40	46	17	5	21	13
Ash, g	158	192	193	58	19	70	9
Gross energy, MJ	21.7	18.6	18.4	20.7	23.2	19.5	23.6
Essential (EAA) [6] and semi essential (SEAA) amino acids, g (16 g N)$^{-1}$							
Arg	5.22	3.98	3.97	7.21	2.93	6.99	3.27
Cys	0.63	0.35	0.36	1.47	1.35	0.98	2.14
His	3.25	4.82	4.77	2.58	2.03	2.41	1.77
Ile	3.79	1.44	1.39	4.63	3.71	4.34	3.56
Leu	7.08	3.35	3.37	7.97	16.50	7.68	6.72
Lys	7.34	4.30	4.27	6.59	1.61	6.14	1.49
Met	2.45	1.24	1.21	1.57	2.06	0.70	1.90
Phe	4.05	1.91	1.87	4.97	5.84	4.82	4.66
Thr	4.14	2.32	2.27	4.36	3.34	3.90	2.45
Val	4.43	2.18	2.14	5.10	4.27	4.42	4.04
Total EAA and SEAA	41.8	25.5	25.3	45.0	42.3	42.4	29.9
Total non-essential AA[6]	41.9	35.4	35.1	53.8	57.6	51.0	65.0
Total AA	83.6	60.9	60.4	98.8	99.8	93.4	94.9

[1] Brown fishmeal, Compania Pesquera Del Pacifico Centro S.A, Lima, Peru

[2] Yihai®, Wilpromil, Glodensea Grain and Oil Industry Co., Ltd, Wilmar, Qinhuangdao, China

[3] Lihua Starch Co., Ltd, Wilmar, Qinhuangdao, China

[4] Zhoushan Good Ocean Grains & Oils Co., Ltd, Zhoushan, China

[5] Golden Mountain, grade two, Dongguan Yihai Kerry Syral Starch Technology Co., Ltd, Dongguan, China.

[6] Trp excluded. Cys and Tyr are semi essential (SEAA)

Table 3 Feed formulation and analyzed chemical composition (on dry matter basis).

Diet	FMC	CSW10	CSW20	CSW40	CSW60	HSW10	HSW20	HSW40	HSW60
Ingredients, g kg^{-1}									
FM	450	405	360	270	180	405	360	270	180
SPC	100.0	114.9	129.7	159.4	189.1	114.9	129.7	159.4	189.1
Wheat gluten	40	40	40	40	40	40	40	40	40
Wheat flour	137.7	134.6	131.7	119.7	110.6	134.6	131.7	119.7	110.6
CSW	0	15.3	30.6	61.2	91.8	0	0	0	0
HSW	0	0	0	0	0	15.3	30.6	61.2	91.8
Soybean meal	50	50	50	50	50	50	50	50	50
CGM	50.0	64.9	79.7	111.4	139.1	64.9	79.7	111.4	139.1
Fish oil	110	113	116	123	130	113	116	123	130
Premix [1]	20	20	20	20	20	20	20	20	20
L-Lys	0	0	0	0	1.0	0	0	0	1.0
DL-Met	0	0	0	0.5	1.1	0	0	0.5	1.1
MCP	10.0	10.0	10.0	12.5	15.0	10.0	10.0	12.5	15
Y_2O_3	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Choline Cl [2]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Soy lecithin [3]	20	20	20	20	20	20	20	20	20
Vitamin C [4]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin E [5]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Analyzed content, kg^{-1}									
Dry matter, g	967	943	930	918	953	924	935	926	938
Crude protein, g	502	505	504	503	500	504	503	503	501
Crude fat, g	143	153	156	151	158	146	146	158	148
Ash, g	116	108	106	103	95	111	106	103	96
Gross energy, MJ	20.8	20.6	20.5	20.5	20.9	20.3	20.6	20.5	20.8
SEAA[6] and EAA, %									
Arg	2.75	2.75	2.72	2.65	2.61	2.74	2.70	2.66	2.59
Cys	0.41	0.46	0.44	0.40	0.39	0.37	0.39	0.43	0.45
His	1.51	1.43	1.48	1.46	1.47	1.37	1.36	1.48	1.44
Ile	1.95	2.01	1.94	1.84	1.79	2.05	2.04	1.87	1.82
Leu	4.02	4.16	4.17	4.22	4.38	4.08	4.11	4.29	4.33
Lys	3.25	3.15	2.98	2.74	2.62	3.13	2.97	2.78	2.57
Met	0.85	0.90	0.90	0.81	0.77	0.81	0.80	0.76	0.79
Phe	2.49	2.40	2.37	2.33	2.34	2.25	2.25	2.37	2.37
Thr	2.01	2.00	1.96	1.86	1.78	1.96	1.92	1.90	1.79
Tyr	1.66	1.73	1.72	1.72	1.71	1.65	1.64	1.70	1.73
Val	2.23	2.26	2.20	2.07	2.01	2.31	2.28	2.12	2.03
∑EAA	23.1	23.2	22.9	22.1	21.9	22.7	22.5	22.4	21.9
∑AA	47.0	47.2	47.0	46.1	46.3	47.1	46.8	47.0	46.3

[1] Vitamin premix (mg kg^{-1} diet): vitamin A 1.95; vitamin B_1 20; vitamin B_2 10; vitamin B_6 30; niacinamide 250; ascorbic acid 5; calcium pantothenate 50; folic acid 20; vitamin E 1200; vitamin K 0.8; vitamin D 0.05; inositol 650; Defatted Rice Bran 150. Mineral premix (mg kg^{-1} diet): $CuSO_4 \cdot 5H_2O$ 10; $FeSO_4 \cdot H_2O$ 300; $ZnSO_4 \cdot H_2O$ 200; $MnSO_4 \cdot H_2O$ 100; KI (10%) 80; Na_2SeO_3 (10% Se) 67; $CoCl_2 \cdot 6H_2O$ (10% Co) 5; NaCl 100; zeolite 638. Vitamin premix: mineral premix = 2: 1.5.

[2] 60% Choline Chloride Powder (Vegetable Carrier), Shandong Aocter Group, Shandong, China

[3] Youlin®, powder (> 95%), Beijing Meiyas Phospholipid Technology Co., Ltd, Beijing, China.

[4] L-Ascorbate-2-Monophosphate, ⩾35%, feed grade, Hangzhou Tiannong Bio-nutrition Technology Co., Ltd., Zhejiang, China

[5] DL-alpha-Tocopherol acetate，50% feed grade, NHU Co., Ltd., Zhejiang, China

[6] Trp excluded. Cys and Tyr are semi essential (SEAA)

Table 4 Feed intake, growth performance and feed conversion ratio (FCR) of hybrid grouper fed diets with CSW or HSW combinations

Parameters	Diet					P-value	Pooled S.E.M. [1]	Regression	R^{2}
	FMC	CSW10	CSW20	CSW40	CSW60				
Final body weight (FBW), g fish^{-1}	671	667	672	662	632	0.07	21.3		
Feed intake (FI), g DM fish^{-1}	213	212	230	216	209	0.21	14.0		
Weight gain (WG), g fish^{-1}	311^{a}	301^{a}	313^{a}	300^{a}	276^{b}	0.026	15.9	$-0.523x+313$	0.46
Feed conversion ratio (FCR), g FI (g WG)$^{-1}$	0.785^{a}	0.805^{ab}	0.802^{ab}	0.825^{b}	0.866^{c}	<0.001	0.0176	$1.25 \times 10^{-3}x + 0.784$	0.81
	Diet								
	FMC	HSW10	HSW20	HSW40	HSW60				
Final body weight (FBW), g fish^{-1}	671	687	710	669	667	0.17	29.1		
Feed intake (FI), g DM fish^{-1}	213	226	236	230	218	0.37	18.4		
Weight gain (WG), g fish^{-1}	311	324	346	310	304	0.22	29.3		
Feed conversion ratio (FCR), g FI (g WG)$^{-1}$	0.785	0.799	0.782	0.809	0.821	0.12	0.0247		

For diet codes see Table 2. Different superscript letters $^{a,\ b,}$ and ^{c} indicate significant ($P < 0.05$) differences among treatments.

[1] Pooled standard error of means.

Table 5 Factorial analysis of data on feed intake, growth performance and feed utilization of hybrid grouper fed diets with different stickwater types and inclusion levels

Factor	FBW, g fish^{-1}	FI, g DM fish^{-1}	WG, g fish^{-1}	FCR, g FI (g WG)$^{-1}$
Stickwater type				
CSW	658^{b}	217	297^{b}	0.824^{b}
HSW	681^{a}	228	321^{a}	0.802^{a}
Inclusion level, %				
10	677^{ab}	219	312^{ab}	0.802^{ab}
20	691^{a}	233	329^{a}	0.792^{a}
40	666^{bc}	223	305^{bc}	0.817^{b}
60	649^{c}	214	290^{cd}	0.844^{c}
Pooled S.E.M[1]	15.5	21.6	26.1	0.026
Factorial ANOVA (P>F)				
Stickwater type	0.006	0.061	0.002	0.005
Inclusion level	0.014	0.132	0.007	<0.001
Type * level	0.493	0.937	0.674	0.232

Different superscript letters $^{a, b, c,}$ and ^{d} indicate significant ($P < 0.05$) differences among treatments.

[1] Pooled standard error of means.

Table 6 Somatic indices of hybrid grouper fed diets with CSW or HSW combinations

Somatic Indices	Diet					*P*-value	Pooled S.E.M.[1]	Regression	R^2
	FMC	CSW10	CSW20	CSW40	CSW60				
HSI [2], %	2.35^{ab}	2.34^{ab}	2.20^{bc}	1.91^{c}	1.90^{c}	0.03	0.244	$-8.77 * 10^{-3}x + 2.37$	0.56
VSI [3], %	12.76	12.46	16.79	12.89	14.74	0.22	3.13	\	\
CF [4], g $(cm)^{-3}$	2.86	2.96	2.83	2.68	2.66	0.16	0.195	\	\
	Diet								
	FMC	HSW10	HSW20	HSW40	HSW60				
HIS, %	2.35	2.11	2.43	2.60	2.18	0.10	0.270	\	\
VSI, %	12.76	13.75	13.62	13.24	13.1	0.18	0.64	\	\
CF, g $(cm)^{-3}$	2.86	2.80	2.89	2.96	2.74	0.71	0.265	\	\

For diet codes see Table 2. Different superscript letters $^{a, b,}$ and ^{c} indicate significant ($P < 0.05$) differences among treatments.

[1] Pooled standard error of means.

[2] Hepatosomatic index.

[3] Viscerosomatic index.

[4] Condition factor.

Table 7 Factorial analysis of somatic indices of hybrid grouper fed diets with different stickwater types and inclusion level

Factor	HSI [1], %	VSI [2], %	CF [3], g (cm)$^{-3}$
Stickwater type			
CSW	2.09^{a}	14.2	2.78
HSW	2.33^{b}	13.4	2.85
Inclusion level, %			
10	2.23	13.1	2.88
20	2.31	15.2	2.86
40	2.25	13.1	2.82
60	2.04	13.9	2.70
Pooled S.E.M. [4]	0.081	0.79	0.065
Factorial ANOVA (P>F)			
Stickwater type	<0.01	0.33	0.33
Inclusion level	0.14	0.23	0.24
Type * Level	<0.01	0.23	0.16

Different superscript letters ^{a} and ^{b} indicate significant ($P < 0.05$) differences among treatments.

[1] Hepatosomatic index.

[2] Viscerosomatic index.

[3] Condition factor.

[4] Pooled standard error of means.

Table 8 Whole body compositions and nutrient retentions of hybrid grouper fed diets with CSW and HSW combinations

Items	Diet					P-value	Pooled S.E.M.[1]	Regression	R^2
	FMC	CSW10	CSW20	CSW40	CSW60				
Dry matter, g kg^{-1}	337	339	332	344	339	0.28	7.9	\	\
Crude protein, g kg^{-1}	169^{b}	174^{ab}	176^{a}	176^{a}	175^{a}	0.04	3.4	$-0.005x^2+0.391x+169.827$	0.57
Crude fat, g kg^{-1}	101^{b}	110^{ab}	109^{ab}	119^{a}	111^{ab}	0.04	7.3	$-0.01x^2+0.786x+100.830$	0.50
Ash, g kg^{-1}	56.1^{a}	44.3^{b}	39.2^{bc}	38.7^{bc}	37.5^{c}	<0.001	4.15	$0.01x^2-0.872x+54.16$	0.79
Protein retention efficiency, %	46.7	48.9	47.4	48.8	46.0	0.47	2.95	\	\
Energy retention efficiency, %	52.6	57.2	52.2	57.9	56.0	0.41	5.62	\	\
	Diet								
	FMC	HSW10	HSW20	HSW40	HSW60				
Dry matter, g kg^{-1}	337	336	338	334	330	0.51	7.7	\	\
Crude protein, g kg^{-1}	169	169	175	173	173	0.53	6.2	\	\
Crude fat, g kg^{-1}	101	107	117	113	108	0.07	7.9	\	\
Ash, g kg^{-1}	56.1^{a}	50.1^{ab}	38.6^{c}	40.5^{c}	43.0^{bc}	<0.01	6.31	$0.012x^2-0.937x+56.17$	0.64
Protein retention efficiency, %	46.7	45.6	52.7	45.0	47.0	0.15	3.87	\	\
Energy retention efficiency, %	52.6	53.0	63.0	51.8	52.8	0.13	7.18	\	\

For diet codes see Table 2. Different superscript letters $^{a, b,}$ and ^{c} indicate significant ($P < 0.05$) differences among treatments.

[1] Pooled standard error of means.

Table 9 Factorial analysis of whole-body compositions and nutrient retentions of hybrid grouper fed diets with different stickwater types and inclusion level

Factor	Dry matter, g kg^{-1}	Crude protein, g kg^{-1}	Crude fat, g kg^{-1}	Ash, g kg^{-1}	Nitrogen retention, %	Energy retention, %
Stickwater type						
CSW	338	175	112	39.9	47.8	56.1
HSW	336	173	111	42.8	47.6	55.1
Inclusion level, %						
10	337	171	108	47.1^{a}	47.2	55.1
20	336	176	113	39.0^{b}	50.0	57.6
40	340	175	116	39.7^{b}	47.0	55.4
60	335	174	109	39.5^{b}	46.5	54.4
Pooled S.E.M. [1]	2.6	1.4	2.7	1.50	1.11	2.24
Factorial ANOVA (P>F)						
Stickwater type	0.3	0.1	0.8	0.07	0.85	0.67
Inclusion level	0.6	0.1	0.2	<0.01	0.17	0.78
Type * level	0.1	0.8	0.3	0.51	0.04	0.07

For diet codes see Table 2. Different superscript letters ^{a} and ^{b} indicate significant ($P < 0.05$) differences among treatments.

[1] Pooled standard error of means.

Table 10 Apparent digestibility coefficient of nutrients of hybrid grouper fed diets with CSW or HSW combinations

ADC, %	Diet					*P*-value	Pooled S.E.M. [1]	Regression	R^2
	FMC	CSW10	CSW20	CSW40	CSW60				
Nitrogen (N)	86.4	88.5	87.5	85.9	86.4	0.14	1.58	\	\
Energy	84.1^{ab}	88.0^{a}	87.3^{ab}	84.6^{ab}	83.9^{b}	0.01	1.81	$-0.003x^2+0.125x+85.313$	0.34
Arg	95.5	96.0	95.4	95.0	94.8	0.18	0.74	\	\
Cys	72.2	88.6	87.2	87.9	81.7	0.05	8.34	\	\
His	91.6	91.9	90.8	89.3	88.8	0.19	2.24	\	\
Ile	93.4^{a}	92.3^{ab}	91.2^{bc}	90.6^{c}	91.8^{bc}	0.02	1.08	$2.04*10^{-3}x^2 -0.152x + 93.5$	0.66
Leu	95.1	95.1	94.7	94.4	94.7	0.55	0.69	\	\
Lys	91.1^{a}	91.7^{a}	89.6^{ab}	87.6^{bc}	86.2^{c}	<0.001	1.52	$-0.0938x + 91.7$	0.77
Met	92.4^{a}	88.1^{b}	87.3^{b}	86.5^{b}	88.1^{b}	<0.01	1.71	$4.39*10^{-3}x^2 – 0.322x +91.8$	0.73
Phe	94.2	94.0	93.4	93.3	94.0	0.57	0.99	\	\
Thr	89.9^{a}	90.2^{a}	88.5^{ab}	86.9^{b}	84.7^{c}	<0.001	1.27	$-0.0925x + 90.4$	0.83
Tyr	93.7	93.3	92.5	92.5	92.9	0.27	0.93	\	\
Val	92.2^{ab}	92.7^{a}	91.7^{ab}	90.7^{b}	90.3^{b}	0.011	0.94	$-0.0394x+92.5$	0.61
∑EAA [2]	93.0^{a}	93.0^{a}	92.0^{ab}	91.3^{b}	91.2^{b}	0.037	0.99	$-0.0338 x + 93.0$	0.53
∑AA [2]	92.3^{ab}	92.6^{a}	91.5^{abc}	90.7^{bc}	90.1^{c}	<0.01	0.91	$-0.0420 x + 92.6$	0.67
	FMC	HSW10	HSW20	HSW40	HSW60				
N	86.4	85.5	87.9	86.3	85.9	0.40	1.95	\	\
Energy	84.1	84.2	87.2	84.8	83.8	0.11	1.95	\	\
Arg	95.5^{ab}	96.0^{a}	96.0^{a}	94.8^{b}	94.8^{b}	0.02	0.60	$-0.0193x + 95.9$	0.39
Cys	72.2	85.7	87.9	86.9	85.1	0.08	8.58	\	\
His	91.6	93.5	91.8	89.4	90.7	0.14	2.25	\	\
Ile	93.4^{a}	92.4^{a}	92.7^{a}	90.7^{b}	89.6^{b}	<0.001	0.81	$-0.0636x + 93.4$	0.84
Leu	95.1^{ab}	95.3^{a}	95.5^{a}	94.4^{bc}	94.0^{c}	<0.01	0.51	$-0.0224x + 95.4$	0.55
Lys	91.1^{a}	91.8^{a}	91.0^{a}	87.9^{b}	86.8^{b}	<0.01	1.72	$-0.0868x + 92.0$	0.68
Met	92.4^{a}	89.5^{b}	88.4^{b}	83.9^{c}	85.8^{c}	<0.001	1.47	$3.75*10^{-3}x^2 – 0.344x + 92.7$	0.86
Phe	94.2	94.0	94.1	93.0	93.2	0.20	0.90	\	\
Thr	89.9^{a}	89.7^{a}	89.8^{a}	86.4^{b}	84.7^{b}	<0.001	1.31	$-0.0947x + 90.6$	0.80
Tyr	93.7^{a}	93.2^{a}	93.5^{a}	91.9^{b}	91.7^{b}	<0.01	0.72	$-0.0356x + 93.7$	0.66
Val	92.2^{a}	92.6^{a}	93.0^{a}	90.9^{b}	89.4^{c}	<0.001	0.73	$-1.44*10^{-3}x^2 + 0.0325x + 92.4$	0.83
∑EAA [2]	93.0^{a}	93.1^{a}	93.1^{a}	91.1^{b}	90.8^{b}	<0.01	0.83	$-0.0456 x + 93.4$	0.69
∑AA [2]	92.3^{a}	92.9^{a}	92.8^{a}	90.5^{b}	89.9^{b}	<0.01	0.88	$-0.0517 x + 93.0$	0.67

For diet codes see Table 2. Different superscript letters $^{a, b,}$ and ^{c} indicate significant ($P < 0.05$) differences among treatments.

[1] Pooled standard error of means. [2] Trp excluded.

Table 11 Factorial analysis of data on apparent digestibility coefficient (ADC, %) of hybrid grouper fed diets with different stickwater type and inclusion level

Factor	N	Energy	Arg	Cys	His	Ile	Leu	Lys	Met	Phe	Thr	Tyr	Val	∑EAA[1]	∑AA[1]
Stickwater type															
CSW	87.1	85.9	95.3	86.3	90.2	91.5	94.7	88.8	87.5	93.7	87.6	92.8	91.3	91.9	91.3
HSW	86.4	85.0	95.4	86.4	91.3	91.3	94.8	89.4	86.9	93.6	87.7	92.6	91.5	92.0	91.5
Inclusion level, %															
10	87.0	86.1^{a}	96.0^{a}	87.2^{a}	92.7^{a}	92.3^{a}	95.2^{a}	91.8^{a}	88.8^{a}	94.0	89.9^{a}	93.2^{a}	92.7^{a}	93.1^{a}	92.8^{a}
20	87.7	87.2^{a}	95.7^{a}	87.5^{a}	91.3^{ab}	92.0^{a}	95.1^{a}	90.3^{a}	87.8^{ab}	93.8	89.1^{a}	93.0^{ac}	92.3^{a}	92.5^{a}	92.2^{a}
40	86.1	84.7^{b}	94.9^{b}	87.4^{a}	89.3^{c}	90.6^{b}	94.4^{b}	87.8^{b}	85.2^{c}	93.1	86.7^{b}	92.2^{b}	90.8^{b}	91.2^{b}	90.6^{b}
60	86.1	82.9^{b}	94.8^{b}	83.4^{b}	89.7^{bc}	90.7^{b}	94.4^{b}	86.5^{b}	87.0^{b}	93.6	84.7^{c}	92.3^{bc}	89.8^{c}	91.0^{b}	90.0^{b}
Pooled S.E.M.[2]	0.54	0.46	0.17	0.98	0.62	0.29	0.14	0.52	0.5	0.23	0.38	0.25	0.27	0.26	0.24
Factorial ANOVA (P>F)															
Stickwater type	0.23	0.06	0.45	0.94	0.08	0.58	0.67	0.25	0.26	0.74	0.82	0.44	0.66	0.59	0.32
Inclusion level	0.14	<0.001	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	<0.01	0.02	<0.01	<0.01	<0.01
Type * level	0.13	0.02	0.28	0.18	0.75	<0.01	0.011	0.80	0.020	0.17	0.34	0.037	0.05	0.20	0.14

Different superscript letters $^{a, b,}$ and ^{c} indicate significant ($P < 0.05$) differences among treatments.

[1] Trp excluded.

[2] Pooled standard error of means.

Conflict of Interest

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author contributions

Yuexing Zhang and Lei Zhang contributed to the design of this study, performed most of experiments and statistical analysis, drafted, and revised the manuscript. Liying Huang designed the study, drafted & revised the manuscript, analyzed samples, and provided reagents and experimental facilities. Zhiyong Dong, Qiang Lu, and Yuan Zou participated in the conduction of feeding trial and analysis of experimental samples and data. Feng Tang, and Shuaibing Zhao participated in feed formulation, edited the manuscript, provided reagents, and analyzed samples. Trond Storebakken contributed to the design of this study, edited, and revised the manuscript. All the authors read and approved this version of the final manuscript and confirm the integrity of this work.

Data availability statement

The data that support the findings of this study and not presented in the figures and tables are available from the corresponding author on reasonable request.

Ethics statement

Hybrid grouper (*Epinephelus fuscoguttatus* ♀×*E. lanceolatus* ♂) is a commercially farmed species in China not the protected species by Chinese law. During the feeding period and sampling procedures, the experimental fish were maintained in compliance with the Laboratory Animal Welfare Guidelines of China (Decree No. 2 of Ministry of Science and Technology, issued in 1988).