Norwegian University of Life Sciences
Faculty of Veterinary Medicine and Biosciences
Department of Animal and Aquacultural Sciences

Master Thesis 2015
60 credits

Evaluation of Vital wheat gluten as a source of protein in extruded diets for juvenile Giant croaker (Nibea japonica): Feed technological properties and biological responses.

Bingyan Lu

Evaluation of Vital wheat gluten as a source of protein in extruded diets for juvenile Giant croaker (*Nibea japonica*): Feed technological properties and biological responses.

List of Abbreviations

AA = Amino acid
ADC = Apparent digestibility coefficient
ANF = Anti-nutritional factor
Ca = Calcium
CP = Crude protein
CF = Crude fat
DP = Digestible protein
DE = Digestible energy
EAA = Essential amino acid
ERE = Energy retention efficiency
FI = Feed intake
FCR = Feed conversion ratio
FM = Fish meal
GE = Gross energy
GWT = Mixture of vital wheat gluten, wheat flour and taurine
LT-FM = Low-temperature dried fish meal
NRE = Nitrogen retention efficiency
NEAA = Non- essential amino acid
NSP = Non-Starch Polysaccharides
P = Phosphorous
SPC = Soy protein concentrate
TAA = Total amino acid
VWG = Vital wheat gluten
WG = Weight gain

Abstract

The aim of present study was to evaluate Vital wheat gluten (VWG) as protein source in comparison with high-quality fish meal (LT-FM) and soy protein concentrate (SPC) in extruded diets for juvenile Giant croaker (*Nibea japonica*). An ingredient blend named GWT was prepared by mixing the VWG, wheat and taurine with the ratio of 77.5%, 20.5% and 2.0%. The dietary treatments consisted of a control diet (V0) based on LT-FM (20%) and SPC (21.4%), six diets (VF1, VF2, VF3, VS1, VS2 and VS3) in which the LT-FM or SPC had been replaced by the GWT at the levels of 1/3, 2/3 and 100%, respectively, and one diet (VFS) which jointly replacing 50% proteins from LT-FM and 50% proteins from SPC by proteins of GWT.

Extrusion parameters were recorded and discussed, the physical quality of feed pellet were also determined. A trend of increased expansion with both increased proportion of FM replaced by VWG, and with increased proportion of SPC replaced by VWG were found.

Each diet was fed to triplicate tanks of 11-g Giant croaker reared in a sea water flow through system at 28℃ for 59 days. The results showed that no significant impact on weight gain (WG), whole body composition and apparent digestibility coefficients (ADC) of individual AA and total AA were found when the LT-FM had been gradually replaced by GWT from 0 to 100% ($P > 0.05$). Feed intake (FI) and feed conversion ratio (FCR) were significantly increased and retentions of N and energy were significantly decreased with the increasing GWT inclusion ($P < 0.05$). No significant effect were seen in whole body composition except for ash content and energy retention when the SPC was gradually replaced by GWT from 0 to 100%. Retention of N and ash content were significantly decreased for all replacement of dietary SPC with GWT. Quadric relationships were found in both FI and WG with increasing GWT inclusion. The ADC of total AA was increased with GWT inclusion, and a linear increase of ADC of Cys was also observed. Crude fat content and gross energy in whole body and retentions of N and energy were significantly decreased when both 50% of LT-FM and SPC were replaced by GWT, but the FI and WG were not significantly affected. To conclude, in extruded practical diet for Giant croaker with both 20.0% inclusion of high quality fish meal and 21.4% inclusion of SPC, one third of the high quality of dietary fish meal or 65% of the SPC can be safely replaced by the taurine supplemented mixture of vital wheat gluten and wheat flour without causing any adverse effect on feed intake, growth rate, feed conversion, whole body compositions, and retentions of N and energy.

Keywords

Wheat gluten, Giant croaker *Nibea japonica,* protein source, extrusion, pellet quality, growth performance

Contents

1 Introduction

1.1 Wheat gluten

1.1.1 Composition and industrial production of wheat gluten

The first article about wheat gluten was published as long back as in in 1728 (Bailey, 1941). Afterwards, in 1907, Thomas has classified wheat protein as gliadin, glutenin, albumin and globulin by their different solubility, meanwhile, he had pointed out that wheat gluten had important impact on dough (Osbome, 1907). The composition of wheat gluten is shown in Table 1. In the past five decades, much research had been done on wheat gluten and it may have several definitions: It can be as simple as 'storage proteins of the wheat grain' (Shewry and Halford, 2002) or it can have a more functional description as 'gliadin proteins coded for by the Gli-1 and Gli-2 loci coding, plus glutenin polypeptides coded for by the Glu-1 and Glu-3 loci in wheat' (Gianibelli et al., 2001).

Table 1.

Chemical composition and content of wheat gluten (Shewry and Halford, 2002)

	%		%		mg/ 100 g
Gliadin	43.0	Lipid	2.8	Calcium	78
Glutenin	39.0	Glucide	3.1	Magnesium	700
Other protein	4.4	Ash	2.0	Iron	62
Starch	6.5				

Normally, dry wheat gluten contains approximately 75-85% protein, 5-10% moisture. Starch, lipid and Non-Starch Polysaccharides (NSP) account for the rest. As shown in Table 1, gliadins (soluble) and glutenins (insoluble) are balanced with equal amounts. Giladins are present as monomers with low molecular weight, responsible for dough viscosity and extensibility (Belton, 1999). However, as polymeric proteins, glutenins have high molecular weight, viscosity and elasticity resulting in the tenacity of dough (Wieser, 2007). Because it's an annually renewable source and available in large quantities at competitive price (Domenek et al., 2004), wheat gluten now is widely used in food as baking, flour, noodles and other food items. When it's used in non-food industry, it performs well in pet food, aquaculture feed and calf-milk replacements.

1.1.2 Vital Wheat Gluten

Vital Wheat gluten (VWG) can be defined as the 'cohesive, visco-elastic proteinaceous material prepared as a by-product of the isolation of starch from wheat flour' (Day et al., 2006). By washing the dough preparation under water and centrifugation, VWG is obtained. After this process, starch granules and water-soluble constituents are removed. Since extensibility is very sensitive to temperature (Grace, 1989), it could be concluded that VWG is a wheat flour protein material that has been separated from the starch, and then dried rapidly with a minimum amount of heat in order to preserve its vitality (functional characteristics including extensibility).

1.1.3 Nutritional properties of wheat gluten for fish feed

As a protein source

Being different from terrestrial vertebrates, fish has poikilothermy and ammonotelism. Therefore, dietary protein is a major component of formulated fish feed. Not only the high protein concentration showed in the above part, but also high digestibility had been observed (Robaina et al., 1999). The protein digestibility of wheat gluten can be higher than that of high-quality fish meal (Sugiura et al., 1998). Similar result have been obtained by Storebakken et al. (2000), showing is that ADC of CP and all amino acids apart from alanine and lysine increased significantly with increasing proportion of wheat gluten in extruded feed for Atlantic salmon. In conclusion, wheat gluten has high nutritional value for fish feed as a protein source.

Amino acids composition

Balanced profile of amino acids in diets is critical for growth and health of fish. The first limiting amino acid of wheat gluten, as shown in Table 2, is lysine (Allan et al., 2000). This is further illustrated by the comparison of some limiting amino acids in three commonly used protein sources, shown in Fig. 1. Deficiency of lysine can lead to reduction of growth and feed efficiency (Cheng et al., 2003). Furthermore, it may cause health issues such as dorsal and caudal fin erosion in rainbow trout (Ketola, 1983). As a result, it is necessary to supply lysine in fish feed if wheat gluten is used as a major protein source. Previous research has shown that wheat gluten can partly replace fish meal (FM) when the diets were supplied free lysine in rainbow trout (Pfeffer et al., 1995). Davies et al. (1997) showed that when 0.29% to 0.58% lysine was added into diets, rainbow trout had improved growth performance at the replacement on 50% of FM with wheat gluten. Apart from the lysine, tryptophan and arginine are the followed limited AA as well.

Table 2.

Amino acid composition of wheat gluten (Woychik et al., 1961)

		Amino acid		Amino acid g (16 g N)$^{-1}$			
Alanine	2.4	Glycine	3.1	Methionine	1.8	Tryptophan	1.0
Arginine	2.4	Histidine	2.2	Phenylalanine	4.9	Tyrosine	3.8
Aspartic acid	2.9	Isoleucine	4.0	Proline	13.7	Valine	4.1
Cysteine	2.1	Leucine	6.8	Serine	5.2		
Glutamic acid	37.3	Lysine	1.2	Threonine	2.5		

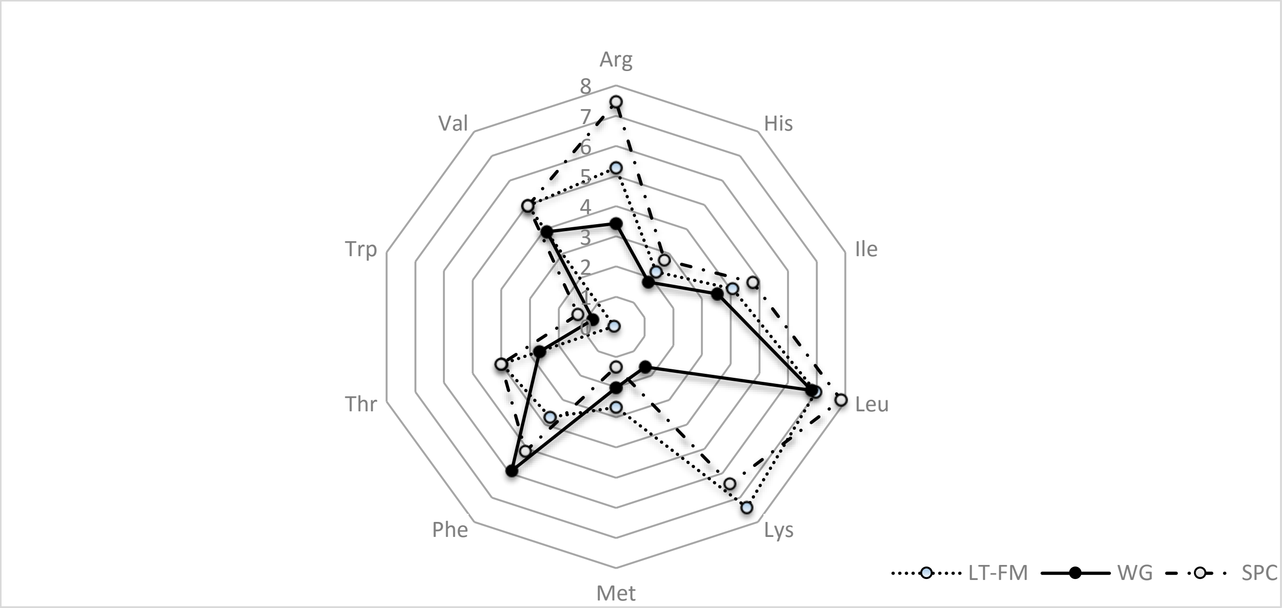

Figure 1. Contents of essential amino acids in three experimental protein sources, g (16 g N)$^{-1}$

Wheat gluten contains a higher concentration of sulfur-containing amino acids (1.8% of CP is methionine and 2.6% of CP is cysteine) than other PP sources (Allan et al., 2000). SBM and SPC respectively contain 1.4 and 1.3 g/ 100 g CP of methionine and 1.3 and 1.4 g/ 100 g CP of cysteine, which both are lower than fish requirements (Apper-Bossard et al., 2013). Thus, a combination of lysine and methionine supplementation was required for fish diets taken wheat gluten as only protein source (N × 6.25 at 445 g/ kg dry matter) (Pfeffer and Henrichfreise, 1994), meaning that the content of methionine in wheat gluten does not satisfy the requirement for maximum fish growth, in spite of being higher than that of LT-FM.

Anti-nutrient Factors (ANF) and Non-Starch Polysaccharides (NSP)

Replacing FM by plant protein (PP) sources may result in higher ANF content in diets. Some important antinutrients found in PP sources are shown in Table 3. ANF are defined as substances which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals. They could be divided into four groups: 1. factors affecting protein utilization and digestion, such as protease inhibitors, tannins, lectins; 2. factors affecting mineral utilization, which include phytates, gossypol pigments, oxalates, glucosinolates; 3. antivitamins; 4. miscellaneous substances such as mycotoxins, mimosine, cyanogens, nitrate, alkaloids, photosensitizing agents, phytoestrogens and saponines (Francis et al., 2001). ANF may reduce feed intake, growth, nutrient digestibility and utilization (Krogdahl et al., 2010). These negative effects have been observed in various species such as carp (Abel et al., 1984), Nile tilapia (Wee and Shu, 1989), rainbow trout (Dabrowski et al., 1989), Atlantic salmon (van der Poel, 1989). Because VWG is derived from wheat, which has low content of ANF, and the washing processing extracts the water-soluble ANF, the VWG almost has no ANF. There has not been observed action similar to ANF has been observed when wheat gluten was used as FM replacement in different species (Tusche et al., 2012). Also, when use wheat gluten replaced FM completely, no ANF effect was observed (Pfeffer et al., 1992).

The replacement of FM with PP sources may also result in increased level of Non-Starch Polysaccharides (NSP) in diets (Gatlin et al., 2007). Aquatic animals don't have a high capacity to digest dietary fibers, and a high dietary level of fibers may reduce digestibility and utilization of other nutrients, acting as ANF (Apper-Bossard et al., 2013). Such negative effects resulted from fibers were observed among others in rainbow trout, tilapia, and European sea bass (Dias et al., 1998; Hilton et al., 1983; Shiau et al., 1989). In SPC or soybean meal (SBM), the level of fibers is 4.5% and 7.0% respectively. For VWG, the amount of NSP is only 0.5 to 1%.

Table 3.

Important antinutrients present in some commonly used fish feed ingredients (Francis et al., 2001)

Plant-derived nutrient source	Antinutrients present
Soybean meal	Protease inhibitors, lectins, phytic acid, saponines, phytoestrogens, antivitamins, allergens
Rapeseed meal	Protease inhibitors, glucosinolates, phytic acid, tannins
Lupin seed meal	Protease inhibitors, saponines, phytoestrogens, alkaloids
Pea seed meal	Protease inhibitors, lectins, tannins, cyanogens, phytic acid, saponines, antivitamins
Sunflower oil cake	Protease inhibitors, saponines, arginase inhibitor
Cottonseed meal	Phytic acid, phytoestrogens, gossypol, antivitamins, cyclopropenoic acid
Leucaena leaf meal	Mimosine
Alfalfa leaf meal	Protease inhibitors, saponines, phytoestrogens, antivitamins
Mustard oil cake	Glucosinolates, tannins
Sesame meal	Phytic acid, protease inhibitors

1.1.4 Research on replacement of fish meal with wheat gluten in fish feed

In feeds for carnivorous fish, fish meal (FM) used to be the most common and important protein source especially for the marine fish species (Tacon et al., 2011). Because of the limited production and increasing price of FM, it is a trend to replace the FM in diets with other plant proteins (PP) (Olsen and Hasan, 2012). Several studies have been undertaken to evaluate the effects of replacement of FM with PP (Gatlin et al., 2007). For example, replacing FM by soy protein concentrate in diets of rainbow trout (Mambrini et al., 1999); Atlantic salmon (Storebakken et al., 1998), in which SPC incubation with phytase resulted in improved protein digestibility, feed conversion, protein retention, and reduced metabolic N-excretion. Evaluating different PP replacement in diets of Atlantic salmon (Bransden et al., 2001; Carter and Hauler, 2000). Recently, Zhang et al. (2012) have shown that the growth performance in rainbow trout can be similar to that obtained with LT-FM as the only source of protein, when using combinations of EAA-supplemented PP concentrates.

Based on the nutritional properties talked above as well as the production and sustainability, wheat gluten is a promising PP source. Some research have found that wheat gluten could replace up to 50% FM in diets without adversely effect of growth performance and feed efficiency, such as rainbow trout (Rodehutscord et al., 1995), Atlantic salmon (Storebakken et al., 2000), and seabass (Tibaldi et al., 2011). In rainbow trout, optimal performance in terms of weight gain and apparent net protein utilization was achieved by the fish fed a wheat gluten-based diet supplemented with lysine (0.58%) yielding a digestible lysine level of 1.9% of the complete feed (Davies et al., 1997), what's more Incorporation of wheat gluten into the diet did not adversely affect flavor of the fillets (Skonberg et al., 1998). In gilthead sea bream, use of 88% CP from wheat gluten had the highest protein and energy digestibility (96%, 91%, respectively) and better growth performance and feed conversion ratio (Kissil and Lupatsch, 2004).

1.2 Extruded feed

1.2.1 Extrusion

Extrusion means exposing the feed mix to high temperature, shear force and pressure, over a short period of time (Aslaksen et al., 2006). The extrusion system consists of a barrel housing with one or two rotating screws (single-or twin screw extruder) and a preconditioner as well as an accompanying machine control system (Sørensen, 2012). An extrusion processing line was shown in Fig. 1. Because of the high physical and nutritional quality of the feed, extrusion processing has become the primary technique used for fish feed production over the past 30 years (Hilton et al., 1981).

The preconditioner is a high speed mixing unit designed for mixing water and steam into the blend of dry ingredients. A precondition chamber may consist of two chambers and rotating shaft. The ingredient mixes with steam under controlled pressure as it turns for uniform addition of moisture and hot air comes in. This gives room for initial gelatinization of the starch and hydration of the protein and reduce the friction. The precondition chamber allows the mash to reach a temperature of up to 90 ℃ and moisture content of about 30%. The temperature generated in preconditioner is known as specific thermal energy dissipation (STE). The mash is then moved into the extruder barrel.

The extruder barrel consists of heads, shear locks and extruder screw which is a long cylinder with helical flight wrapped around it. Under the extrusion process, the temperature (120 - 130℃) and pressure (20 – 30 bar) come to a high point, which makes the blend of ingredients into a melt. It is made up of screws element mounted round a shaft and a shear lock which helps in proper mixing by reducing flow. The screws are configure to facilitate repeated mixing and conveying of mash, and also to generate pressure need to move the extrudate. The retention time is around 15 to 50 seconds. It should be noted that immediately after extruding the material, venting opening removes the steam thereby dropping the temperature within seconds to 35℃ and also reduces moisture content by moisture flash off. In extrusion, specific mechanical energy (SME) is generated by friction as the mash is moved forward by the rotating screw.

At the end of the barrel is the extruder die that prevents the outright flow of material which helps in pressure build-up and shear force that is needed for the mash to be plasticised. These are connected directly to a rotating knife that cuts the pellets at an appropriate length. In addition, oil could be added by using a vacuum coating machine if it needed.

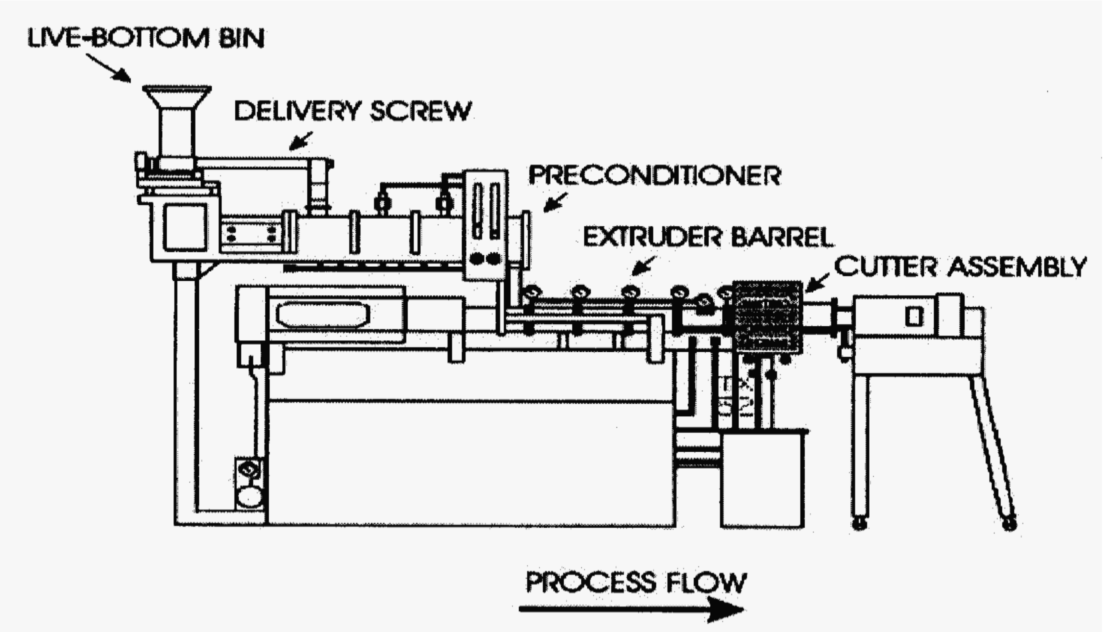

Figure 2. An extruded processing line

1.2.2 Effect of extrusion on fish feed

Extrusion facilitates starch gelatinization (Kim et al., 2006; Stone et al., 2003), which makes the feed durable in water and renders the starch digestible (Henrichfreise and Pfeffer, 1992). Meanwhile, extruded pellets had superior water stability and absorbed more water than steam pellets (Hilton et al., 1981). Therefore, extruded pellets has higher prehension, which lead to the increasing feed efficiency and reducing wastage of water. Extrusion also inactivates heat-labile ANF, such as inhibitors of digestive enzymes (Pfeffer et al., 1995). Thus, as a complex result, extrusion positively influences the digestibility of different nutrients. Mild extrusion processing could enhance the digestibility of plant proteins (Srihara and Alexander, 1984). Take rainbow trout as an example, the apparent digestibility coefficients of nutrients in test ingredients processed with or without extrusion are shown in Table 4. More than that, it could increase utilization of nitrogen-free extracts or other components and better growth performance would come from that (Bangoula et al., 1992; Burel et al., 2000).

Table 4.

Apparent digestibility coefficients of nutrients in test ingredients processed with or without extrusion for rainbow trout (Cheng and Hardy, 2003).

	Soybean meal		Barely		Corn gluten meal		Whole wheat	
Item	without	With	without	With	without	with	without	with
Dry matter	75.4	78.4	43.6	67.2*	74.2	86.0	46.7	71.1*
Crude protein	98.1	98.1	95.6	94.3*	87.4	75.4***	95.6	90.2*
Crude fat	73.0	86.1*	72.6	80.7*	76.0	75.7	77.3	74.4*
Gross energy	79.1	81.9	48.5	69.9	80.0	88.9*	54.0	77.1**
Ca	7.4	8.6	29.4	24.4	1.5	7.7	20.9	19.8
K	99.8	99.7	99.3	99.4	99.6	99.5	99.1	99.5
Mg	78.9	78.4	89.7	89.4	76.6	75.3	87.9	85.9*
P	63.2	60.6	76.3	70.6**	65.6	64.7	71.1	67.4*
S	98.1	97.9	96.5	96.4	94.7	91.8*	96.7	94.8**
Cu	94.9	94.2	88.3	81.7***	85.2	77.3***	86.6	79.4***
Fe	77.2	54.0**	55.9	53.4	78.7	33.4*	54.4	47.5
Mn	30.6	32.3	43.8	42.7	42.5	42.2	36.5	26.9
Zn	6.7	58.1*	55.4	48.0**	53.0	45.4**	56.0	40.9***

Asterisks show significant effect between same ingredients processed with and without extrusion. * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$).

1.2.3 Characteristics of VWG in extruded fish feed

VWG is visco-elastic, making it an efficient pellet binder in extruded fish feed. Under the back ground that most carnivorous fish have very low ability of hydrolyzing starch and regulating blood glucose concentration when the digestible starch level is high (Stone, 2003; Wilson, 1994), VWG can partly replace starch or indigestible binders in fish feed (Storebakken et al., 2000).

After hydration, mixing, shearing and heating, gliadins and glutenins interact in the dough through forces of various natures linked to their compositions: non-covalent bonds and covalent disulfide bonds (Wieser, 2007). Then, gluten forms a strong cohesive network to entrap the other ingredients, providing adapted physical characteristics to the pellet in term of binding: improvement of the pellet hardness and pellet durability (Apper-Bossard et al., 2013). Elevated breaking force was observed in diet FM was partly replaced by VWG compared to diets used FM or SPC as protein source (Draganovic et al., 2011). Furthermore, the water insolubility of

VWG reduces pellet breakdown (Day et al., 2006),which could be useful in feeds where water stability must be high, such as feeds for eals and shrimp.

1.3 Giant croaker, *Nibea japonica*

1.3.1 Classification, distribution and value

Giant croaker is a carnivorous and benthopelagic marine species and is classified as Actinopterygii (ray-finned fishes) > Perciformes (perch-likes) > Sciaenidae (drums or croakers) > Nibea. It lives in southwest Pacific, Southern Japan and East China sea and its depth range is 25-80m. It has desirable characteristics for aquaculture such as tolerance to widely varying salinity (18-30 ppt) and spawns in captivity. The Giant croaker is an important commercial farming fish along the coast of East China Sea. However, for commercial farming of Giant croaker raw trashfish and shrimps is the current commercially used feed, leading to high cost and nitrogen waste output (Wang et al., 2006a). Therefore, it is urgent to develop formulated feed for Giant croaker.

1.3.2 Previous research on Giant croaker

There has not been abundant research on the nutrition of this species. One study has found that the optimal dietary protein requirement for juvenile Giant croaker was determined to be 45% of a dry diet fed to fish in seawater. The conclusion was based on weight gain, feed efficiency, protein efficiency ratio values and muscle ratio (Lee et al., 2001). Results from previous studies on the nutrition of Giant croaker was are summarized in Table 5.

Table 5.

Some research on Giant croaker with focus on nutrition

Topic of study	Initial weight	Methods	Main results	Reference
Effect of feeding different dietary protein and lipid levels on growth, feed utilization, body composition and swimming performance	44.6 g	Feeding fish by diets with protein levels at 360, 400, 440, 480, 520 g kg^{-1} and lipid level 90, 150 g kg^{-1}	Suitable dietary crude protein and lipid levels are 480 g kg^{-1} and 90 g kg^{-1}. At same protein level, no significant difference in growth between fish fed diets with different lipid level.	Chai et al. (2013)
Effect of dietary lipid level on growth, feed utilization and body composition	6.7 g	Feeding fish by isoproteic diets with lipid level 5%-21%	Lipid of body and liver had linear increase with increase lipid level. Optimal dietary lipid level was 9%.	Han et al. (2014)
Effects of dietary starch level on growth and body composition	7.4 g	Feeding fish by diets with corn starch level 0-30%	12.2 –12.7% dietary starch level provided maximum growth.	Li et al. (2014)
Dietary calcium requirement	0.55 g	Feeding them with purified diets containing different levels of Ca for 10 weeks	At the level of rearing water containing 400 mg Ca l^{-1}, a minimum Ca level of 0.10% of the dry diet was required to maintain the normal growth.	Hossain (1999)
Effects of different dietary amino acid patterns on growth performance and body composition	10.7 g	Feeding fish by diets simulated the dietary amino acid profiles of juvenile Giant croaker whole body protein	The amino acid (AA) patterns of juvenile whole body protein could be used as a guideline in the formulation The juvenile Giant croaker could utilize high amounts (20%) of CAA in coated form for growth.	Chen et al. (2015)
Effects of different feeding rates on growth performance and body composition	26.6 g	Feeding fish by different feeding rates at 1%, 2%, 3%, 4% and 5%	Significant difference of growth performance and body composition were observed under different feeding rates. 3% was suitable feeding rate	Huilai et al. (2007)

2 Objectives of the research

The main objective of the research was to determine the nutritional value of Vital wheat gluten as the protein source in comparison with high-quality fish meal and soy protein concentrate in extruded diets for juvenile Giant croaker (*Nibea japonica*). The main response criteria were growth performance, feed utilization, nutrient retention and apparent digestibility of amino acids.

3 Materials and methods

3.1 Ingredients and diets

Fish meal (FM), Vital wheat gluten (VWG) and soy protein concentrate (SPC) were used as main experimental protein sources. Meanwhile, soybean meal (SBM), peanut meal (PM) and krill meal (KM) were used as supporting protein sources. The composition and amino acid analysis of feed ingredients used in the experimental diets (based on dry matter) are shown in Table 6.

VWG, wheat flour and taurine was premixed into a gluten, wheat and taurine blend (GWT), formulated to contain the same amount of crude protein (CP) as the FM and SPC. One control diet (V0) and seven experimental diets were formulated to be isonitrogenous (45% CP), isoenergetic (21.7 MJ kg^{-1}), and balanced for phosphorus. The measured crude protein levels varied from 44.1% to 45.6%. Three groups of diets were set: Diets VF1, VF2 and VF3 were formulated by substituting FM by increasing amounts of GWT. In diets VS1, VS2 and VF3, GWT gradually substituted SPC. The rate of replacement was 1/3, 2/3 and 1 in both groups. Finally, half FM and half SPC were replaced by GWT in group VFS. Feed formulation and analyzed chemical composition are shown in Table 7.

The experimental diets were produced in Feed Technology Laboratory of the Feed Research Institute, Chinese Academy of Agricultural Science, in Beijing. All the dry ingredients were ground in a hammer mill through a 0.2 mm screen and mixed. Then the mixed mesh were fed into a co-rotating four-section-barrel twin-screw extruder (MY56X2A, Muyang, Jiangsu, China) with a double shaft conditioner and die plate (MY56A 12-03/02 XL 09 11) containing the dies of 2 mm in diameter. The obtained extruded pellets were air-dried in a bed dryer fixed with electrical fans to final dry matter (DM) contents of 920–950 g kg^{-1} and top-dressed with the water bath heated (50 °C) blend of fish oil and soy lecithin. All the diets except Diet V0 were supplemented with first 4 limiting essential amino acids (EAA), and mono calcium phosphate, to the levels of EAA, Ca, and P in the V0 diet. Yttrium oxide (0.1 g kg^{-1}) was added to the diets as inert marker in order to determine apparent digestibility coefficients.

Table 6.

Composition of feed ingredients used in the experimental diets.

Ingredient	Fish meal[1]	Vital wheat gluten [2]	Soy protein concentrate[3]
Composition, g kg^{-1}			
Dry matter (DM), g	913	935	925
In dry matter, g kg^{-1}			
Crude protein, g	748	837	694
Crude fat, g	117	44	47
Starch, g	-	74	-
Ash, g	122	10	58
Gross energy, kJ g^{-1}	21.8	22.0	20.1
Essential amino acids (EAA), g (16 g N)$^{-1}$			
Arg	5.5	3.6	7.4
His	1.7	2.0	2.5
Ile	3.8	3.5	4.6
Leu	7.0	7.0	8.1
Lys	7.3	1.8	6.7
Met	2.6	1.4	0.9
Phe	3.8	5.2	5.3
Thr	4.1	2.5	4.1
Tyr	3.2	3.3	3.5
Val	4.5	3.8	4.7
Total EAA	43.3	34.1	47.9
Non-essential amino acids (NEAA), g (16 g N)$^{-1}$			
Ala	6.1	2.7	4.4
Asp	8.4	3.2	11.6
Cys	0.8	1.8	0.3
Glu	13.1	35.2	19.1
Gly	5.8	3.4	4.2
Pro	4.2	12.8	5.0
Ser	4.0	4.6	5.2
Total NEAA[4]	42.3	63.8	49.8
Total AA[5]	85.6	97.9	97.6

[1] Triple 9®, low-temperature dried fish meal, Esbjerg, Denmark.

[2] AMYGLUTEN 110, Syral Belgium N.V, Belgium.

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

[4,5] Trp excluded.

Table 7.

Feed formulation and analyzed chemical composition

Diet	V0	VF1	VF2	VF3	VS1	VS2	VS3	VFS
Ingredients, g kg^{-1}								
GWT[1]	-	69.0	138.0	207.0	69.0	138.0	207.0	207.0
Fish meal[2]	200.0	133.0	66.0	-	200.0	200.0	200.0	100.0
Soy protein concentrate[3]	214.0	214.0	214.0	214.0	142.0	71.0	-	107.0
Soybean meal[4]	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0
Peanut meal[5]	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0
Krill meal[6]	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Wheat flour	266.4	250.4	234.6	217.8	262.9	259.1	255.4	236.3
Fish oil[7]	84.0	89.0	94.0	99.0	84.0	84.0	84.0	92.0
Soy lecithin[8]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Premix[9]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Mono calcium phosphate[10]	14.0	17.0	20.0	23.0	14.5	14.5	14.5	19.0
Choline Cl[11]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Y_2O_3[12]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lysine[13]	-	3.7	7.4	11.0	3.1	6.1	9.1	10.0
Methionine[14]	-	0.4	0.8	1.2	-	-	-	0.2
Arginine[15]	-	1.1	2.1	3.1	2.1	4.2	6.2	4.7
Threonine[16]	-	0.8	1.5	2.3	0.8	1.5	2.2	2.2
Analyzed content, kg^{-1}								
Dry matter, g	951	954	957	956	957	957	957	955
Crude Protein, g	441	447	444	449	447	451	456	454
Crude Fat, g	135	136	139	142	140	141	131	140
Ash, g	76	69	61	54	72	68	64	59
Gross Energy, KJ g^{-1}	21.5	21.6	21.6	22.0	21.5	21.7	21.8	22.0
Amino Acid g (100 g CP)$^{-1}$								
Arg	6.5	6.5	6.6	6.4	6.5	6.3	6.8	6.2
Ile	4.1	3.9	3.9	3.8	3.9	3.7	3.7	3.5
Leu	7.2	7.0	7.2	7.0	7.0	6.7	6.9	6.6
Lys	6.1	6.0	6.1	6.1	6.1	5.9	6.2	5.9
Met+Cys	2.1	2.0	2.1	2.1	2.0	2.0	2.5	1.9
Phe+Tyr	7.4	7.3	7.7	7.7	7.1	6.7	7.3	6.8
Thr	3.8	3.8	3.8	3.7	3.8	3.7	3.8	3.5
Val	4.4	4.2	4.1	4.0	4.2	4.0	4.1	3.8
Total EAA	41.7	40.7	41.5	40.7	40.6	39.0	41.4	38.2
Ala	4.7	4.3	4.0	3.5	4.6	4.2	4.3	3.7
Asp	9.9	8.8	8.6	7.8	8.8	7.8	7.1	6.9
Glu	16.3	18.7	21.0	22.9	18.0	19.1	22.1	21.4
Gly	5.0	4.5	4.3	3.9	4.7	4.5	4.6	4.0
Pro	4.7	5.4	6.1	7.0	5.5	6.0	7.2	7.2
Ser	4.4	4.4	4.6	4.5	4.3	4.2	4.3	4.2
Total AA	88.8	88.7	92.1	92.4	88.6	86.6	93.0	87.4

[1] Mixture of vital wheat gluten, wheat flour and taurine (mixing ratio: 77.5%, 20.5% and 2%).
Wheat gluten, AMYGLUTEN 110, Syral Belgium N.V, Belgium.
Wheat flour, 7 BLUEKEY®, Beijing Grain and Oil Industry Co., Ltd, Wilmar, Beijing, China.
Taurine-JP8, Qianjiang Yongan Pharmaceutical Co., Ltd., Hubei, China.

[2] Triple 9®, low-temperature dried fish meal, Esbjerg, Denmark.

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

[4] FENGYUAN®, Glodensea Grain and Oil Industry Co., Ltd, Wilmar, Qinhuangdao, China.

[5] FENGYUAN®, Glodensea Grain and Oil Industry Co., Ltd, Wilmar, Shijiazhuang, China.

[6] QRILL™, Antarctic Krill Meal, Aker BioMarine, Oslo, Norway.

[7] Wilpromil, Glodensea Grain and Oil Industry Co., Ltd, Wilmar, Qinhuangdao, China.

[8] Aidayufen Co., Ltd, Rongcheng, China

[9] Vitamin premix (mg kg^{-1}diet): vitamin A 20; vitamin B_1 12; vitamin B_2 10; vitamin B_6 15; vitamin B_{12} 8; niacinamide 100; ascorbic acid 1000; calcium pantothenate 40; biotin 2; folic acid 10; vitamin E 400; vitamin K_3 20; vitamin D3 10; inositol 200; corn protein powder 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

[10] MCP22, mono calcium phosphate, feed grade, Suntran Industrial Group Ltd., Anhui, China.

[11] Be-long corporation, Nanjing, China

[12] Metal Rare Earth Limited, Shenzhen, China.

[13, 14, 15, 16] Siwei Development Group LTD, Hangzhou, China.

3.2 Physical feed quality assessment

The length (50 measurements per diet) and diameter (50 measurements per diet) were measured by a digital caliper (K14C, Guanglu CO., LTD, GuangXi, China). Expansion was calculated as the percent-wise difference between the pellet diameter and the die diameter.

Durability was determined with a pellet tester (ST-136, Shengtai CO., LTD, Ji'nan, China), consists of 2 circular boxes. 50*2 grams of pre-sieved pellets were weighted and loaded in the circular boxes respectively. The speed was 50 rounds per min and the treatment was continued for 10 min. Afterwards, the pellets were sieved again and weighted, the durability (% pellet remaining) was calculated.

Breaking force was measured with a tester (ST-120B, Shengtai CO., LTD, Ji'nan, China). This device has a load cell with 20 kg maximum and 0.1 kg precision. The pellet was individually placed at radial direction to load arm. The forces used to break the pellets were recorded automatically in the device (50 repeats per diet).

Water stability was tested by the loss of dry matter from pellets in a water bath (Kexi CO., LTD, Jintan, China). 10 g pellets (precision 0.1 g) was weighted and set into a cylindrical wire-mesh basket (6.5 cm-height, 6.5 cm-diameter, 0.85 mm-mesh size). Then the pellets contained in the basket were soaked in a 5.5 cm deep water bath at 25℃ for 20 min. Afterwards, the baskets were carefully lifted and submerged three times, dried at 105℃ in a drying oven till constant weight and then weighted, in addition, another 10 g pellets were dried and weighted through the same treatment as control group.

Sinking rate was assessed through dropping single pellets into a 150 cm long transparent plastic tube (50 measurements for each diet). The tube was filled with water at the 100 cm level. Time was monitored from dropping until sinking to the bottom.

3.3 Fish and feeding trial

Giant croaker juveniles were obtained from Marine Fisheries Research Institute of Zhejiang Province (Putuo, Zhoushan, China), acclimated in an indoor pond for 2 weeks, and fed by a commercial diet (Fujian Haid Feed Co., Ltd, Fuzhou, China; crude protein 45%, crude fat 8%).

The 59-day feeding experiment was conducted in Joint Laboratory of Nutrition and Feed for Marine Fish, which belongs to same institute. Before the experiment, the juveniles were starved for 24 h. Thereafter, 624 fish were picked with similar size (initial weight 11 g) and anaesthetized with MS-222 (90 mg l^{-1}). Twenty six juveniles were assigned in each of 21 tanks and batch-weighed. Each dietary treatment was allocated randomly to three tanks randomly. The tank size was 250 l, and were supplied with sand-filtered seawater at a flow of 2 l/min. To make sure that dissolved oxygen was higher than 6.0 mg/l in tank, one nano-pore size air stone (Guizu Co., Ltd, ShanDong, China) was used to support aeration. The photoperiod (13h light and 11h dark) was natural throughout the feeding period. The temperature of water is 28 ± 1℃ during the feeding period.

All fish were fed by hand four times per day at 06:40, 09:50, 13:00 and 16:10 respectively. Each feeding lasted for 30 min. The daily feeding rate was tentatively set 10% in excess based on the average feed intake over the last 3-day feeding. Uneaten feed was collected immediately after feeding. Uneaten feed was collected into strainer bags by syphoning and weighted after 30 min runoff time, before being kept at -16℃. Moisture content of uneaten feed (pooled by tank) was determined when the experiment was finished.

For calculating feed intake, leaching rate was determined by weighting 5 g of each feed into a tank without fish, but with the same quantity of water as experimental tank, for 30 min. The feed was then collected and weighed by the same procedure as when feeding the fish. The amount of leaching was used to correct the values obtained when calculating the daily feeding rate, in accordance with Helland et al. (1996).

3.4 Sampling

Before setting the experiment, 30 juveniles, starved for 24 h, were taken randomly from the acclimation pond and killed by an overdose of MS-222 (Hangzhou DongBao, China) and pooled as triplicate initial samples. The samples were weighted and frozen at -20℃ for subsequent whole body chemical analysis.

At end of the experiment, all 26 Giant croakers per tank were anaesthetized with MS-222 (90 mg l^{-1}) and batch-weighted. Contents of stomachs content and intestines were removed from 5 fish from each tank, and the fish were kept at -20℃ for whole body analysis. The remaining fish were killed by over-dose MS-222 and cut open, the digesta in distal intestine (from the start of the last fold of intestine until the anus) were obtained by careful stripping. Digesta were frozen at -20℃ for subsequent analysis.

3.5 Chemical analyses

The initial and final fish whole body samples were cut up and autoclaved at 120℃ (YXQ-LS, Xunbo, Shanghai, China) for 30min. Afterwards, they were homogenized and oven-dried (Jinghong, Zhejiang, China) at 70℃ for 24h. Lyophilization (LABCONCO Freezon 4.5, Kansas City, USA, -50℃ for 60h) was used to dry feces samples. Feed ingredients, feeds, initial and final whole body samples and freezedried faeces were analyzed for the following parameters: Dry matter was determined through constant temperature drying (GB6435-86) in the same drying oven. Crude protein was determined by the Kjeldahl method (GB6432-86). Crude fat was determined by Soxhlet extraction (GB6433-86) in a Soxhlet machine (Jingke, Shanghai, China). Gross energy was tested by bomb calorimetry (Phillipson Microbomb Calorimeter; Gentry Instruments Inc., Aiken, SC, USA).Ash was determined by the method of combustion at 550^{o}C temperature (GB6438-86) in a muffle (Daoxu, Shangyu, Zhejiang, China). Amino acids were tested by using an amino acids analyzer (L-8900, Hitachi, Japan).

3.6 Calculations and statistical analysis

Expansion (%) was calculated as 100 x [(pellet diameter- die diameter) x die diameter^{-1}]. Water stability (%) was calculated as 100 x [(weight of water bath treated pellets) / (weight of untreated pellets)]. Feed intake (FI) was calculated by subtracting uneaten feed from feed fed on a dry matter basis. Recovery of uneaten feed was estimated as described by (Helland et al., 1996). Weight gain (WG, g / fish): WG=100 x (FBW - IBW) x IBW^{-1}, FBW represents final body weight and IBW represents initial body weight. Feed conversion ratio (FCR) was calculated as: FI x (FBW - IBW)$^{-1}$, where FI means feed intake. Apparent digestibility coefficients (ADC_N) was calculated as: $100 \times [1 - (Y_d \times Y_f^{-1} \times N_f \times N_d^{-1})]$, where Y_d and Y_f stand for the concentration of yttrium in the diet and faeces, N_d and N_f represent the concentration of individual nutrients or energy in the diet and faeces, respectively. Nutrient and energy retentions (R_N) was calculated as: $100 \times (N_1 \times FBW - N_0 \times IBW) \times (N_d \times FI)^{-1}$, where N_1 and N_0 represent the nutrient or energy concentration in the initial and final whole fish samples.

All the data were statistically analyzed by one-way analysis of variance. Each tank was considered an experimental unit (n=3 replicates). Linear and polynomial regression were used for evaluation of relationship between different parameters and GWT inclusion in feed. Polynomial regression up to 2nd order and model were chosen based on P-value and R^2. Pair-Sample t-Test was used to analysis the significant difference when both LT-FM and SPC were replaced by GWT. Level of significance was $P < 0.05$. All statistical analyses were conducted with the Origin (8.0) software (Originlab, Co., Ltd, Nothampton, USA).

4 Results

4.1 Extrusion parameters and physical pellet quality

Water addition to the preconditioner and extruder were used to adjust bulk density to a level tentatively above 520 g l^{-1} (Table 8). This resulted in total water addition levels ranging from 16 to 28% of the extrudate. The revolution of screws was also reduced from 280 to 259 rpm for the Diets V0 and VF1 that required lowest water addition to the extrudate.

Diet V0 had a lower bulk density and sinking rate compared to other experimental diets. Expansion was gradually increased with the increasing GWT inclusion, both for the LT-FM and SPC replacement groups (Fig. 3). As to breaking force, the values decreased in diets with LT-FM replaced by increasing GWT inclusion while increased in diets with SPC replaced by increasing GWT inclusion. The highest water stability is 89.1% with diet VS1 and the lowest is 79.7% of diet VF1, the sinking rate ranged from 7.94 to 9.47 cm s^{-1}.

The content of EAA in eight diets is shown in Table 7, and the EAA profiles of gluten containing diets with the first-four limiting AAs supplementations were nearly to that of the fish meal control (Fig. 4).

Table 8.

Extrusion processing parameters and feed pellet physical quality.

Diet	1	2	3	4	5	6	7	8
	V0	VF1	VF2	VF3	VS1	VS2	VS3	VFS
Extruder parameters								
Feeding rate, kg h^{-1}	125	125	125	125	125	125	125	125
Water addition in conditioner, kg h^{-1}	10.0	15.0	20.0	20.0	18.0	20.0	20.0	20.0
Water addition in extruder, kg h^{-1}	10.0	10.0	15.0	15.0	15.0	15.0	15.0	15.0
Total water addition, %	16.0	20.0	28.0	28.0	26.4	28.0	28.0	28.0
Revolution screws, rpm	259	259	280	280	280	280	280	280
Die temperature, °C	117	87	86	86	88	85	85	86
Cutter speed, rpm	2300	2400	2350	2350	2400	2450	2400	2300
Bulk density, g l^{-1}	518	561	571	563	561	561	550	558
Physical quality								
Length, mm	4.6	4.0	4.3	4.3	3.9	3.8	3.8	4.4
Diameter, mm	3.5	3.5	3.6	3.8	3.5	3.6	3.7	3.8
Expansion, %	74.9	74.1	79.3	87.9	76.7	78.7	83.4	91.8
Durability, %	99.8	99.9	99.5	99.5	99.8	99.7	99.6	99.3
Breaking force, N	24.7	37.1	35.0	34.7	32.4	34.0	35.4	35.8
Water stability, %	88.8	79.7	86.0	84.7	89.1	86.4	85.1	83.1
Sinking rate, cm sec^{-1}	7.9	9.0	8.4	8.2	9.3	9.8	9.5	8.8

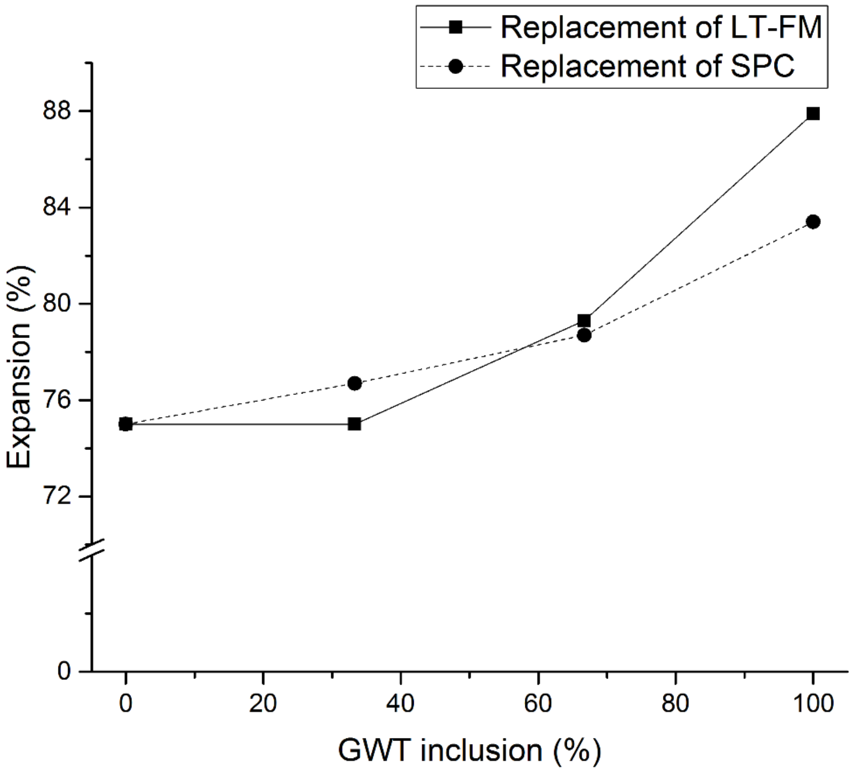

Figure 3. Expansion (%) of experiment diets

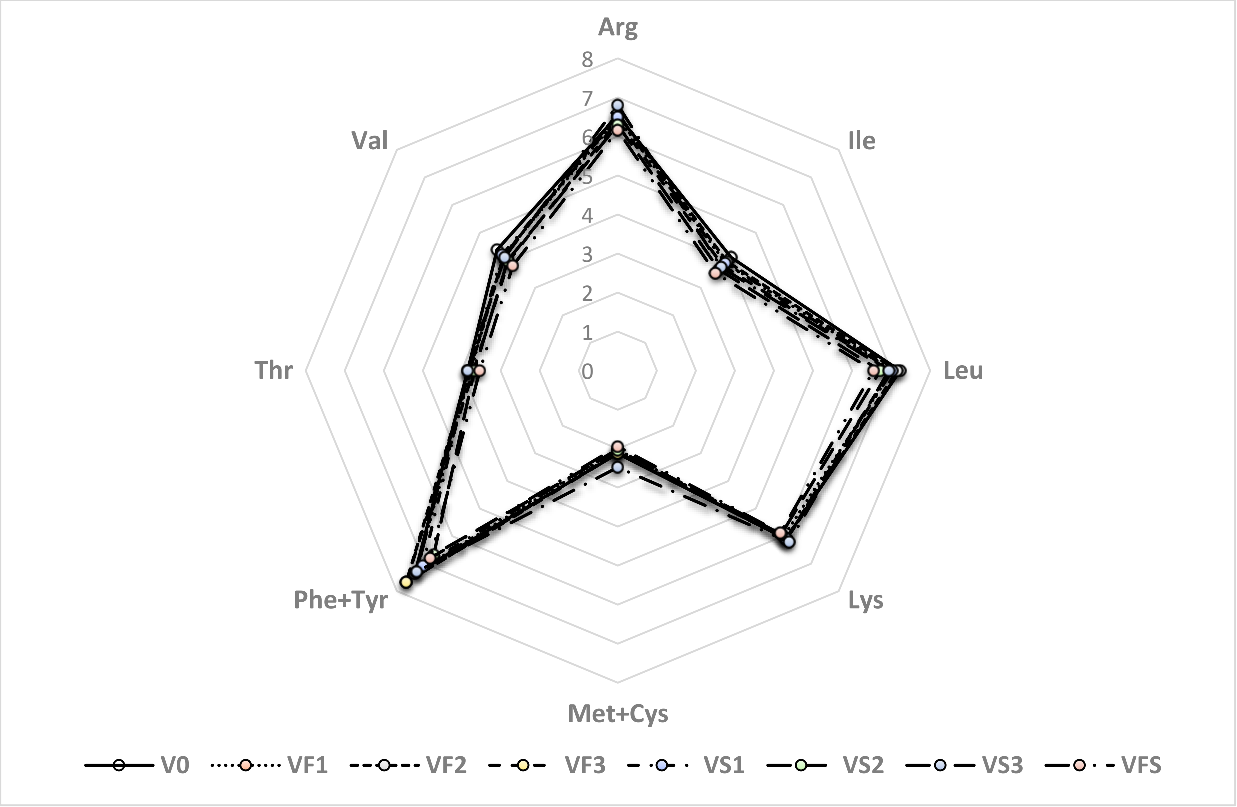

Figure 4. Essential amino acid profiles of the 8 Diets (g (16 g N)$^{-1}$)

4.2 Effects of replacing LT-FM by GWT

One tank of fish fed the diet VF1 were lost during the last stage of the feeding trial because of parasite infection. Otherwise, one pooled fecal sample from another tank of the same treatment was destroyed during analysis. Thus resulted in two sets of data on growth, feed utilization, whole body composition and nutrient retentions and one set of data on ACDs of AA were adopted for statistical analysis in this treatment.

The feed intakes of Giant croakers fed diet with increasing inclusion of GWT ranged from 44.4 g to 68.5 g (Table 9), and were significantly affected by the dietary inclusion of GWT ($P < 0.001$). A strong quadric relationship was also found between FI and GWT inclusion ($R^2 = 0.72$) (Fig. 5). After the 8-week feeding, an average WG of 59.4 g had been achieved. However, the dietary inclusion of GWT had no significant impact on the growth performance of fish ($P > 0.05$). FCR was significantly affected by the dietary inclusion of GWT ($P < 0.01$). A strong quadric relationship was also found between FCR and GWT inclusion ($R^2 = 0.70$) (Fig. 6) and control group had the lowest FCR value. The dietary inclusion of GWT had no significant impact on the body composition of fish ($P > 0.05$). Both Nitrogen (N) and energy retentions were significantly decreased with increasing level of GWT in the diet ($P < 0.01$, $P < 0.05$ respectively), and quadric relationships between GWT inclusion and both N retention ($R^2 = 0.76$) and energy retention ($R^2 = 0.61$) were observed (Fig. 7 and Fig. 8). The average ADC of total EAA and total AA were 83.4% and 83.8%, respectively. No significant effects of GWT inclusion on ADC of individual EAA, total EAA, and total AA were observed.

Table 9.

Effects of Giant croaker fed diet with decreasing LT-FM replaced by GWT[a]

	Replacing level %				R^2	*P*-value	Regression model [c]	Pooled S.E.M
	0	33.3[b]	66.7	100				
Diet	V0	VF1	VF2	VF3				
Growth and feed utilization								
Feed intake, g	44.4	68.5	64.3	66.2	0.72	< 0.001	$45.79 + 0.64x - 0.0045x^2$	5.0
Weight gain, g fish^{-1}	53.6	71.0	54.2	58.7		0.10		9.6
FCR, g DM ingested (g gain)$^{-1}$	0.83	0.97	1.20	1.13	0.70	< 0.01	$0.81 + 0.0087x – (5.32E-5)x^2$	0.10
Whole body composition, g kg^{-1}								
Crude protein	172.4	175.9	173.7	170.0		0.63		5.6
Crude fat	58.4	62.1	49.5	52.1		0.43		13.5
Ash	34.2	35.5	36.3	35.1		0.11		1.0
Moisture	732.0	725.8	739.4	738.3		0.4		12.7
Gross energy, KJ g^{-1}	6.47	6.64	6.10	6.18		0.37		0.5
Nutrient retention, %								
N	45.4	39.7	31.7	32.2	0.76	< 0.01	$45.87 - 0.3x + 0.0016x^2$	3.8
Energy	36.4	32.0	23.4	24.6	0.61	< 0.05	$36.97- 0.27x + 0.0014x^2$	5.5
Apparent digestibility coefficients of amino acid , %								
Arg	93.0	92.7	91.3	92.0		0.90		3.5
Cys	32.1	37.3	35.9	54.3		0.37		31.6
His	85.0	84.0	82.9	82.5		0.88		6.4
Ile	84.6	83.7	81.4	80.6		0.81		8.3
Leu	85.3	85.4	83.0	82.1		0.81		7.6
Lys	86.0	85.3	84.8	84.5		0.97		6.0
Met	69.1	67.0	62.8	63.6		0.31		11.4
Phe	85.7	85.8	84.3	84.8		0.98		7.1
Thr	79.9	79.1	78.1	78.0		0.96		8.2
Tyr	84.3	84.2	81.6	82.2		0.91		9.4
Val	83.0	82.2	80.0	78.7		0.80		8.7
Total EAA	84.5	84.2	82.3	82.4		0.91		7.3
Total AA	84.4	84.1	83.1	83.7		0.98		6.8

[a] n = 3, triplicates per dietary treatment.

[b] n = 2, duplicates in treatment of fish fed diet VF1 were analyzed for growth and feed utilization, whole body composition and nutrient retentions. Only one replicate in treatment of fish fed diet VF1 was analyzed for ADC of AA.

[c] x, the percentage of protein from LT-FM replaced by protein from GWT.

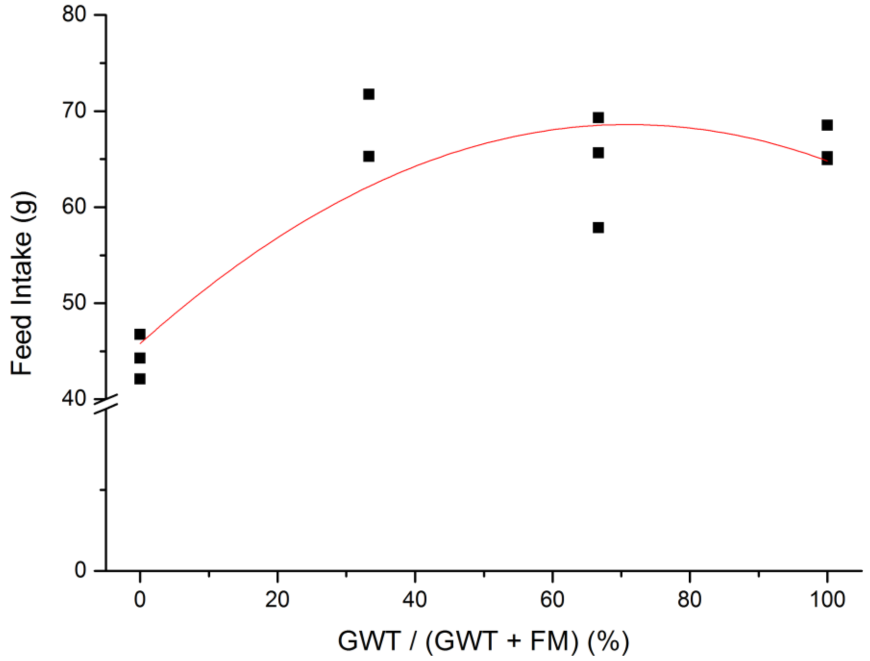

Figure 5. Feed intake (FI) of Giant croakers fed diets where LT-FM is replaced by GWT

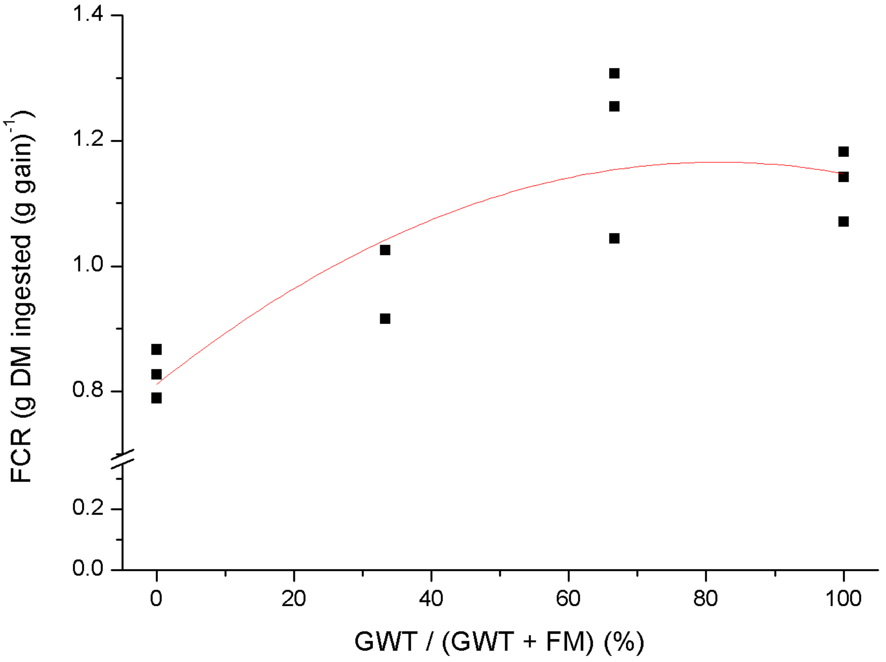

Figure 6. Feed conversion ratio (FCR) of Giant croakers fed diets where LT-FM is replaced by GWT

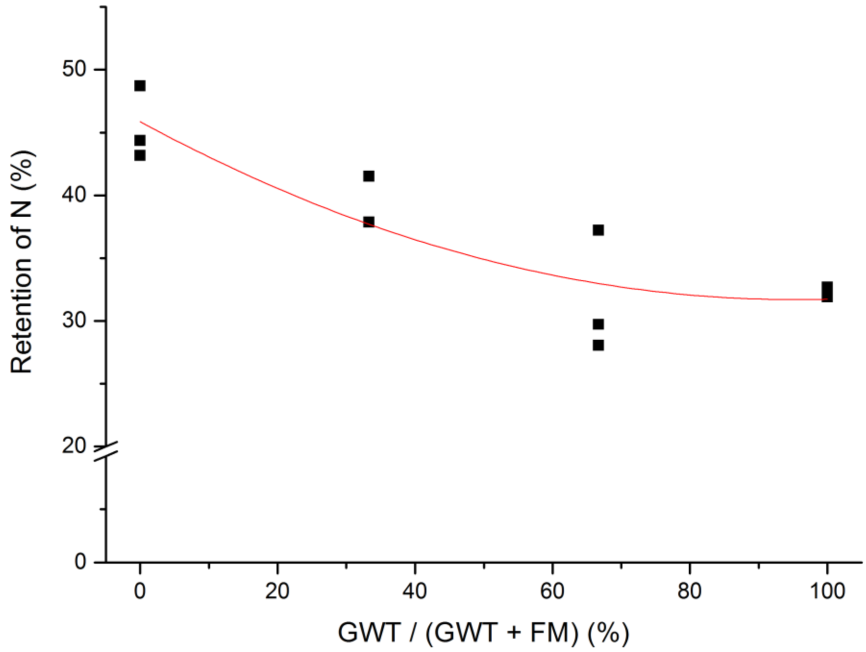

Figure 7. Retention of nitrogen (NRE) of Giant croakers fed diets where LT-FM is replaced by GWT

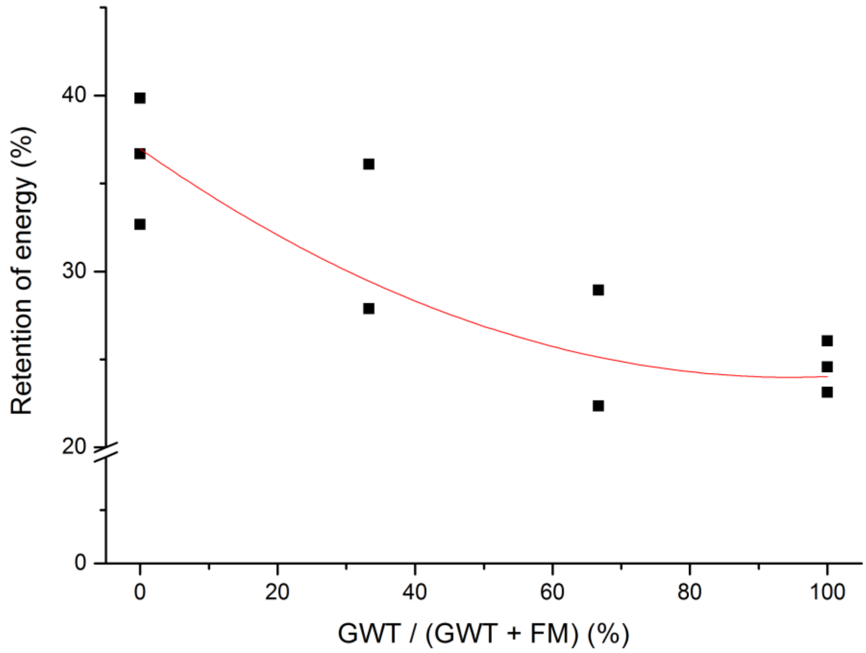

Figure 8. Retention of energy (ERE) of Giant croakers fed diets where LT-FM is replaced by GWT

4.3 Effects of replacing SPC by GWT

The feed intake of Giant croakers fed diet with increasing inclusion of GWT ranged from 44.4 g to 72.6 g (Table 10), and were significantly affected by the dietary inclusion of GWT ($P < 0.01$). A strong quadric relationship was also found between FI and GWT inclusion ($R^2 = 0.62$) (Fig. 9). After 8-week feeding, an average WG of 67.9 g had been achieved. WG increased at first then decreased with increasing GWT inclusion ($P < 0.05$, $R^2 = 0.44$) (Fig. 10). Meanwhile, FCR was significantly affected by the dietary inclusion of GWT ($P < 0.05$). A quadric relationship was also found between FCR and GWT inclusion ($R^2 = 0.55$) (Fig. 11).

The dietary inclusion of GWT had no significant impact on whole body composition of fish except for the ash content ($P < 0.05$). Furthermore, a quadric relationship was found between ash content and GWT inclusion ($R^2 = 0.53$) (Fig. 12). The dietary inclusion of GWT had no significant impact on energy retention. However, N retention was significantly decreased with increasing level of GWT in diet ($P < 0.05$, $R^2 = 0.53$) (Fig. 13).

The ADC of cysteine was linearly increased from 32.1% to 61.0% when GWT inclusion increased from 0 to 100% in diet ($P < 0.01$, $R^2 = 0.76$) (Fig. 14). The average ADC of total amino acids were around 87%, which was significantly increased with the increase of GWT in diet ($P < 0.05$, $R^2 = 0.53$) (Fig. 15). However, the dietary inclusion of GWT had no significant impact on the ADC of other EAAs and total EAA ($P > 0.05$).

Table 10.

Effects of Giant croaker fed diet with decreasing SPC replaced by GWT[a]

	Replacing level %				R^2	*P*-value	Regression model [b]	Pooled S.E.M
	0	33.3	66.7	100				
Diet	V0	VS1	VS2	VS3				
Growth and feed utilization								
Feed intake, g	44.4	72.6	68.8	68.2	0.62	< 0.01	$46.14 + 0.85x - 0.0065x^2$	8.3
Weight gain, g fish^{-1}	53.6	75.8	72.8	69.3	0.44	< 0.05	$54.88 + 0.71x - 0.0058x^2$	9.7
FCR, g DM ingested (g gain)$^{-1}$	0.83	0.96	0.95	0.98	0.55	< 0.05	$0.84 + 0.0037x – (2.25E-5)x^2$	0.06
Whole body composition, g kg^{-1}								
Crude protein	172.4	173.2	176.1	172.8		0.60		4.2
Crude fat	58.4	63.8	63.9	51.8		0.45		11.6
Ash	34.3	35.0	33.9	32.4	0.53	< 0.05	$34.33 + 0.03x – (5.07E-4)x^2$	1.0
Moisture	732.0	723.4	723.9	740.2		0.30		13.2
Gross energy, KJ g^{-1}	6.47	6.74	6.75	6.20		0.36		0.5
Nutrient retention, %								
N	45.4	39.3	40.1	37.4	0.47	< 0.05	$44.92 – 0.15x + (7.72E-4)x^2$	3.0
Energy	36.4	33.0	33.0	28.7		0.15		4.1
Apparent digestibility coefficients of amino acid, %								
Arg	92.9	94.1	94.0	93.8		0.15		0.69
Cys	32.1	40.2	52.0	61.0	0.76	< 0.01	$31.58 + 0.3x$	8.15
His	85.0	86.1	87.3	86.6		0.49		2.03
Ile	84.6	86.4	87.2	86.2		0.37		2.00
Leu	85.5	87.6	88.8	88.2		0.17		1.90
Lys	85.9	87.5	88.2	88.0		0.35		1.85
Met	69.1	70.2	70.1	71.7		0.84		4.08
Phe	85.7	88.0	88.9	88.2		0.11		1.66
Thr	79.9	82.5	84.4	84.4	0.51	0.0501		2.10
Tyr	84.4	85.1	85.1	86.2		0.79		2.51
Val	83.0	84.9	86.0	85.3		0.34		2.20
Total EAA	84.5	86.4	87.2	87.0		0.20		1.75
Total AA	84.4	86.6	87.7	87.8	0.53	< 0.05	$84.41 + 0.08x – (4.57E-4)x^2$	1.53

[a] triplicates per dietary treatment.

[b] x, the percentage of proteins from SPC replaced by proteins from GWT

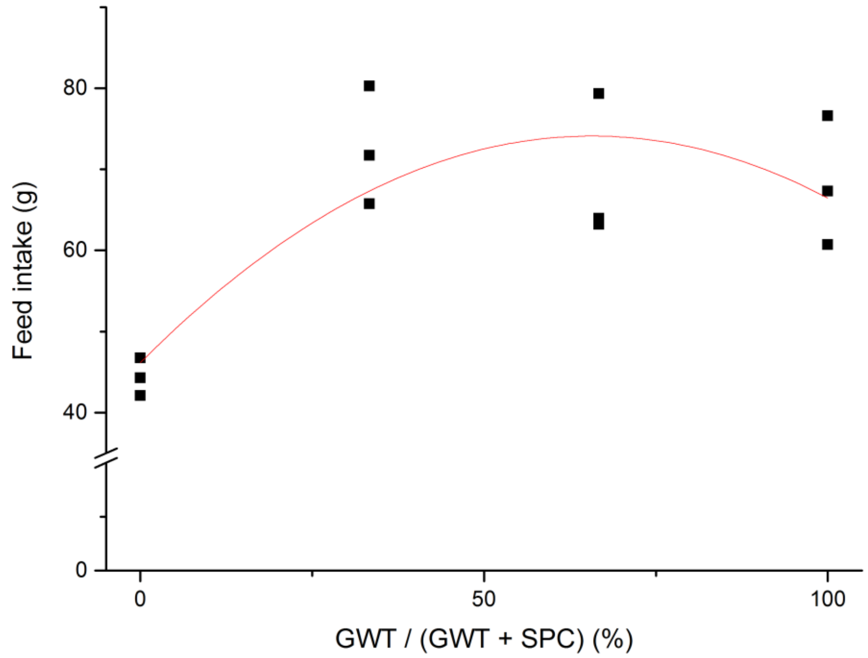

Figure 9. Feed intake (FI) of Giant croakers fed diets where SPC is replaced by GWT

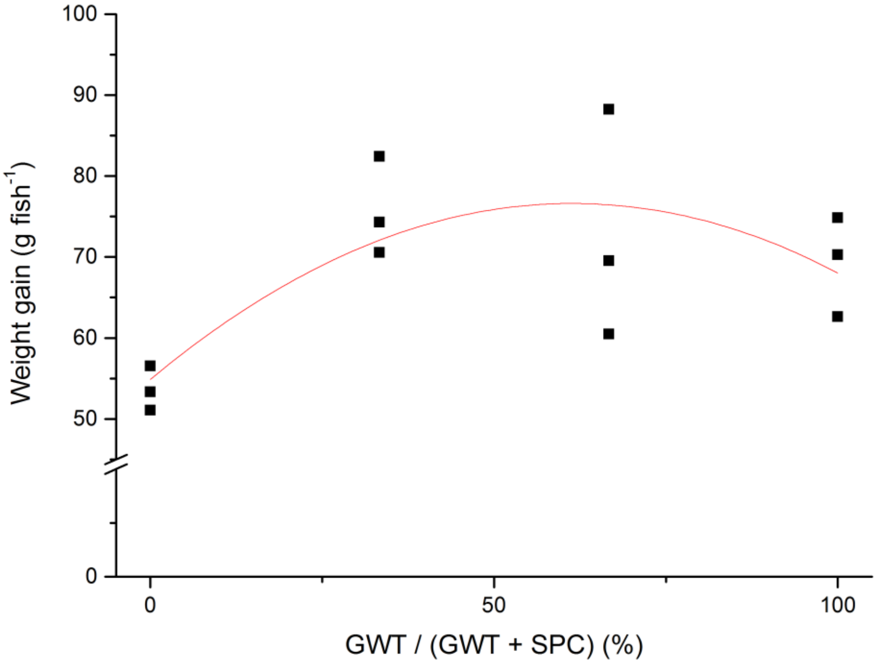

Figure 10. Weight gain (WG) of Giant croakers fed diets where SPC is replaced by GWT

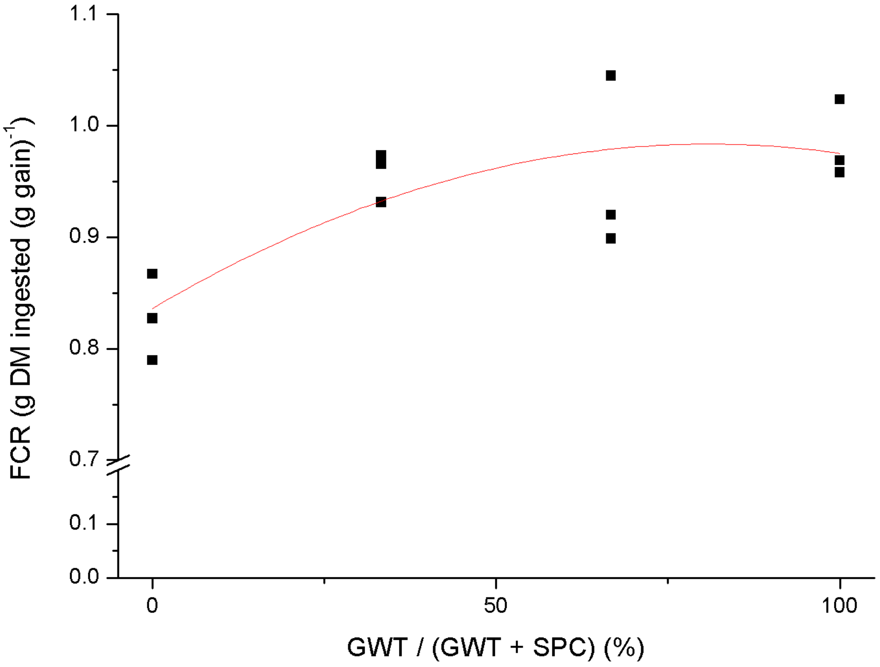

Figure 11. Feed Conversion ratio (FCR) of Giant croakers fed diets where SPC is replaced by GWT

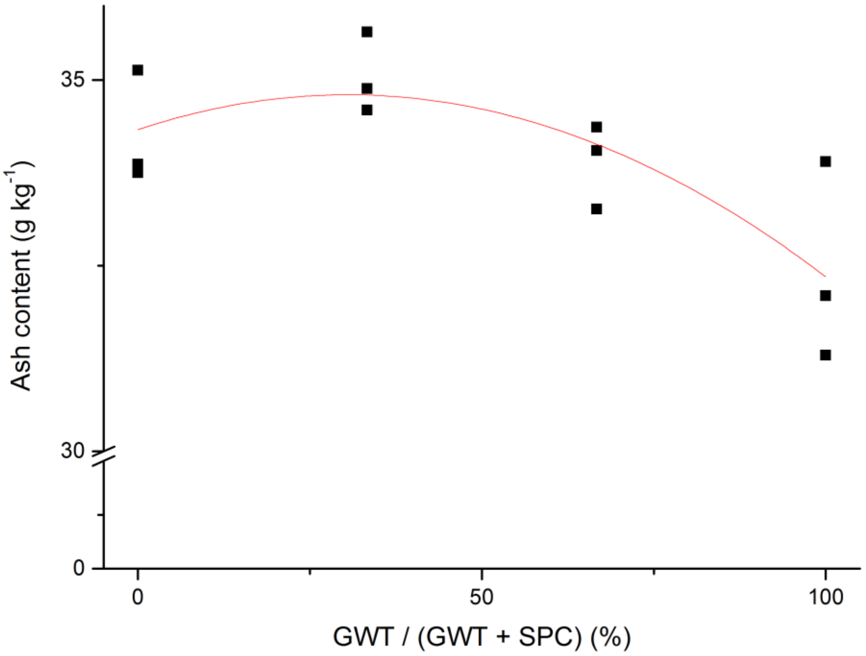

Figure 12. Ash content of Giant croakers fed diets where SPC is replaced by GWT

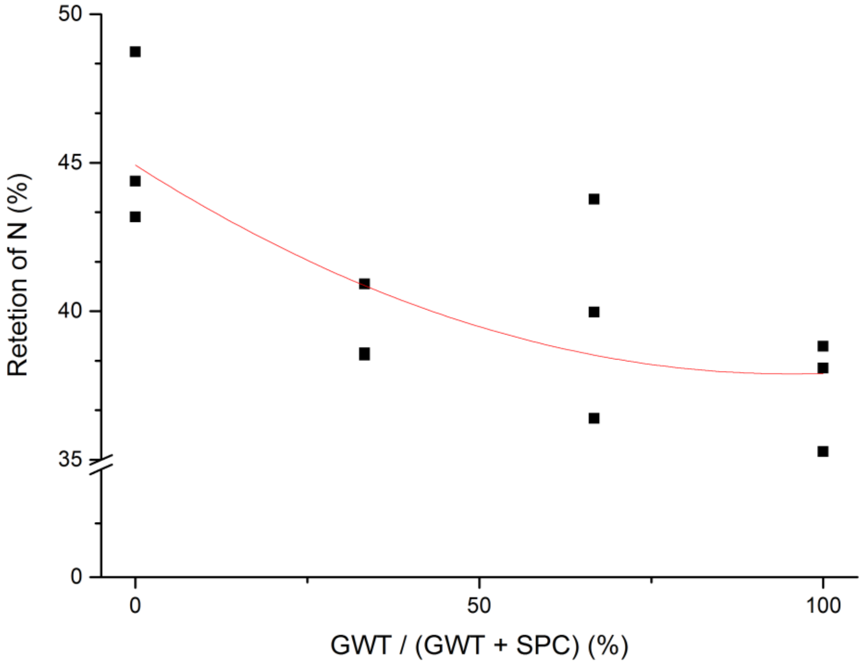

Figure 13. Retention of nitrogen (NRE) of Giant croakers fed diets where SPC is replaced by GWT

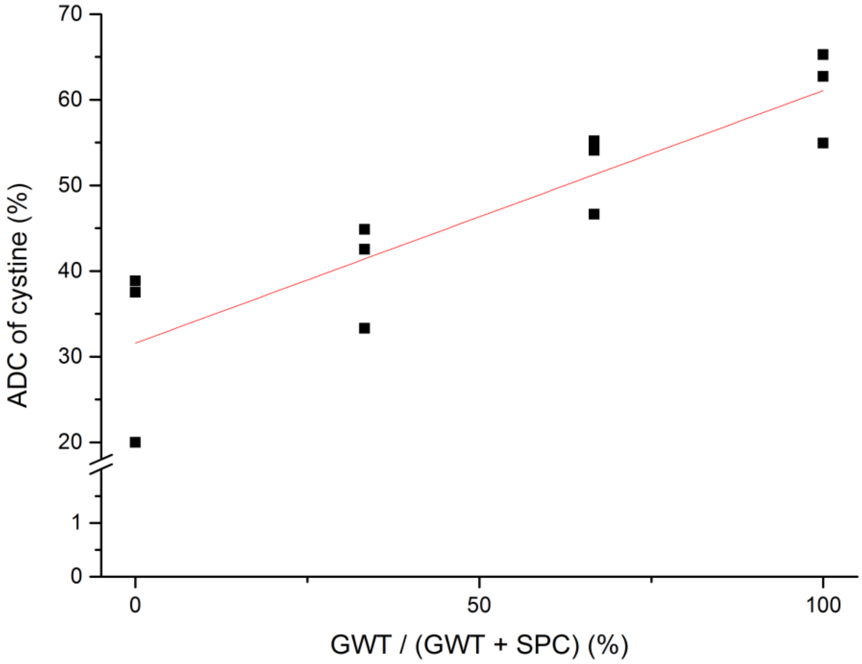

Figure 14. Apparent digestibility coefficients (ADC) of Cys for Giant croakers fed diets where SPC is replaced by GWT

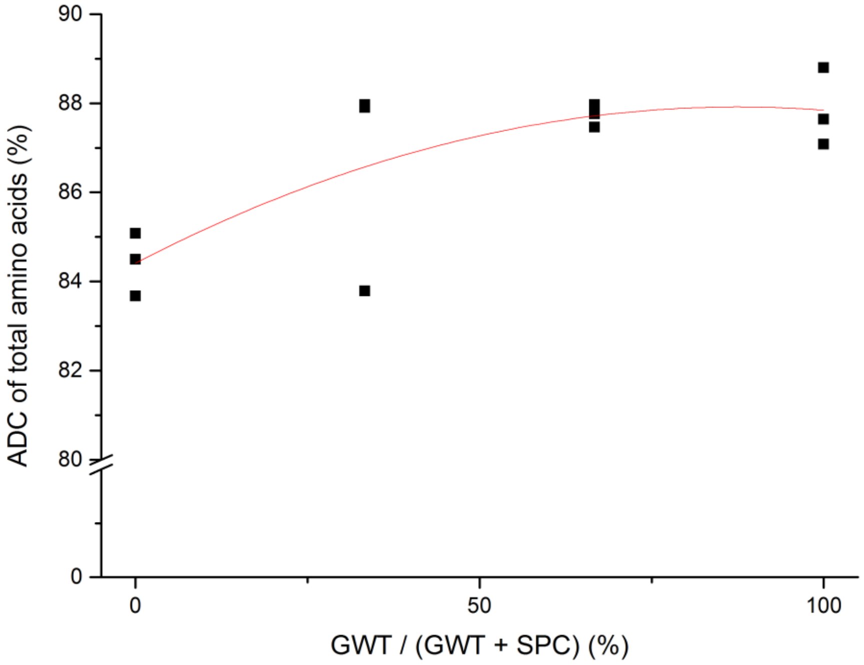

Fig. 15. Apparent digestibility coefficients (ADC) of total amino acids of Giant croakers fed diets where SPC is replaced by GWT

4.4 Effects of jointly replacing LT-FM and SPC by GWT

The results of jointly replacing 50% proteins from LT-FM and 50% proteins from SPC by proteins of GWT are shown in Table 11. When LT-FM and SPC were jointly replaced by GWT, both FCR and whole body moisture content of fish were significant increased. In contrast, fat content and gross energy in whole body, and both N and energy retentions were significantly reduced. No significant difference was found in FI, WG, whole body crude protein and ash content, and ADC of individual EAA, total EAA and total AA.

Table 11.

Effects of Giant croaker fed diet with both 50% LT-FM and SPC replaced by GWT

Diet	V0	VFS	*P* > ltl
Growth and feed utilization			
Feed intake, g	44.4 ± 2.3	62.1 ± 16.4	0.17
Weight gain, g fish^{-1}	53.6 ± 2.7	49.2 ± 9.7	0.4
FCR, g DM ingested (g gain)$^{-1}$	0.83 ± 0.04	1.25 ± 0.10	< 0.05
Whole body composition, g / kg			
Crude protein	172.4 ± 3.8	170.8 ± 3.7	0.73
Crude fat	58.4 ± 7.7	37.7 ± 5.7	< 0.05
Ash	34.2 ± 0.8	36.1 ± 2.4	0.27
Moisture	732.0 ± 8.7	750.6 ± 4.0	< 0.05
Gross energy, kJ g^{-1}	6.5 ± 0.3	5.7 ± 0.2	< 0.05
Nutrient retention, %			
N	45.4 ± 2.9	28.8 ± 2.8	< 0.05
Energy	36.4 ± 3.6	19.7 ± 0.7	< 0.05
Apparent digestibility coefficients of amino acid, %			
Arg	92.9 ± 0.7	91.8 ± 2.8	0.53
Cys	32.1 ± 10.5	49.7 ± 19.1	0.28
His	85.0 ± 0.9	85.2 ± 3.9	0.94
Ile	84.6 ± 1.5	83.4 ± 4.6	0.68
Leu	85.5 ± 1.5	85.3 ± 4.0	0.93
Lys	85.9 ± 1.3	87.0 ± 2.8	0.54
Met	69.1 ± 2.7	63.9 ± 7.5	0.24
Phe	85.7 ± 1.4	86.3 ± 3.9	0.81
Thr	79.9 ± 1.1	82.1 ± 3.7	0.33
Tyr	84.4 ± 1.6	82.4 ± 5.1	0.53
Val	83.0 ± 1.5	82.7 ± 4.4	0.89
Total EAA	84.5 ± 1.0	84.5 ± 4.1	0.99
Total AA	84.4 ± 0.7	86.0 ± 3.5	0.47

5 Discussion

5.1 Feed technological properties

Because of the extruder parameter settings (lower water addition and higher die temperature) during the feed manufacturing of diet V0 resulted in a lower bulk density, and in a slower sinking rate than what was observed for the other diets. It had been demonstrated that either replacing LT-FM or SPC with VWG affected the physical quality of the feed. The gradual increased pellet expansion when more of GWT was included might be attributed to the visco-elastic properties of VWG, in keeping with the results reported by Draganovic et al. (2011). Draganovic et al. (2011) found that the dietary inclusion level of VWG was one of four independent factors that determines the diet expansion rate (Radial expansion (%) = -0.05FM + 0.47VWG + 0.03SPC + 0.27Moisture, $R^2 = 0.77$). However, when the replacing level of LT-FM or SPC was increased from 66.6% to 100% based on dietary protein (dietary inclusion of VWG were from 107 g kg^{-1} to 160 g kg^{-1} indeed), the increase of expansion rate continued, which could be explained by research of Draganovic et al. (2013), whose finding shown the greatest expansion could be obtained when extruded diet pellet containing 150 g kg^{-1} gluten.

As to the breaking point, the values decreased in diets with LT-FM replaced by increasing GWT inclusion, while it increased in diets with SPC replaced by increasing GWT inclusion, this different results might be caused by the combined action of expansion and visco-elastic property of VWG. Previous studies have found the higher expansion was associated with a reduction in hardness (Aarseth et al., 2006; Hansen and Storebakken, 2007; Morken et al., 2012). However, Draganovic et al. (2011) had found the visco-elastic property of VWG would result in an increasing of breaking point of pellets. The opposite trend of breaking point found in present study need to be further studied.

5.2 Biological response

5.2.1 GWT replacing LT-FM

VWG has lower content of lysine, arginine and threonine than LT-FM, and the content of methionine in these three ingredients is highly different. These four amino acids were supplemented gradually in diets VF1, VF2, VF3 and VFS, and added in VS1, VS2 VS3 as well, apart from methionine. As a result, the experimental diets had similar amino acids profiles, and that the supplement of first-four limiting AAs was successful. The analyzed contents of protein and lipid in diets were around 45% and 14%, respectively, which were similar to the reported optimal requirements for this species (Chai et al., 2013; Lee et al., 2001).

This experiment has clearly demonstrated the possibility of VWG as a protein source to replace LT-FM in extruded diets for Giant croaker. The Giant croaker reared in marine water (IBW = 11 g, 59-day reared, 28℃) obtained an average weight gain of 440% after a feeding period of 59 days. This growth ratio is lower than that obtained in a previous study, in which a WG around 700% was obtained (IBW = 10.7 g, 8-week reared, 28℃) (Cheng et al., 2015). This was the highest WG based on this IBW up to now. However, the dietary of that study contained at least 22.3% FM and only one diet containing plant protein. Taking the high concentration of PP in this experiment into consideration, such growth performance may be considered acceptable.

The increasing GWT inclusion had significant effect on FI and FCR of Giant croakers fed diet with dietary protein of LT-FM replaced by GWT, while no significant effect on WG. Thus, it might be concluded that Giant croaker didn't have the same good ability to utilize VGW as other species which have been reported (Davies et al., 1997; Schneider et al., 2004; Storebakken et al., 2015; Sugiura et al., 1998; Tibaldi et al., 2011). Another experiment showed that protein from soybean meal (SBM) can successfully replace protein of FM, as reported by Wang et al. (2011). They also found the FCR of same species fed diet with protein of FM replaced by SBM was significant higher than control group which FM was the sole protein source. Similarly, in the same study, FI of Giant croakers fed diet with protein of FM partially replaced by SBM was higher than control group while the SBM inclusion hasn't shown any significant impact on WG. Another possibility of the low FI of fish fed diet V0 might be attributed to its low bulk density which subsequently resulted in a slower sinking rate in sea water. However, Giant croakers were observed to be extremely sensitive to illumination, so they preferred to take the feed at bottom. Thus the "apparent" over feeding has been conducted based on the uneaten feed collected after each meal, however, the FI of Giant croakers fed the diet V0 might be still under optimal.

A decreasing of N retention (NRE) of cuneate drum also was observed when FM in diet was replaced by other protein source (Wang et al., 2006b; Wang et al., 2006c). The decreased of energy retention (ERE) might be caused by the high FI of fish fed diet with FM replaced by GWT.

5.2.2 GWT replacing SPC

The Giant croaker reared in marine water obtained an average weight gain of 520% after a feeding period of 59 days. Because of the concentration of LT-FM in these diets was 200 g kg^{-1}, it was a reasonable WG value. FI and WG showed a curve that their values increased and then decreased, furthermore, the top value of these two curve was obtained when GWT inclusion was 65.4% and 61.2%, respectively. It could be concluded that a highest FI and WG could be obtained when 61% ~ 65% protein of SPC was replaced by GWT. The curve of FCR showed that value of FCR increased immediately when 33.3% of protein of SPC was replaced by GWT and then became stable, which might indicate VWG affected FCR negatively but not closely related to the content of VWG.

As to the decrease of ash content in whole body observed in fish fed diets where SPC been replaced by increasing GWT inclusion, it could be explained by the decreased amount of available calcium and phosphorus in diet VS1, VS2 and VS3, in which supplementations of MCP was almost the same as diet V0, but the ash content in SPC is almost 5 - fold higher than that in VWG which may implies the differences of Ca and P content in these two ingredients.

The linear increase of ADC of Cys also has been observed in trout fed diets with increasing dietary proportion of protein from hydrolyzed wheat gluten (Storebakken et al., 2015). The decreased digestibility of Cys in diet with SPC replaced by increasing GWT inclusion probably was involved of thermo-treatment during the production of SPC which may stimulate the formation of S-S bonds from -SH groups and reduce the Cys digestibility (Opstvedt et al., 1984), and such treatment was absent during the production of VWG.

5.2.3 GWT replacing LT-FM and SPC

The Giant croakers fed diet jointly replacing 50% proteins from LT-FM and 50% proteins from SPC by proteins of GWT (diet VFS) obtained a weight gain of 350%. When considering the protein digestibility (based on the ADC of total AA) and FI of fish fed diet VFS were not significantly different from that of fish fed diet V0, the higher FCR of fish fed diet V0 could be attributed to its lower energy digestibility. Thus may consequently led to fact that the fish had to increased FI to ensure the abundant energy intake to achieve the similar growth as fish fed the diet V0, as no significant difference were observed in WG between these two groups, and which followed the lower retentions of energy and N consequently. In addition, the lower digestible energy intake might also result in the decreased content of crude fat and gross energy, and increased content of moisture in whole body. Such result had also been found in rainbow trout (D'Souza et al., 2006) and cobia(Zhou et al., 2005).

6 Conclusion

Extrusion parameters and visco-elastic property of VWG can influence the physical properties of pellets of VWG containing feed.

In extruded practical diet of giant croaker with both 20.0% inclusion of high quality fish meal and 21.4% inclusion of SPC, that one third of the high quality of dietary fish meal or 65% of the SPC can be safely replaced by the taurine supplemented mixture of vital wheat gluten and wheat flour. The replacement does not cause adverse effects on feed intake, growth, feed conversion, whole body compositions, or retentions of nitrogen and energy.

7 Acknowledgments

First thanks are given to Syral Belgium N.V who provide Vital wheat gluten and Yihai Kerry Group who provide other ingredients.

Thanks are given to IHA, NMBU where I study, and my adviser Marit.
Thanks also given to Marine Fisheries Research Institute of Zhejiang Province, and to Xuejun Chai and Yuebing Wang for providing us giant croakers.

My personal thanks to my supervisor Trond storebakken, co-supervisor Yuexing Zhang, and my mentor Zhiyong Dong.

Also thanks my parents for supporting and understanding.

8 References

Aarseth, K., Sørensen, M., Storebakken, T., 2006. Effects of red yeast inclusions in diets for salmonids and extrusion temperature on pellet tensile strength: Weibull analysis. Animal feed science and technology. 126, 75-91.

Abel, H., Becker, K., Meske, C., Friedrich, W., 1984. Possibilities of using heat-treated full-fat soybeans in carp feeding. Aquaculture. 42, 97-108.

Allan, G., Parkinson, S., Booth, M., Stone, D., Rowland, S., Frances, J., Warner-Smith, R., 2000. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. Aquaculture. 186, 293-310.

Apper-Bossard, E., Feneuil, A., Wagner, A., Respondek, F., 2013. Use of vital wheat gluten in aquaculture feeds. Aquatic biosystems. 9, 1-13.

Aslaksen, M., Romarheim, O., Storebakken, T., Skrede, A., 2006. Evaluation of content and digestibility of disulfide bonds and free thiols in unextruded and extruded diets containing fish meal and soybean protein sources. Animal feed science and technology. 128, 320-330.

Bailey, C., 1941. A translation of Beccari's lecture 'Concerning grain'(1728). Cereal chemistry. 18, 555-561.

Bangoula, D., Parent, J., Vellas, F., 1992. Nutritive value of white lupin (*Lupinus albus var Lutop*) in rainbow trout (*Oncorhynchus mykiss*). Effect of extrusion cooking. Reproduction, nutrition, development. 33, 325-334.

Belton, P., 1999. Mini review: On the elasticity of wheat guten. Journal of cereal science. 29, 103-107.

Bransden, M., Carter, C., Nowak, B., 2001. Effects of dietary protein source on growth, immune function, blood chemistry and disease resistance of Atlantic salmon (*Salmo salar* L.) parr. 73, 105-113.

Burel, C., Boujard, T., Kaushik, S., Boeuf, G., van der Geyten, S., Mol, K., Kühn, E., Quinsac, A., Krouti, M., Ribaillier, D., 2000. Potential of plant-protein sources as fish meal substitutes in diets for turbot (*Psetta maxima*): growth, nutrient utilisation and thyroid status. Aquaculture. 188, 363-382.

Carter, C., Hauler, R., 2000. Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar L*. Aquaculture. 185, 299-311.

Chai, X., Ji, W., Han, H., Dai, Y., Wang, Y., 2013. Growth, feed utilization, body composition and swimming performance of giant croaker, *Nibea japonica* Temminck and Schlegel, fed at different dietary protein and lipid levels. Aquaculture nutrition. 19, 928-935.

Cheng, C., Wang, J., Han, T., Li, X., Jiang, Y., Wen, X., 2015. Effects of different dietary amino acid patterns on growth performance and body composition of juvenile giant croaker (*Nibea japonica)*. Aquaculture research, 1-8.

Cheng, Z., Hardy, R., 2003. Effects of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture nutrition. 9, 77-83.

Cheng, Z., Hardy, R., Usry, J., 2003. Plant protein ingredients with lysine supplementation reduce dietary protein level in rainbow trout (*Oncorhynchus mykiss*) diets, and reduce ammonia nitrogen and soluble phosphorus excretion. Aquaculture. 218, 553-565.

D'Souza, N., Skonberg, D., Stone, D., Brown, P., 2006. Effect of soybean meal ‐ based diets on the product quality of rainbow trout fillets. Journal of food science. 71, 337-342.

Dabrowski, K., Poczyczynski, P., Köck, G., Berger, B., 1989. Effect of partially or totally replacing fish meal protein by soybean meal protein on growth, food utilization and proteolytic enzyme activities in rainbow trout (*Salmo gairdneri*). New *in vivo* test for exocrine pancreatic secretion. Aquaculture. 77, 29-49.

Davies, S., Morris, P., Baker, R., 1997. Partial substitution of fish meal and full-fat soya bean meal with wheat gluten and influence of lysine supplementation in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture research. 28, 317-328.

Day, L., Augustin, M., Batey, I., Wrigley, C., 2006. Wheat-gluten uses and industry needs. Trends in food science & technology. 17, 82-90.

Dias, J., Huelvan, C., Dinis, M., Métailler, R., 1998. Influence of dietary bulk agents (silica, cellulose and a natural zeolite) on protein digestibility, growth, feed intake and feed transit time in European seabass (*Dicentrarchus labrax*) juveniles. Aquatic living resources. 11, 219-226.

Domenek, S., Brendel, L., Morel, M., Guilbert, S., 2004. Swelling behavior and structural characteristics of wheat gluten polypeptide films. Biomacromolecules. 5, 1002-1008.

Draganovic, V., van der Goot, A., Boom, R., Jonkers, J., 2011. Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed. Animal feed science and technology. 165, 238-250.

Draganovic, V., van der Goot, A., Boom, R., Jonkers, J., 2013. Wheat gluten in extruded fish feed: effects on morphology and on physical and functional properties. Aquaculture nutrition. 19, 845-859.

Francis, G., Makkar, H., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture. 199, 197-227.

Gatlin, D., Barrows, F., Brown, P., Dabrowski, K., Gaylord, T., Hardy, R., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture research. 38, 551-579.

Gianibelli, M., Larroque, O., MacRitchie, F., Wrigley, C., 2001. Biochemical, genetic, and molecular characterization of wheat glutenin and its component subunits. Cereal chemistry. 78, 635-646.

Grace, G., 1989. Preparation of vital wheat gluten, [Proc. World congress on vegetable protein utilization in human foods and animal feedstuffs]. TH Applewhite, ed. Am. Oil chemistry. Soc.: Champaign, IL, pp. 112-114.

Han, T., Li, X., Wang, J., Hu, S., Jiang, Y., Zhong, X., 2014. Effect of dietary lipid level on growth, feed utilization and body composition of juvenile giant croaker (*Nibea japonica)*. Aquaculture. 434, 145-150.

Hansen, J., Storebakken, T., 2007. Effects of dietary cellulose level on pellet quality and nutrient digestibilities in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 272, 458-465.

Helland, S., Grisdale-Helland, B., Nerland, S., 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. Aquaculture. 139, 157-163.

Henrichfreise, B., Pfeffer, E., 1992. Wheat and wheat starch as potential sources of digestible energy for rainbow trout (*Oncorhyrwhus mykiss*). Journal of animal physiology and animal nutrition. 67, 143-147.

Hilton, J., Cho, C., Slinger, S., 1981. Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (*Salmo gairdneri R.*). Aquaculture. 25, 185-194.

Hilton, J., Atkinson, J., Slinger, S., 1983. Effect of increased dietary fiber on the growth of rainbow trout (*Salmo gairdneri*). Canadian journal of fisheries and aquatic sciences. 40, 81-85.

Hossain, M., 1999. Dietary calcium requirement of giant croaker *Nibea japonica*. Journal of the faculty of agriculture, Kyushu university. 44, 99-104.

Huilai, S., Bao, L., Jian, L., 2007. Effect on the growth and biochemical element of body compositionof juvenile *Nibea japonica* by different feeding rates. Marine fish, 53-56.

Ketola, H., 1983. Requirement for dietary lysine and arginine by fry of rainbow trout (*Salmo gairdneri*) Journal of animal science. 56, 101-107.

Kim, J., Tanhehco, E., Ng, P., 2006. Effect of extrusion conditions on resistant starch formation from pastry wheat flour. Food chemistry. 99, 718-723.

Kissil, G., Lupatsch, I., 2004. Successful replacement of fishmeal by plant proteins in diets for the gilthead seabream, *Sparus aurata* L. The Israeli journal of aquaculture bamigeh. 56(3), 188-199.

Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S., Bakke, A., 2010. Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. Aquaculture research. 41, 333-344.

Lee, H., Cho, K., Lee, J., Yang, S., 2001. Dietary protein requirement of juvenile giant croaker, *Nibea japonica* Temminck & Schlegel. Aquaculture research. 32, 112-118.

Li, X., Wang, J., Han, T., Hu, S., Jiang, Y., 2014. Effects of dietary carbohydrate level on growth and body composition of juvenile giant croaker *Nibea japonica*. Aquaculture research. 46, 1-8.

Mambrini, M., Roem, A., Carvedi, J., Lalles, J., Kaushik, S., 1999. Effects of replacing fish meal with soy protein concentrate and of DL-methionine supplementation in high-energy, extruded diets on the growth and nutrient utilization of rainbow trout, *Oncorhynchus mykiss*. Journal of animal science. 77, 2990-2999.

Morken, T., Kraugerud, O., Sørensen, M., Storebakken, T., Hillestad, M., Christiansen, R., Øverland, M., 2012. Effects of feed processing conditions and acid salts on nutrient digestibility and physical quality of soy‐based diets for Atlantic salmon (*Salmo salar*). Aquaculture nutrition. 18, 21-34.

Olsen, R., Hasan, M., 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. Trends in food science and technology. 27, 120-128.

Opstvedt, J., Miller, R., Hardy, R., Spinelli, J., 1984. Heat-induced changes in sulfhydryl-groups and disulfide bonds in fish-protein and their effect on protein and amino- acid digestiblity in rainbow-trout (*Salmo gairdneri*). Journal of agricultural and food chemistry. 32, 929-935.

Osbome, T., 1907. [The proteins of the wheat kenel]. Publication of the Carnegie institute, Washington.

Pfeffer, E., Henrichfreise, B., 1994. Evaluation of potential sources of protein in diets for rainbow trout (*Oncorhynchus mykiss*). Archives of animal nutrition. 45, 371-377.

Pfeffer, E., Sabty, H., Haverkamp, R., 1992. Studies on lysine requirements of rainbow trout (*Oncorhynchus mykiss*) fed wheat gluten as only source of dietary protein. Journal of animal physiology and animal nutrition. 67, 74-82.

Pfeffer, E., Kinzinger, S., Rodehutscord, M., 1995. Influence of the proportion of poultry slaughter by‐products and of untreated or hydrothermically treated legume seeds in diets for rainbow trout, *Oncorhynchus mykiss* (*Walbaum*), on apparent digestibilities of their energy and organic compounds. Aquaculture nutrition. 1, 111-117.

Robaina, L., Corraze, G., Aguirre, P., Blanc, D., Melcion, J., Kaushik, S., 1999. Digestibility, postprandial ammonia excretion and selected plasma metabolites in European sea bass (*Dicentrarchus labrax*) fed pelleted or extruded diets with or without wheat gluten. Aquaculture. 179, 45-56.

Rodehutscord, M., Mandel, S., Pack, M., Jacobs, S., Pfeffer, E., 1995. Free amino acids can replace protein-bound amino acids in test diets for studies in rainbow trout (*Oncorhynchus mykiss*). The Journal of nutrition. 125, 956.

Sørensen, M., 2012. A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. Aquaculture nutrition. 18, 233-248.

Schneider, O., Amirkolaie, A., Vera‐Cartas, J., Eding, E., Schrama, J., Verreth, J., 2004. Digestibility, faeces recovery, and related carbon, nitrogen and phosphorus balances of five feed ingredients evaluated as fishmeal alternatives in Nile tilapia, *Oreochromis niloticus* L. Aquaculture research. 35, 1370-1379.

Shewry, P., Halford, N., 2002. Cereal seed storage proteins: structures, properties and role in grain utilization. Journal of experimental botany. 53, 947-958.

Shiau, S., Kwok, C., Chen, C., Hong, H., Hsieh, H., 1989. Effects of dietary fibre on the intestinal absorption of dextrin, blood sugar level and growth of tilapia, *Oreochromis niloticmx O. aureus*. Journal of fish biology. 34, 929-935.

Skonberg, D., Hardy, R., Barrows, F., Dong, F., 1998. Color and flavor analyses of fillets from farm-raised rainbow trout (*Oncorhynchus mykiss*) fed low-phosphorus feeds containing corn or wheat gluten. Aquaculture. 166, 269-277.

Srihara, P., Alexander, J., 1984. Effect of heat treatment on nutritive quality of plant protein blends. Canadian institute of food science and technology Journal. 17, 237-241.

Stone, D., 2003. Dietary carbohydrate utilization by fish. Reviews in Fisheries Science. 11, 337-369.

Stone, D., Allan, G., Anderson, A., 2003. Carbohydrate utilization by juvenile silver perch, *Bidyanus bidyanus* (Mitchell). III. Digestibility and utilization of starch and its breakdown products. Aquaculture research. 34, 109-121.

Storebakken, T., Shearer, K., Roem, A., 1998. Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. Aquaculture. 161, 365-379.

Storebakken, T., Shearer, K., Baeverfjord, G., Nielsen, B., Åsgård, T., Scott, T., de Laporte, A., 2000. Digestibility of macronutrients, energy and amino acids, absorption of elements and absence of intestinal enteritis in Atlantic salmon, *Salmo salar*, fed diets with wheat gluten. Aquaculture. 184, 115-132.

Storebakken, T., Zhang, Y., Ma, J., Øverland, M., Mydland, L.T., Kraugerud, O.F., Apper, E., Feneuil, A., 2015. Feed technological and nutritional properties of hydrolyzed wheat gluten when used as a main source of protein in extruded diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 448, 214-218.

Sugiura, S., Dong, F., Rathbone, C., Hardy, R., 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture. 159, 177-202.

Tacon, A., Hasan, M., Metian, M., 2011. Demand and supply of feed ingredients for farmed fish and crustaceans: Trends and prospects. [Fao Fisheries and Aquaculture Technical Paper]

Tibaldi, E., Tulli, F., Piccolo, G., Guala, S., 2011. Wheat gluten as a partial substitute for fish meal protein in sea bass (*D. labrax*) diets. Italian journal of animal science. 2, 613-615.

Tusche, K., Arning, S., Wuertz, S., Susenbeth, A., Schulz, C., 2012. Wheat gluten and potato protein concentrate — Promising protein sources for organic farming of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 344–349, 120-125.

van der Poel, A., 1989. Effects of processing on antinutritional factors (ANF) and nutritional value of legume seeds for non-ruminant feeding, International Workshop on Antinutritional Factors (ANF) in Legume Seeds', Wageningen (Netherlands), 23-25. Pudoc, pp. 213-229.

Wang, J., Han, T., Hu, S., 2011. The influence of replacing fish meal partially in the diet with soybean meal on growth, body composition and feed utilization of juvenile giant croaker, *Nibea japonica*. Journal of Zhejiang Ocean University (Natural science), 279-285.

Wang, Y., Guo, J., Li, K., Bureau, D., 2006a. Effects of dietary protein and energy levels on growth, feed utilization and body composition of cuneate drum (*Nibea miichthioides*). Aquaculture. 252, 421-428.

Wang, Y., Guo, J., Bureau, D.P., Cui, Z., 2006b. Replacement of fish meal by rendered animal protein ingredients in feeds for cuneate drum (*Nibea miichthioides*). Aquaculture. 252, 476-483.

Wang, Y., Kong, L., Li, C., Bureau, D., 2006c. Effect of replacing fish meal with soybean meal on growth, feed utilization and carcass composition of cuneate drum (*Nibea miichthioides*). Aquaculture. 261, 1307-1313.

Wee, K., Shu, S., 1989. The nutritive value of boiled full-fat soybean in pelleted feed for Nile tilapia. Aquaculture. 81, 303-314.

Wieser, H., 2007. Chemistry of gluten proteins. Food microbiology. 24, 115-119.

Wilson, R., 1994. Utilization of dietary carbohydrate by fish. Aquaculture. 124, 67-80.

Woychik, J., Boundy, J., Dimler, R., 1961. Wheat gluten proteins, amino acid composition of proteins in wheat gluten. Journal of agricultural and food chemistry. 9, 307-310.

Zhou, Q., Mai, K., Tan, B., Liu, Y., 2005. Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (*Rachycentron canadum*). Aquaculture nutrition. 11, 175-182.

Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no