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Reducing dietary wild derived fishmeal inclusion levels in production diets for large yellowtail kingfish (*Seriola lalandi*)

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ABSTRACT

Further research to understand the effect of dietary wild derived fishmeal (WD-FM) substitution with commercially relevant alternative ingredients for large yellowtail kingfish (*Seriola lalandi*; YTK) was investigated. This 36-week study was designed to replace dietary inclusions of WD-FM with alternative protein ingredients including poultry meal, soy protein concentrate and by-product fishmeal (PM, SPC and BP-FM) and measure the effect on the growth performance, feed utilisation, and health of large YTK (2.5 kg initial weight) at ambient water temperatures (average 16.6 °C). Six diets were formulated on a digestible basis to contain 39% digestible protein (~45–46% crude protein), 23% digestible lipid (~24–25% crude lipid), and a digestible energy level of 17 MJ kg^{-1} (~19 MJ kg^{-1} gross energy level). Fish were fed to apparent satiation once daily at 10:00 h. Substitution of fish meal with alternative ingredients did not significantly impact fish growth, feed utilisation, gastrointestinal health, blood haematology or measured biochemistry indices. Results from the current study will allow reductions to the dietary WD-FM inclusion levels, with tangible sustainability benefits. The inclusion of the alternative protein sources resulted in improvements in the fish in-fish out ratios of up to 35.1%. This study suggests formulation criteria for large YTK should include a minimum of 10% WD-FM. Further to this, at least 30% of the diet should consist of a combination of poultry meal, soy protein concentrate and fishmeal (both wild and by-product). Our data further support the use of BP-FM up to ~20% inclusion, while PM and SPC should be limited to ~10% inclusion until further data is available on these raw materials in YTK feeds. These recommendations will facilitate formulation flexibility for large YTK feeds, enabling formulators to adapt to changes to extrinsic factors such as raw material availability, and sustainability while minimising cost and performance impacts.

1. Introduction

As aquaculture production increases, demand for wild derived fishmeal (WD-FM) and fish oil may result in constrained production and substantial increases in price (Gatlin III et al., 2007; Rocker et al., 2022). Yellowtail kingfish (*Seriola lalandi*; YTK) are large carnivorous pelagic species, with a vast natural distribution and are cultured globally. Anecdotally, during growout they are fed commercial diet preparations that can contain >30% WD-FM, with some literature suggesting even higher levels as standard (Booth and Pirozzi, 2021). To improve the sustainability and potentially reduce diet costs, validation of commonly available alternative protein ingredients is required for YTK growout diets (Gatlin III et al., 2007; Stone and Bowyer, 2013; Stone et al., 2016). Currently, little published information is available relating to reducing the use of WD-FM for large YTK during the growout phase, which requires the greatest volume of feed (Stone et al., 2016).

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A vast number of studies have investigated the potential of various dietary ingredients to reduce WD-FM levels in aquaculture diets for fish, including land animal protein, plant proteins, novel algal or bacterial proteins and fishmeal by-products, with many recent comprehensive reviews on the subject now available (Glencross et al., 2019; Hua et al., 2019; Turchini et al., 2019; Boyd et al., 2020; Naylor et al., 2021). Studies on fishmeal replacement have met with varying levels of success in many aquatic species. Ingredients including poultry meal (PM), soy protein concentrate (SPC) and by-product fishmeal (BP-FM) were identified to have great potential to partially replace dietary inclusions of WD-FM in production diets for large YTK. These ingredients have the added benefits of being mostly comparable in terms of their amino acid profile, they are commonly available, and they will assist in improving sustainability targets for farms. There are some obvious exceptions to this like a distinct lack of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). Poultry meal is high in protein (~65%), has an excellent amino acid profile and has been successfully used to reduce dietary WD-FM inclusions for several aquaculture species (Sealey et al., 2011; Zhou et al., 2011; Davidson et al., 2016; Simon et al., 2019). For example, juvenile Cobia (*Rachycentron canadum*; 5.8 g) fed a diet with 35% WD-FM combined with 15% PM grew similarly to a 50% WD-FM control diet (Zhou et al., 2011). In contrast, juvenile Atlantic Salmon (*Salmo salar*; 281 g) fed a 0% WD-FM + 30% PM diet exhibited inferior growth, compared to those fed a 19.5% WD-FM diet (Davidson et al., 2016). While these studies have successfully used PM as protein source, a species-dependent response to replacing WD-FM with PM is apparent. Dam et al. (2019) determined that in sub-adult (~574 g) YTK poultry meal was highly digestible and similar to other high-quality ingredients. Current commercial YTK diets contain varying levels of PM as a protein source; however, the long-term effect on growth performance when replacing WD-FM with PM in diets for large YTK is not yet understood (Stone and Bowyer, 2013).

Dietary inclusions of soy products in aquafeeds for a range of finfish species including YTK has received considerable attention also (Barrows et al., 2007; Bowyer et al., 2013a; Bowyer et al., 2013b; Trushenski et al., 2014; Bansemer et al., 2015; Stone et al., 2018). Dietary inclusions of solvent extracted soybean meal (SE-SBM) in YTK diets reduced the growth performance and feed utilisation also leading to the development of sub-acute enteritis and the hind gut (Bowyer et al., 2013a; Bansemer et al., 2015; Stone et al., 2018). As such, recommendations from the previous studies have suggested that SE-SBM should be excluded from YTK diets (Stone and Bowyer, 2013), In contrast, Bowyer et al. (2013b) reported that the growth rate and nutrient utilisation of juvenile YTK (initial weight 22 g) fed a 20% dietary inclusion of SPC was similar to a fishmeal control diet. SPC, a highly refined and more expensive soy product, has undergone extensive processing via heat and alcohol extraction to remove and reduce certain types of antinutritional factors and concentrate the protein (Gatlin III et al., 2007). While the inclusion of SPC in diets for fingerling Seriola has met with success (Bowyer et al., 2013b; Trushenski et al., 2014; Bansemer et al., 2015), the effect of replacing dietary WD-FM with SPC for large YTK (> 1.5 kg) over a longer time frame is unknown.

One key difference between the alternatives mentioned above and WD-FM is that it contains the essential n-3 LC-PUFAs, eicosapentaenoic acid (20:5n-3, EPA), docosapentaenoic acid (22:5n-3, DPA) and docosahexaenoic acid (22:6n-3, DHA). Most alternative protein ingredients derived from terrestrial animal or plant sources typically lack appreciable levels of n-3 LC-PUFA (Hua et al., 2019). Production of BP-FM obtained from seafood processing is increasing and this valuable resource also contains appreciable levels of n-3 LC-PUFA and other compounds only found in fish (Shepherd and Jackson, 2013). Fishmeal by-products are typically less expensive than WD-FM and they also have the added benefit of being considerably more sustainable (Tacon and Metian, 2008; Shepherd and Jackson, 2013). Replacement of WD-FM with tuna BP-FM has met with considerable success in several species including juvenile amberjack (*Seriola dumerili*) (Uyan et al., 2009), Japanese flounder (*Paralichthys olivaceus*) (Uyan et al., 2006), spotted rose snapper (*Lutjanus guttatus*) (Hernández et al., 2014) and juvenile Korean rockfish (*Sebastes schlegeli*) (Kim et al., 2018). The authors of these studies generally demonstrated acceptable performance and high protein digestibility, while noting that the higher ash content of BP-FM may be problematic in some situations (Hernández et al., 2014).

Recently, Stone et al. (2022) reported the optimal crude dietary protein and lipid to be ~43% and ~ 25% respectively in large YTK grown over summer, and fed diets with 30% WD-FM. However, it remains to be seen how much of the WD-FM can be replaced with alternative ingredients while maintaining performance using the previously determined optimal protein and lipid macronutrients. Research has shown that alternative ingredients can replace WD-FM for juvenile YTK and other aquaculture species. However, little published information is available regarding the reduction of WD-FM levels in commercial diets for large YTK (> 1.5 kg). Therefore, the aim of the present experiment was to investigate the growth performance, feed utilisation, and health of YTK fed diets where dietary inclusions of WD-FM were replaced with PM, SPC and BP-FM.

2. Methods

2.1. Experimental design and diets

Wild derived fishmeal (WD-FM) and three alternative protein ingredients, poultry meal, soy protein concentrate, and by-product fishmeal (PM, SPC and BP-FM) were investigated in this study. The biochemical composition of the four protein ingredients is presented in Table 1. The PM, SPC and BP-FM ingredients were formulated into a control diet on a digestible protein basis by reducing wild derived fishmeal levels to either 10 or 20% of the diet. Diet 'WD-FM 100%' included 30% wild derived fishmeal; diet 'BP-FM 33%' replaced 10% of the total WD-FM with BP-FM (equal to 10.7% inclusion); diet 'BP-FM 66%' replaced 20% of the total WD-FM with BP-FM (equal to 21.4% inclusion); diet 'PM 33%' replaced 10% of the total WD-FM with PM (equal to 11.3% inclusion); diet 'BP-FM 33% + PM 33%' replaced 20% of the total WD-FM with BP-FM (equal to 10.7% inclusion) and PM (equal to 11.3% inclusion); diet 'SPC 33%' replaced 10% of the WD-FM with SPC (equal to 10.9% inclusion). This resulted in six separate diets in this study, with formulation and compositional data presented in Table 2.

The six diets were formulated to contain 39% digestible protein and 17 MJ kg^{-1} digestible energy, based on previous coefficients measured for the same species (Booth et al., 2010; Stone and Bowyer, 2013). The diets were also formulated to contain other highly palatable and digestible ingredients at realistic commercial inclusion levels, including other terrestrial animal and plant meals which were not changed between the diets.

The sinking experimental diets (a 9 mm kernel) were manufactured by Skretting Australia (Cambridge, Tasmania, Australia) using extrusion technology. The oils were applied by vacuum coating post-extrusion. The fish were fed the diets to apparent satiation daily at 10:00 h. Apparent satiation feeding was achieved by providing feed to the tank and monitoring feed intake of fish over a period of four min $tank^{-1}$. Care was taken to minimise waste by dispersing feed evenly and slowly across each tank. Once small quantities of uneaten feed were observed on the tank bottom, fish were judged to have reached apparent satiation. Feed inputs were recorded daily, which included a weight adjustment made after the removal of uneaten pellets.

2.2. Experimental fish, experimental system, and stocking

Experimental fish were maintained according to the procedures described for the care and use of laboratory animals (National Research Council (NRC), 2011a). Experimental work was conducted in the pool-farm facility at the South Australian Research and Development

Table 1
The biochemical composition of the four protein test ingredients used in the current experiment.

Item	Wild derived fish meal	Fish meal by-product	Poultry meal	Soy protein concentrate
Analysed proximate composition (as is basis: g 100 g^{-1})				
Moisture	7.9	5.1	5.6	7.9
Crude protein	64.4	60.2	65.0	59.4
Crude lipid	7.8	11.0	11.3	2.2
Ash	17.0	20.2	14.1	6.4
Carbohydrate [1]	3.0	4.0	4.0	24.0
Gross energy (MJ kg^{-1})	14.3	15.0	15.9	15.0
Analysed essential amino acids (g 100 g^{-1}) [2]				
Arginine	3.62	3.60	4.08	4.02
Histidine	1.47	1.79	1.12	1.33
Isoleucine	2.47	2.50	2.22	2.53
Leucine	4.23	4.18	4.06	4.09
Lysine	4.30	4.09	3.13	3.22
Methionine	1.70	1.59	1.01	0.54
Phenylalanine	2.44	2.37	2.37	2.84
Threonine	2.38	2.40	2.24	2.05
Valine	3.01	2.99	3.07	2.71
Total amino acids [2]	51.41	50.59	52.08	49.77
Analysed minerals (mg kg^{-1})				
Calcium (g 100 g^{-1})	4.5	6.8	4.4	0.4
Copper	5.2	4.9	5.2	6.5
Iodine (I)	1.8	1.1	1.1	<0.1
Iron	540.0	350.0	470.0	130.0
Magnesium (g 100 g^{-1})	0.3	0.2	0.1	0.4
Manganese	15.0	4.8	11.0	36.0
Phosphorus (g 100 g^{-1})	3.1	3.9	2.6	0.7
Potassium (g 100 g^{-1})	0.8	0.3	0.6	2.2
Selenium	1.8	7.2	0.9	<0.1
Sodium (g 100 g^{-1})	0.1	0.1	<0.1	<0.1
Zinc	83.0	170.0	95.0	44.0
Analysed fatty acids (mg 100 g^{-1}) [2]				
16:0 Palmitic	1771	2497	2610	295
18:0 Stearic	460	924	881	84
18:1n-9 Oleic	811	1452	4622	411
18:2n-6 Linoleic	133	143	1435	1188
18:3n-3 alpha-Linolenic	55	33	147	143
20:4n-6 (ARA)	109	275	124	<10
20:5n-3 (EPA)	1037	550	11	<10
22:6n-3 (DHA)	1537	2992	34	<10
Total SFA	2863	4279	3684	407
Total MUFA	1435	2178	5368	418
Total PUFA	3104	4224	1865	1333
Total Omega-3	2816	3718	226	143
Total Omega-6	289	506	1639	1190
Total n-3 LC-PUFA	2746	3674	68	0

[1] Carbohydrate = 100 - (moisture + lipid + protein + ash).
[2] non-essential amino and complete fatty acid profiles are presented in the supplementary table.

Institute, South Australian Aquatic Science Centre (SARDI SAASC; West Beach, South Australia, Australia). YTK ($n = 306$; 2.52 ± 0.25 kg; 546 ± 20 mm (fork length; mean ± standard deviation) were obtained from Clean Seas Seafood (Port Lincoln, South Australia, Australia). Upon arrival at the SARDI SAASC facility, YTK were transferred to 5000 L outdoor undercover tanks supplied with partially recirculating (100% system water exchange d^{-1}) sea water at ambient temperature and held for 1 month. Supplemental fluorescent lighting was also provided above

Table 2
The formulation and biochemical composition of the six test diets used.[1]

Diet [2]	WD-FM 100%	BP-FM 33%	BP-FM 66%	PM 33%	BP-FM 33% + PM 33%	SPC 33%
Summary of ingredients (g 100 g^{-1})						
Wild derived fishmeal	30.0	20.0	10.0	20.0	10.0	20.0
By-product fishmeal	0.0	10.7	21.4	0.0	10.7	0.0
Poultry meal	0.0	0.0	0.0	11.3	11.3	0.0
Soy protein concentrate	0.0	0.0	0.0	0.0	0.0	10.9
Terrestrial animal meals [3]	17.0	17.0	17.0	17.0	17.0	17.0
Terrestrial plant meals [4]	29.9	29.5	29.2	28.8	28.5	28.3
Fish oil	6.9	6.4	5.9	8.1	7.6	8.1
Poultry oil	13.4	13.5	13.6	11.8	11.9	12.7
Methionine	0.4	0.4	0.4	0.5	0.5	0.6
Taurine	1.0	1.0	1.0	1.0	1.0	1.0
Choline Chloride (60%)	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin/mineral [5]	1.2	1.2	1.2	1.2	1.2	1.2
Analysed proximate composition (as is basis: g 100 g^{-1})						
Moisture	8.7	7.5	7.4	7.7	7.2	7.8
Crude protein	45.4	45.7	46.0	44.9	46.1	46.1
Crude lipid	24.1	24.8	23.9	24.7	25.0	24.3
Ash	8.9	9.0	9.8	8.4	8.8	7.8
Carbohydrate [6]	13.0	13.0	13.0	14.0	13.0	14.0
Gross energy (MJ kg^{-1})	18.8	19.1	18.9	19.1	19.3	19.2
Rancidity test						
p-Anisidine value	5.3	5.2	3.7	4.5	5.9	5.4
Peroxide value (mEqO2 kg^{-1})	6.3	5.9	6.5	7.5	8.3	9.1
Analysed essential amino acids (g 100 g^{-1}) [6]						
Arginine	2.26	2.26	2.31	2.31	2.32	2.30
Histidine	1.28	1.24	1.28	1.31	1.27	1.31
Isoleucine	1.41	1.39	1.42	1.39	1.40	1.44
Leucine	3.06	2.99	3.09	3.07	3.11	3.12
Lysine	2.41	2.34	2.38	2.34	2.40	2.35
Methionine	1.08	1.04	1.05	1.01	1.13	1.05
Phenylalanine	1.86	1.82	1.89	1.86	1.88	1.95
Threonine	1.47	1.44	1.48	1.46	1.48	1.47
Taurine	1.02	0.99	1.03	1.08	0.98	0.98
Total Amino Acids	35.60	35.20	35.90	36.00	36.60	36.30
Analysed fatty acids (mg 100 g^{-1}) [7]						
16:0 Palmitic	4989	5208	5043	5014	5075	4909
18:0 Stearic	1542	1612	1601	1655	1675	1555
18:1n-9 Oleic	6965	7266	7098	7459	7150	6926
18:2n-6 Linoleic	2531	2678	2677	2841	2725	2722
18:3n-3 alpha-Linolenic	458	446	454	469	450	462
20:4n-6 (ARA)	193	198	215	222	250	194
20:5n-3 (EPA)	1542	1463	1267	1433	1600	1652
22:6n-3 (DHA)	1615	1637	1577	1482	1700	1604
Total SFA	7664	7936	7696	7706	7875	7582
Total MUFA	8628	8928	8676	9065	8800	8554
Total PUFA	6724	6820	6501	6842	7150	7023
Total Omega-3	3880	3794	3489	3606	4000	3985
Total Omega-6	2844	3026	3011	3211	3150	3062
Total n-3 LC-PUFA	3398	3323	3035	3137	3550	3499

[1] Values are mean ± SE; $n = 3$, a significance level of $P < 0.05$ was used for all statistical tests.
[2] For diet details, please refer to methods Section 2.1 Experimental design and diets.
[3] Includes blood meal and meat meal.

[4] Includes wheat flour, wheat gluten and lupin meal.
[5] Includes vitamin and mineral premix (a proprietary product supplied by DSM Nutritional Products, Basal, Switzerland), stabilised vitamin C, vitamin E adsorbate, natural astaxanthin and phosphate.
[6] Carbohydrate = 100 - (moisture + lipid + protein + ash).
[7] non-essential amino acids and complete fatty acid profile presented in full supplementary table. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n-3 LC-PUFA: omega-3 long chain polyunsaturated fatty acids, ARA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid.

each 5000 L tank on a 12 L: 12D routine. The ambient sea water was settled, and sand filtered prior to entry to the system. The sea water was passed through a rotating drum filter and returned through the biological filter followed by UV sanitation. Each tank had a flow rate of approximately 10 L m^{-1}. A more detailed description of the same system was previously reported by Crowe et al. (2021). During the acclimation period fish were fed Ridley Pelagica Sink 9 mm with target protein of 44% and target fat of 24% (Ridley Agriproducts, Narangba, Australia). Upon arrival at SARDI SAASC, YTK were inspected, and were observed to have a low burden of skin flukes (*Benedenia seriola*) and gill flukes (*Zeuxapta seriola*). Treatment was deemed necessary and was prescribed by a veterinarian (Future Fisheries Veterinary Service Pty Ltd., Ballina, New South Wales, Australia). At the commencement of the experiment, YTK were anaesthetised using AQUI-S® (AQUI-S® New Zealand Ltd., Lower Hutt, New Zealand) at a concentration of 14 mg L^{-1} of seawater. Seventeen fish were then individually measured, weighed, and stocked into one of three replicate 5000 L tanks.

2.3. Water quality analyses and maintenance

Tanks were continuously supplied with partially recirculating sea water as described earlier. All tanks were supplied with continuous aeration and supplemental oxygenation throughout the study. The tanks were cleaned by manually brushing the sides as required, typically every second day. Water quality parameters were measured daily at 12:00 h and maintained at appropriate levels for acceptable growth of YTK throughout the experiment. Water temperature was measured using a thermometer and ranged from 13.0 to 23.5 °C (average ± SD; 16.6 ± 2.8). Dissolved oxygen (% saturation) was measured using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark) and averaged 102.9 ± 5.3% (average ± SD). The pH was measured daily using a meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, Illinois, USA) and averaged 7.8 ± 0.2 (average ± SD). Salinity was measured weekly using a portable salinity refractometer (model RF20, Extech Instruments, Nashua, New Hampshire, USA) and averaged 38.0 ± 0.0 g L^{-1} (average ± SD).

2.4. Intermediate checks and final harvest sampling

At 4-, 8-, 12-, 16-, 20-, 24-, 28- and 32-weeks post-stocking, all fish were anaesthetised then individually measured, weighed, visually inspected for skin and gill flukes, and returned back to their respective tanks. At 36 weeks (252 days), all fish were anaesthetised and weighed and measured. Three fish from each tank were euthanised by lethal overdose of AQUI-S® (n = 3 fish $tank^{-1}$; n = 18 tanks; n = 54 fish) and stored frozen at −20 °C for biochemical analysis. In addition, a total of twelve initial fish (n = 12 fish) were collected and stored frozen at −20 °C. Whole blood was extracted from a further three euthanised fish per tank (n = 3 fish $tank^{-1}$; n = 18 tanks; n = 54 fish) using a 19 G needle with a 5 mL syringe, 24 h post last feeding, and placed in two separate Vacuette® or BD vacutainer ® tubes (Z serum clot activator or EDTA tubes). Serum was analysed for blood biochemistry and whole blood was analysed for blood haematology conducted by IDEXX (Unley, South Australia, Australia). The blood sampled fish were then dissected, and the viscera, liver and visceral fat was weighed in order to calculate visceral index (VSI; %), hepatosomatic index (HSI; %) and intraperitoneal fat (%), respectively. The stomach from these fish were opened longitudinally, and were subjectively scored for gastric dilation (Chown, 2015). Briefly, Stage 0 is defined as having pronounced/well defined folds throughout the pylorus, anterior and distal stomach, while Stage 1 is defined as having minimal or absent folds throughout the pylorus and anterior stomach but has pronounced/well defined folds in the distal stomach (Chown, 2015). In addition, a one-cm^2 longitudinally opened hindgut section was collected from each blood-sampled fish for histology. In brief, hindgut samples were fixed in 10% seawater formalin for >48 h, processed, and embedded in paraffin wax. Tissue sections were cut using a microtome and floated on to onto Starfrost® glass slides and dried for >24 h at room temperature before being stained with hematoxylin and eosin (H and E) and periodic acid-schiff alcian blue (PAS/AB pH 2.5). Gastrointestinal morphological parameters in the hindgut including muscularis and submucosa thickness, villus length and thickness, lamina propria thickness, total goblet cell number, eosinophilic droplets in epithelial cells and melanomacrophage centres were measured.

2.5. Biochemical and histological analyses

The analysed chemical composition of diets and whole-body tissue were conducted by the National Measurement Institute (NMI; Port Melbourne, Victoria, Australia). Methods are certified by the National Association of Testing Authorities (NATA), Australia and conducted under the ISO/IEC 17025 accreditation standards. A one kg sample of each diet was collected, split, ground and analysed for proximate composition (moisture gravimetric method VL298, Kjeldahl nitrogen x 6.25 method VL299, fat gravimetric method VL300 in method VL302, ash gravimetric method VL286, carbohydrate and energy by calculation method VL412), rancidity (p-anisidine value (outsourced) and peroxide value titration method VL311), amino acid profile including taurine method HPLC-PDA-MS-MS method VL450, cholesterol GC-FID method VL288, minerals ICP-MS method VL247 and fatty acid profile GC-FID method VL289 following in-house protocols. Whole fish samples were partially thawed, homogenised, and analysed for proximate composition, fatty acids profile, amino acids profile, taurine and mineral composition following the methods described above.

2.6. Apparent digestibility coefficients

Following the final weight and sampling events, the eleven remaining fish were returned to their respective tanks and fed their respective diet for a further 6 days. After this point the fish were lightly anesthetised with AQUI-S®, and faeces removed from the anus by applying gentle abdominal pressure and performing two stripping movements in a posterior direction. Samples from each tank were pooled into plastic vials and frozen at −20 °C until analysis. The ADC (%) was calculated following standard methods (Maynard and Loosli, 1969; Miegel et al., 2010):

$$\mathrm{ADC}\ (\%) = 100 - \left(100 \times \left[\%M_{\mathrm{feed}} / \%M_{\mathrm{faeces}}\right] \times \left[\%N_{\mathrm{faeces}} / \%N_{\mathrm{feed}}\right]\right)$$

where M refers to the inert marker (in this case acid insoluble ash (AIA)) and N refers to the nutrient of interest.

2.7. Performance indices

All data reported for each treatment were based on the mean of the three replicate tanks. All calculations using fish weight and diets were based on wet or as fed values, respectively:

$$\text{Biomass gain}\ \left(\mathrm{kg\ tank^{-1}}\right) = \text{final weight} - \text{initial weight}$$

$$\text{Specific growth rate } \left(\text{SGR}, \%\text{d}^{-1}\right) = \left(\left[ln \text{ final weight}–ln \text{ initial weight}\right]/\text{d}\right) \times 100$$

$$\text{Length growth rate } \left(\text{mm d}^{-1}\right) = \left(\text{final fish fork length}–\text{initial fish fork length}\right)/\text{d}$$

$$\text{Condition factor} = \left(\text{fish weight [g]}/\text{fish fork length [cm]}^3\right) \times 100$$

$$\text{Apparent feed conversion ratio (FCR)} = \text{feed consumed}/\text{fish weight gain}$$

$$\text{Apparent protein deposition} = \left(\left[\text{final body protein}–\text{initial body protein}\right]/\text{protein intake} \times 100\right.$$

$$\text{Apparent energy deposition} = \left(\left[\text{final body energy}–\text{initial body energy}\right]/\text{energy intake} \times 100\right.$$

$$\text{Intraperitoneal fat } (\%) = \text{wet intraperitoneal fat weight} \times 100/\text{final wet fish weight}$$

$$\text{Visceral index (VSI; \%)} = \text{wet visceral weight} \times 100/\text{final wet fish weight}$$

$$\text{Hepatosomatic index (HSI; \%)} = \text{wet liver weight} \times 100/\text{final wet fish weight}$$

$$\text{Fish in fish out ratio (FIFO)} = \text{FCR} \times 0.75 \times 0.5 \times [(\%\text{fish meal in feed}/22.5) + ((\%\text{fish oil in feed}–0.08 \times \%\text{fish meal in feed})/5)]$$

where the FIFO ratio is expressed in reduction fish equivalent and FCR is the feed conversion ratio (kg feed kg^{-1} fish). The yield of reduction fish is 22.5% WD fish meal and 5% fish oil. The factor 0.75 considers that approximately 25% of the WD fishmeal and fish oil is produced from non-wild sources, and the factor 0.08 considers that WD fish meal typically contains approximately 8% fish oil (Terpstra, 2015).

2.8. Statistical analyses

The IBM SPSS software package (version 24 for Windows; IBM SPSS Inc., USA) was used for all statistical analyses. Data were assessed using Levene's test for equality of variance errors and Shapiro-Wilk test for normal distribution. Data were compared across all treatments using a one-factor ANOVA. When significant effects were observed, the Student-Newman-Keuls (SNK) pairwise comparison test was chosen to detect significant differences between all treatments in this experiment (Zar, 1999). A significance level of $P < 0.05$ was used for all statistical tests. All values are presented as means ± standard error (SE) of the mean unless otherwise stated.

3. Results

3.1. Growth performance and feed utilisation

There were no significant differences in the initial weight and fork length of YTK between treatments (Table 3). YTK fed actively during the experiment, and final weight, biomass gain, specific growth rate (SGR), final fork length, length growth rate and final condition factor of YTK were not significantly influenced by diet (Table 3). The average weight of all YTK progressed well and responded to the temperature profile as expected, with a plateau in growth observed over the cooler period (Fig. 1). Apparent feed consumption (kg $tank^{-1}$) and apparent feed intake (% BW d^{-1}) were not significantly affected by diet (Table 3). Feed conversion ratio (FCR) of YTK was also not significantly influenced by diet (Table 3). Fish fed the BP-FM 66% diet (10% WD-FM + 21.4% BP-FM) had numerically the highest FCR however this was not statistically different.

3.2. Nutrient retention

The diets had no significant effect on apparent protein deposition (range 20.6 to 22.7%) or apparent energy deposition (range 21.0 to 23.0%) in the fish (Table 3).

3.3. Whole fish compositional analysis

Carcass moisture (range 62.4 to 64.5%), protein (range 19.1 to 19.8% wet), lipid (range 15.2 to 15.8% wet) ash (range 1.9 to 2.5% wet), carbohydrate (< 1% wet) and energy (range 9.0 to 9.2 MJ kg^{-1} wet) contents of fish were not significantly different between diets (Table 3). The fatty acid and amino acid composition of fish were also not significantly influenced by diet (Table 4). The potassium content of fish fed BP-FM 66% diet (10% WD-FM + 21.4% BP-FM) was significantly lower than those fish fed the control WD-FM 100% (30% WD-FM), the BP-FM 33% (20% WD-FM + 10.7% BP-FM) and the SPC 33% (20% WD-FM + 10.8 SPC) diets (Supplementary table). The diets did not significantly influence other mineral levels measured (calcium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, zinc (Supplementary table).

Table 3
Growth performance and feed utilisation of yellowtail kingfish fed different fish meal replacement diets for 252 days.[1]

Diet [2]	WD-FM 100%	BP-FM 33%	BP-FM 66%	PM 33%	BP-FM 33% + PM 33%	SPC 33%	ANOVA
Growth performance							
Initial weight (kg)	2.52 ± 0.01	2.52 ± 0.02	2.53 ± 0.02	2.52 ± 0.01	2.53 ± 0.01	2.52 ± 0.01	$P = 0.981$
Final weight (kg)	4.31 ± 0.04	4.29 ± 0.05	4.28 ± 0.07	4.31 ± 0.07	4.33 ± 0.01	4.44 ± 0.04	$P = 0.321$
Biomass gain (kg tank^{-1})[4]	30.45 ± 0.52	30.23 ± 0.75	29.76 ± 0.77	30.40 ± 1.10	30.71 ± 0.29	32.66 ± 0.65	$P = 0.157$
SGR (% day^{-1})	0.21 ± 0.00	0.21 ± 0.00	0.21 ± 0.00	0.21 ± 0.01	0.21 ± 0.00	0.22 ± 0.00	$P = 0.120$
Initial fork length (mm)	544 ± 2	545 ± 1	545 ± 1	549 ± 1	547 ± 2	546 ± 1	$P = 0.165$
Final fork length (mm)	630 ± 4	630 ± 2	629 ± 3	635 ± 3	637 ± 5	636 ± 1	$P = 0.368$
Length growth rate (mm day^{-1})	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.00	0.16 ± 0.01	0.18 ± 0.00	0.17 ± 0.00	$P = 0.163$
Final Condition factor	1.72 ± 0.02	1.72 ± 0.01	1.72 ± 0.00	1.68 ± 0.02	1.68 ± 0.04	1.73 ± 0.01	$P = 0.272$
Feed utilisation							
Apparent feed consumption (kg tank^{-1})	68.90 ± 0.93	69.99 ± 2.25	72.62 ± 0.73	70.84 ± 2.10	71.69 ± 0.73	74.02 ± 1.18	$P = 0.235$
Apparent feed intake (% bw d^{-1})	0.50 ± 0.01	0.51 ± 0.01	0.53 ± 0.00	0.52 ± 0.01	0.52 ± 0.01	0.53 ± 0.01	$P = 0.409$
Apparent FCR (as fed)	2.26 ± 0.04	2.32 ± 0.04	2.44 ± 0.05	2.33 ± 0.05	2.33 ± 0.01	2.27 ± 0.08	$P = 0.193$
Proximate composition[3]							
Moisture (%)	63.9 ± 0.8	63.6 ± 0.4	63.4 ± 0.4	62.4 ± 1.1	64.5 ± 0.7	63.8 ± 0.7	$P = 0.494$
Protein (% wet)	19.8 ± 0.2	19.1 ± 0.7	19.8 ± 0.1	19.5 ± 0.3	19.3 ± 0.3	19.7 ± 0.1	$P = 0.647$
Lipid (% wet)	15.5 ± 0.2	15.8 ± 0.3	15.4 ± 0.7	15.8 ± 0.8	15.4 ± 0.7	15.2 ± 0.5	$P = 0.958$
Ash (% wet)	2.0 ± 0.2	2.0 ± 0.1	2.3 ± 0.3	2.5 ± 0.3	2.4 ± 0.1	1.9 ± 0.3	$P = 0.378$
Carbohydrate (% wet; by difference)	<1	<1	<1	<1	<1	<1	*N/A*
Energy (MJ kg^{-1} wet)	9.10 ± 0.10	9.07 ± 0.03	9.10 ± 0.25	9.20 ± 0.31	8.97 ± 0.27	8.97 ± 0.18	$P = 0.967$
Nutrient retention[4]							
Apparent PD	22.7 ± 0.2	20.6 ± 1.8	21.1 ± 0.5	21.7 ± 0.4	20.7 ± 0.7	22.0 ± 0.9	$P = 0.546$
Apparent ED	23.0 ± 0.4	22.0 ± 0.5	21.3 ± 1.5	22.6 ± 1.2	21.0 ± 1.4	21.7 ± 0.8	$P = 0.758$
Fish in fish out ratio [5]	2.3	1.9	1.5	2.2	1.7	2.1	*N/A*
Difference to control	–	−17.9%	−35.1%	−4.8%	−25.4%	−7.1%	*N/A*

[1] Values are mean ± SE; $n = 3$, a significance level of $P < 0.05$ was used for all statistical tests.
[2] For diet details, please refer to methods Section 2.1 Experimental design and diets.
[3] Initial fish proximate composition (wet basis): Moisture 65.1%, protein 17.2%, lipid 15.4%, ash 1.9%, carbohydrate (by difference) <1%, energy 8.60 MJ kg^{-}.
[4] PD = protein deposition, ED energy deposition.
[5] Fish in fish out ratio (FIFO) = FCR × 0.75 × 0.5 × [(% fish meal in feed / 22.5) + ((% fish oil in feed - 0.08 × % fish meal in feed) / 5)].

3.4. Blood haematology and biochemistry

None of the measured blood haematology or biochemistry parameters were significantly affected by diet (Supplementary table).

3.5. Apparent digestibility coefficients

Apparent digestibility coefficients (ADC) for diet dry matter and protein were significantly affected by diet (Table 5). Dry matter and protein ADCs were significantly higher for fish fed the BP-FM 33% diet

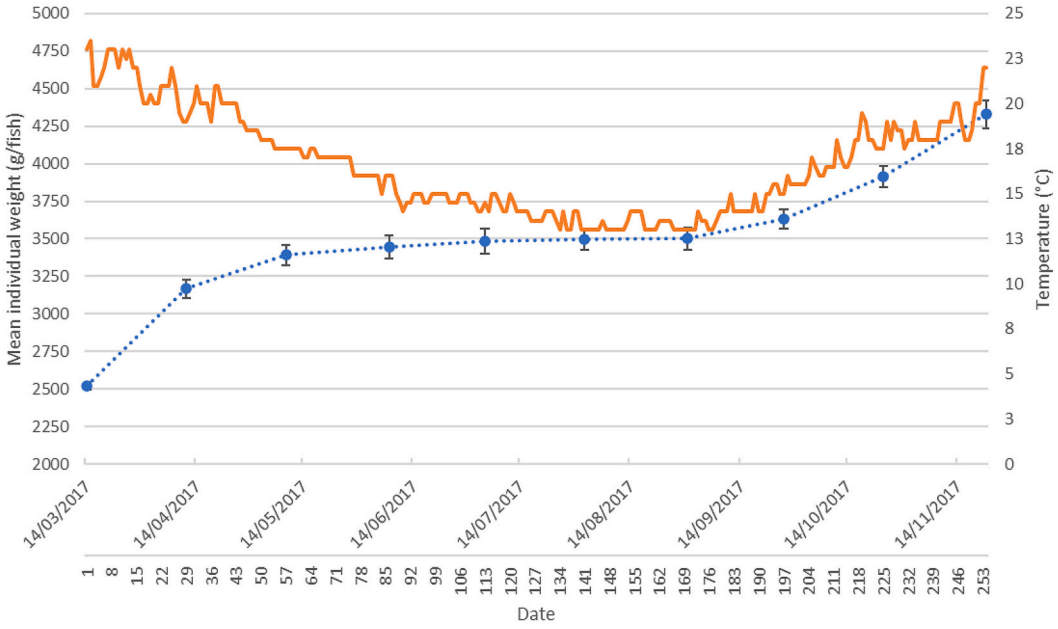

Fig. 1. Mean weight of yellowtail kingfish fed different fishmeal replacement diets ($n = 18$ tanks, mean +/− pooled SE) at 0, 28, 56, 85, 112, 140, 169, 196, 224 and 252 days. Water temperature profile between stocking and final weight check at harvest (average water temperatures was 16.6 °C [range 23.5–13.0 °C]).

Table 4
Fatty acid (mg 100 g^{-1}) and essential amino acid composition (g 100 g^{-1}) of whole yellowtail kingfish fed different fish meal replacement diets for 252 days.[1]

		Diet [2]						
	Initial	WD-FM 100%	BP-FM 33%	BP-FM 66%	PM 33%	BP-FM 33% + PM 33%	SPC 33%	ANOVA
Fatty acids[3]								
16:0 Palmitic	3295.6	2661.6 ± 87.1	2711.7 ± 61.1	2567.5 ± 119.9	2692.2 ± 136.7	2652.7 ± 171.6	2631.4 ± 70.5	$P = 0.958$
18:0 Stearic	954.8	827.0 ± 30.3	853.1 ± 21.3	816.5 ± 20.5	849.2 ± 36.1	842.8 ± 50.6	812.5 ± 15.1	$P = 0.901$
18:1n-9 Oleic	5590.2	5199.3 ± 138.6	5376.1 ± 99.0	5303.3 ± 229.9	5411.3 ± 280.0	5168.6 ± 358.7	5076.6 ± 126.3	$P = 0.885$
18:2n-6 Linoleic	1386.0	1834.1 ± 24.5	1859.0 ± 32.2	1872.5 ± 83.5	1904.0 ± 79.7	1830.7 ± 68.3	1804.2 ± 53.2	$P = 0.883$
18:3n-3 alpha-Linolenic	154.0	211.8 ± 5.3	216.0 ± 7.8	216.1 ± 9.6	221.7 ± 10.8	210.0 ± 5.3	202.2 ± 8.2	$P = 0.651$
20:4n-6 (ARA)	107.8	113.5 ± 3.7	121.0 ± 9.8	123.5 ± 5.5	120.9 ± 1.9	117.6 ± 1.8	111.8 ± 13.2	$P = 0.843$
20:5n-3 (EPA)	261.8	583.0 ± 44.2	542.8 ± 47.9	519.9 ± 26.6	553.9 ± 25.8	551.3 ± 26.5	548.3 ± 59.7	$P = 0.931$
22:6n-3 (DHA)	770.0	980.2 ± 66.6	1006.3 ± 89.1	1060.7 ± 58.0	986.5 ± 47.0	948.9 ± 62.1	910.3 ± 133.6	$P = 0.843$
Total SFA [4]	5005.0	4072.5 ± 131.6	4149.4 ± 101.3	3929.0 ± 160.7	4100.5 ± 195.6	4086.9 ± 262.5	4034.3 ± 98.6	$P = 0.957$
Total MUFA [4]	6699.0	6790.9 ± 172.3	7014.5 ± 143.3	6846.5 ± 298.2	7026.4 ± 361.4	6756.2 ± 449.3	6652.8 ± 175.5	$P = 0.926$
Total PUFA [4]	2987.6	4512.7 ± 143.3	4498.5 ± 205.8	4532.7 ± 208.5	4567.5 ± 189.4	4430.7 ± 105.0	4351.7 ± 311.4	$P = 0.977$
Total Omega-3 [4]	1355.2	2410.0 ± 141.6	2360.5 ± 177.7	2377.6 ± 116.2	2373.5 ± 99.7	2322.8 ± 107.3	2279.2 ± 253.0	$P = 0.993$
Total Omega-6 [4]	1617.0	2056.1 ± 23.4	2085.4 ± 36.2	2108.9 ± 90.3	2135.8 ± 85.6	2066.7 ± 77.7	2027.2 ± 69.3	$P = 0.892$
Total n-3 LC-PUFA [4]	1185.8	1795.5 ± 117.7	1781.0 ± 151.9	1812.0 ± 94.7	1777.9 ± 79.3	1725.0 ± 96.8	1676.8 ± 216.4	$P = 0.979$
Essential amino acids[3]								
Arginine	0.82	0.80 ± 0.12	1.00 ± 0.05	0.91 ± 0.07	0.96 ± 0.03	0.93 ± 0.04	0.98 ± 0.00	$P = 0.388$
Histidine	0.69	1.00 ± 0.05	1.15 ± 0.13	0.99 ± 0.06	0.98 ± 0.06	1.04 ± 0.06	0.98 ± 0.06	$P = 0.574$
Isoleucine	0.67	1.09 ± 0.13	1.06 ± 0.12	1.13 ± 0.17	1.14 ± 0.16	1.00 ± 0.15	0.94 ± 0.14	$P = 0.903$
Leucine	1.20	1.30 ± 0.06	1.30 ± 0.10	1.20 ± 0.00	1.20 ± 0.00	1.27 ± 0.07	1.20 ± 0.06	$P = 0.875$
Lysine	1.60	1.58 ± 0.34	1.43 ± 0.28	1.12 ± 0.19	1.28 ± 0.22	1.35 ± 0.28	1.37 ± 0.22	$P = 0.624$
Methionine	0.44	0.59 ± 0.05	0.59 ± 0.04	0.54 ± 0.01	0.53 ± 0.00	0.56 ± 0.02	0.54 ± 0.02	$P = 0.623$
Phenylalanine	0.59	0.70 ± 0.04	0.76 ± 0.08	0.65 ± 0.06	0.69 ± 0.04	0.72 ± 0.05	0.69 ± 0.06	$P = 0.832$
Threonine	0.69	0.75 ± 0.01	0.80 ± 0.04	0.70 ± 0.05	0.73 ± 0.01	0.75 ± 0.01	0.68 ± 0.04	$P = 0.259$
Valine	0.71	1.16 ± 0.18	1.12 ± 0.13	0.98 ± 0.06	0.95 ± 0.08	1.05 ± 0.10	0.96 ± 0.12	$P = 0.719$
Taurine	0.14	0.22 ± 0.02	0.22 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.25 ± 0.04	$P = 0.764$

[1] Values are mean ± SE; $n = 3$, a significance level of $P < 0.05$ was used for all statistical tests.
[2] For diet details, please refer to 2.1 Experimental design and diets.
[3] Includes other fatty acids and non-essential amino acids not in the table. For full table, please refer to complete profiles in full supplementary table.
[4] SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n-3 LC-PUFA: omega-3 long chain polyunsaturated fatty acids, ARA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid.

Table 5
Apparent digestibility and somatic indices of yellowtail kingfish fed different fish meal replacement diets for 252 days.[1]

Diet [2]	WD-FM 100%	BP-FM 33%	BP-FM 66%	PM 33%	BP-FM 33% + PM 33%	SPC 33%	ANOVA
Apparent digestibility coefficient							
Dry matter	44.4 ± 9.8^{ab}	56.5 ± 0.8^{a}	33.3 ± 0.0^{b}	40.4 ± 4.2^{ab}	59.2 ± 2.4^{a}	49.0 ± 5.0^{ab}	$P = 0.023$
Protein	73.7 ± 5.4^{ab}	84.5 ± 2.4^{a}	68.4 ± 3.4^{b}	80.7 ± 1.4^{ab}	86.2 ± 0.3^{a}	81.3 ± 3.7^{ab}	$P = 0.016$
Energy	61.4 ± 6.6	65.5 ± 2.5	49.0 ± 2.1	53.5 ± 3.1	67.3 ± 4.3	64.6 ± 3.2	$P = 0.055$
Visceral somatic parameters							
Intraperitoneal fat (%)	1.7 ± 0.2	1.5 ± 0.3	1.8 ± 0.1	1.6 ± 0.1	1.8 ± 0.2	1.7 ± 0.3	$P = 0.944$
Visceral index (VSI; %)	5.9 ± 0.2	5.9 ± 0.3	6.7 ± 0.3	6.2 ± 0.2	6.4 ± 0.1	6.2 ± 0.4	$P = 0.303$
Hepatosomatic index (HSI; %)	0.9 ± 0.1	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.0	0.9 ± 0.1	$P = 0.890$
Stomach morphology							
Gastric dilation score [3]	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$P = 0.458$

[1] Values are mean ± SE; $n = 3$, a significance level of $P < 0.05$ was used for all statistical tests.
[2] For diet details, please refer to method Section 2.1 Experimental design and diets.
[3] Gastric dilation score based on Chown (2015).

(20% WD-FM + 10.7% BP-FM) and the BP-FM 33% + PM 33% diet (10% WD-FM + 10.7% BP-FM + 11.3% PM) than those fed the BP-FM 66% diet (10% WD-FM + 21.4% BP-FM). Dry matter and protein ADC for fish fed the WD-FM 100% diet (30% WD-FM), the PM 33% diet (20% WD-FM + 11.3% PM) and the SPC diet (20% WD-FM + 10.9 SPC) were statistically similar. Energy ADC was not significantly affected by any diet although the same trend was apparent (Table 5).

3.6. Visceral somatic parameters and gastrointestinal morphology

Intraperitoneal fat (1.5 to 1.8%), visceral index (VSI; 5.9 to 6.7%) and hepatosomatic index (HSI; 0.8 to 0.9%) of fish were not significantly influenced by diet (Table 5). Diet did not affect gastric dilation (Table 5). A single fish fed WD-FM 100% was determined to have a Stage 1 gastric dilation score, with all remaining fish determined to be Stage 0 (healthy/no gastric dilation). Muscularis and submucosa thickness, villi length and thickness, lamina propria thickness, total mucus cells, eosinophilic droplets in epithelial cells and melanomacrophage centres in the hindgut were not significantly affected by diet (Supplementary table).

4. Discussion

The aim of the current experiment was to investigate the potential for replacing dietary inclusions of WD-FM with alternative ingredients (PM, SPC and BP-FM) on the growth performance, feed utilisation, and some

indicators of health on large YTK. The study was designed to be commercially relevant in many aspects, using ~2.5 kg initial fish and a long culture period, subject to natural seasonal variations. The lack of significant differences observed in production performance or health indicators demonstrates that there is flexibility in formulation strategies for on-growing YTK. This research provides a new framework for raw material selection, while meeting sustainability and economic criteria for commercial YTK production.

The inclusion levels of PM (11.3%) and SPC (10.9%) used in the diet for large YTK in the current study resulted in good growth, which supports the use of these levels in commercially produced diets. This is consistent with appropriate inclusion levels established for a range of other carnivorous freshwater and marine species, such as rainbow trout (Sealey et al., 2011), Atlantic salmon (Davidson et al., 2016), cobia (Zhou et al., 2011) and juvenile YTK (Bowyer et al., 2013b). While no significant differences for growth performance or feed utilisation were observed in this experiment, the diet containing the highest inclusion of tuna by-product fish meal (BP-FM 66%) resulted in the poorest mean FCR and biomass gain values. This may indicate that this diet is near the limits of practical inclusion for BP-FM. This finding is consistent with results from Kim et al. (2018) who reported the growth and feed utilisation of Korean rockfish (*Sebastes schlegeli*) also tended to be reduced as high-ash tuna by-product fishmeal replaced WD-FM. The ash content of the tuna by-product fishmeal in their experiment was 21.4% (Kim et al., 2018), compared to 20.2% used in the present experiment, suggesting some consistency between the meals used.

As BP-FM is derived from fish which have been processed to recover the edible portion of flesh, bone and hence ash contents are typically higher than WD-FM (Aksnes and Mundheim, 1997; Caballero et al., 1999; Kim et al., 2018). Gatlin III et al. (2007) report that most alternative plant proteins contain lower ash content compared to fishmeal (~2 to 8%), and Galkanda-Arachchige et al. (2020) emphasised that poultry meal should be a promising alternative to fishmeal because of many features including its low ash content. In addition to the high ash content in BP-FM, the protein quality is potentially lower, as it is comprised of a large proportion of connective tissue and viscera (Aksnes and Mundheim, 1997; Caballero et al., 1999; Kim et al., 2018). Kim et al. (2018) reported lower content of the first two limiting amino acids, lysine (4.3 vs 5.5%) and methionine (1.8 vs 2.2%) in tuna BP-FM compared to WD-FM. In the current study the lysine (4.1 vs 4.3%) and methionine (1.6 vs 1.7%) levels in the BP-FM were also lower than in the WD-FM (Table 1).

Upon closer examination of results in the current study (Table 5), the apparent digestibility for dry matter and protein were significantly lower for the YTK fed the BP-FM 66% which contained the highest proportion of BP-FM (21.4% of the diet), compared to the diets BP-FM 33% and BP-FM 33% + PM 33%. High ash levels have been reported to interfere with nutrient digestion in a range of fish species. Stone et al. (2000) reported a reduction in dry matter, energy and nitrogen apparent digestibility in silver perch (*Bidyanus bidyanus*) fed high ash meat by-products. Protein digestibility is reported to be negatively correlated with high ash content in meat and fish meals for rainbow trout (*Oncorhynchus mykiss*) (Watanabe and Pongmaneerat, 1991), Gilthead Seabream (*Sparus aurata*) (Nengas et al., 1995) and Olive flounder (*Paralichthys olivaceus*) (Rahman et al., 2016). Reduced nutrient digestibility may have contributed to the numerically poorer feed utilisation parameters of the YTK fed the diet BP-FM 66%. These findings together suggest that BP-FM inclusion may be limited to around 10% in commercial diets for YTK that contain 20% wild derived fishmeal. Additionally, consideration must always be given to the ash and protein quality of ingredients derived from processing waste streams when selecting ingredients for commercial YTK diets.

Many parameters were measured in this experiment, and for the vast majority there were no significant differences detected among the treatment groups (please refer to the supplementary tables for full details). It is important to note that the amino acid composition of the flesh did not vary in response to the diets, despite the different ingredients tested. Moreover, there were no aberrations to the fatty acid profiles of the flesh, consistent with expectations. The measured potassium content was significantly different with diet BP-FM 66% fed fish having lower levels than the WD-FM 100%, BP-FM 33% and SPC 33% fed fish. However, the levels were all within normal ranges for similar size fish observed from previous experiments (Stone et al., 2016). The BP-FM used had less potassium (2900 ppm) compared to the WD-FM (8400 ppm) however these levels were much lower than the SPC (22,000 ppm) therefore, with the available information we cannot interpret this data further.

It is important for all aquaculture producers to reduce their reliance on marine derived ingredients to improve the sustainable production of fish, commonly measured by the fish in fish out ratio (FIFO) (Tacon and Metian, 2008; Jackson, 2009; Terpstra, 2015). The fish in fish out ratio is calculated based on the mass of wild derived marine ingredients required per unit mass of fish production and considers the FCR of the production unit (Tacon and Metian, 2008; Jackson, 2009; Terpstra, 2015). All alternative protein sources used in this study, including the BP-FM, result in reduced FIFO ratio. The inclusion of the alternative protein sources in this experiment resulted in improvements in the FIFO ratio of up to 35.1% without negatively impacting any production or health measurements (Table 3). An added advantage of the alternative ingredients tested was that they were more cost effective than WD-FM at the time of diet production (proprietary information from the fish feed companies involved), which may also result in improved economics of the farm. Given, there were no significant differences in growth and FCR, actual savings realised by producers may be considerable in terms of sustainability and economics, however on-farm validation may still be warranted prior to full adoption.

The growth performance of the large YTK fed diets containing the alternative protein sources may be further enhanced with specific amino acid fortification. The diets used in the current study were formulated with specific nutritional information to contain methionine at 1.0%, with analysed levels ranging from 1.01 to 1.13% (Table 2) (Stone and Bellgrove, 2013; National Research Council (NRC), 2011b). The methionine and cystine requirements for juvenile YTK (~53 g initial weight) were recently investigated (Candebat et al., 2020; Candebat et al., 2021). Based on growth performance and feed utilisation the authors estimated the methionine requirement to be ~2.0% of the diet. It is known that faster growing juvenile fish have higher nutrient demands (National Research Council (NRC), 2011b) and therefore it is possible that the levels of dietary methionine provided in the current experiment may be satisfactory for optimal growth performance. However, in light of this newer information, future studies may be warranted to investigate amino acid requirements in larger YTK as WD-FM levels are gradually reduced in growout diets. This demonstrates the importance of incremental improvements to the nutrient requirements for YTK at all stages of development.

5. Conclusion

In conclusion, results from the current study provide valuable, commercially relevant information to reduce the dietary WD-FM inclusion levels in production diets for large YTK. There were no significant negative effects on growth performance or feed utilisation noted with any of the diets evaluated, and there were only minor changes to the apparent digestibility coefficients noted. Reducing dietary WD-FM inclusions in current commercial diets with commonly available alternative ingredients may lead to improved diet sustainability. Sustainability, as measured by the fish in-fish out ratio, was improved by up to ~35% by the incorporation of PM, SPC and BP-FM ingredients. This may provide YTK producers with major advantages in terms of accreditation, market access and improved consumer perception. In addition, information pertaining to the replacement of WD-FM with alternative protein sources will provide flexibility for feed manufacturers to select raw

materials that most economically meet the nutrient criteria in diet formulations for YTK. This is particularly advantageous, as availability and prices for fish feed ingredients remain volatile. Based on results from the current investigation, we recommend that when using SPC in diets for large YTK, formulations contain at least 20% WD-FM. We recommend that WD-FM substitution with PM, SPC and BP-FM in diets be followed up with further pilot scale commercial trials before full diet formulation flexibility is realised.

CRediT authorship contribution statement

Matthew S. Bansemer: Conceptualization, Investigation, Formal analysis, Writing – review & editing. **Michael J. Salini:** Conceptualization, Methodology, Writing – review & editing. **Leo Nankervis:** Conceptualization, Methodology, Writing – review & editing. **David A. J. Stone:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Funding acquisition, Project administration.

Declaration of Competing Interest

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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