Journal of Sustainability Science and Management
Volume 18 Number 5, May 2023: 1-15
eISSN: 2672-7226

PROTEIN SPARING USING SAGO STARCH ON THE PERFORMANCE OF TIGER GROUPER, *EPINEPHELUS FUSCOGUTTATUS* ♀ × GIANT *GROUPER, E. LANCEOLATUS* ♂ HYBRID

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Submitted final draft: 26 January 2023 *Accepted: 8 March 2023* http://doi.org/10.46754/jssm.2023.05.001

Abstract: Starch is an important non-protein energy source in the practical diets for fish and its optimal inclusion can maximise the use of dietary protein for growth. In the present study**,** a feeding trial was conducted to investigate the effects of protein sparing with carbohydrates on growth and diet digestibility in hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*). Diets were formulated with lowered levels of protein (50% to 45% and 40%) and increased levels of starch (20% to 25% and 30%). At the end of the feeding trial, growth performance and body indices (except for viscerosomatic index) were not significantly influenced ($P > 0.05$) by the dietary treatments. In contrast, the whole-body proximate fish and liver compositions depended on the test diets. The dietary starch influences the haematological parameters, even though no clear trend was observed. Meanwhile, the apparent digestibility coefficient of protein and lipid was considered high in all experimental diets. Overall, the diet with protein level lowered to 45% and starch level increased to 30% performed as well as the control diet, indicating some ability of hybrid grouper to utilise sago starch as an energy source to spare protein.

Keywords: Hybrid grouper, *Epinephelus* spp., protein-sparing effect, sago starch.

Abbreviations:
40P25S – Diet with 40% protein & 25% starch
40P30S – Diet with 40% protein & 30% starch
45P25S – Diet with 45% protein & 25% starch
45P30S – Diet with 45% protein & 30% starch
50P20S – Diet with 50% protein & 20% starch
CMC – Carboxymethyl Cellulose
ADC – Apparent Digestibility Coefficient
BWG – Body Weight Gain
SGR – Specific Growth Rate
FI – Feed Intake
FCR – Feed Conversion Ratio
PER – Protein Efficiency Ratio
NPU – Net Protein Utilisation
CF – Condition Factor
HSI – Hepatosomatic Index
VSI – Viscerosomatic Index
S.E.M. – Standard Error Mean
IPF – Intraperitoneal Fat
RBC – Red Blood Cell
MCH – Mean Corpuscular Haemoglobin
WBC – White Blood Cell
MCHC – Mean Cell Haemoglobin Concentration

Introduction

Protein is an important and the most expensive ingredient in the fish diet. Hybrid grouper (tiger grouper, *Epinephelus fuscoguttatus* (Forsskål, 1775) ♀ × giant grouper, *E. lanceolatus* (Bloch, 1790) ♂) is a carnivorous fish that naturally has a high requirement for dietary protein which normally based on the expensive fish meal. Grouper species require 40–50% protein and 8–16% lipid (Teng & Chua, 1978; Tuan & Williams, 2007; Shapawi *et al*., 2014; Yong *et al*., 2019). Meanwhile, the recommended inclusion levels of carbohydrates in marine fish species fall within 15–25% (NRC, 2011).

In general, carnivorous fish including grouper tends to use protein as the source of energy apart from being used to form body tissue (FAO, 1980). This portion is considered wasteful when excess protein is broken down to energy. Therefore, the diet formulation's major aim is to convert dietary protein to tissue protein for fish biomass. Starch is the least expensive form of dietary non-protein component. Incorporating starch in the diets of grouper species has been reported to be beneficial for the fish growth and physical quality of the pelleted diets (Kraugerud *et al*., 2011; Shapawi *et al*., 2011; Li *et al*., 2016). Incorporation of optimum levels of carbohydrates in the fish diet will help to increase protein retention by preventing the catabolism of protein. Therefore, knowing an optimum amount of the required protein and non-protein nutrients to spare some protein for maintaining good growth is crucial to formulating cost-effective and balanced diets.

At present, information on the potential of carbohydrates to spare protein in aquaculture species including grouper is scarce. In the past, a few studies have been done on the protein-sparing of carbohydrates in marine species such as Senegalese sole, *Solea senegalensis* (Guerreiro *et al*., 2012), juvenile turbot, *Scophthalmus maximus* (Zeng *et al*., 2015), tiger prawn, *Penaeus monodon* (Shiau & Peng, 1992) and white-leg shrimp, *Litopenaeus vannamei* (Wang *et al*., 2014). Research on carbohydrate nutrition in groupers is mainly on the comparison of the ability of the fish to use different carbohydrate sources and their complexity (Wilson, 1994). Starch is the most common carbohydrate form used in formulated feeds for aquatic species. In our study on utilising different dietary carbohydrate sources in hybrid grouper (Ismail *et al*., 2018), starches in the forms of sago, corn, and tapioca included at a 20% inclusion level performed better than dextrin.

In a study by Li *et al.* (2016), reducing lipid from 22.6 to 14.79%, with a concomitant increase in CHO level from 3.05 to 19.23%, did not negatively affect the growth of giant grouper (*E. lanceolatus*) early juveniles, a finding like those reported in humpback grouper (Valenciennes, 1828) (Shapawi *et al*., 2011). More information is needed to determine the protein-sparing effect in this carnivorous species using cheaper alternatives. Most starch forms are relatively cheaper than dietary lipids, thus the ability of starch to spare some protein is worth further investigation. The sago plant grows mostly in Southeast Asia and Southern Pacific islands, where the grouper aquaculture industry is well-developed. Therefore, the present study was conducted to determine the effects of different levels of protein and starch in the diets on fish performance to develop cost-effective diets for the sustainability of grouper farming.

Materials and Methods

Experimental diets

Fishmeal and fish oil were used as protein and lipid sources, respectively, and sago starch was selected as the sole source of carbohydrates. Five experimental diets were formulated with different protein and starch levels; 40% protein & 25% starch; 40% protein & 30% starch; 45% protein & 25% starch; 45% protein & 30% starch, which yielded diets 45P25S, 45P30s, 40P25S, and 40P30S. The control diet was formulated with 50% crude protein and 20% carbohydrate (50P20S). The diets were prepared by mixing all dry ingredients and adding the wet ingredients (about 40% of the total ingredient) to form a moist dough. The dough was screw-pressed through a 3 mm die and the strands of moist feeds formed were oven dried at 40°C for 4 hours. The dried diets were stored in sealed plastic bags and kept at 4°C until feeding. The diet formulation and proximate composition of experimental diets are shown in Table 1.

Fish Rearing and Management

The feeding trial was conducted at the Fish Hatchery of the Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS) for 57 days. Hybrid grouper juveniles were purchased from a local fish farmer in Sabah,

Table 1: Formulation and proximate composition of each experimental diet (% dry matter basis)

Ingredients/diets	Protein/Carbohydrate (100 g dry basis)				
	50P20S	**45P25S**	**45P30S**	**40P25S**	**40P30S**
Fish meal[1]	68.11	61.30	61.30	54.49	54.49
Dietary lipid[2]	1.23	2.10	2.10	2.98	2.98
Sago starch[3]	20.0	25.0	30.0	25.0	30.0
CMC[4]	1.10	1.10	1.10	1.10	1.10
Vitamin premix[5]	3.00	3.00	3.00	3.00	3.00
Mineral premix[6]	2.00	2.00	2.00	2.00	2.00
Alpha cellulose[7]	4.06	5.00	0.00	10.93	5.93
Chromic Oxide[8]	0.50	0.50	0.50	0.50	0.50
Proximate composition (% of dry matter basis)					
Moisture	10.23 ± 0.24	9.84 ± 0.07	9.69 ± 0.10	10.18 ± 0.04	9.99 ± 0.09
Crude Protein	52.66 ± 0.24	47.45 ± 0.15	45.21 ± 0.07	42.74 ± 0.04	42.95 ± 0.25
Crude Lipid	10.80 ± 0.16	9.48 ± 0.41	9.49 ± 0.44	10.02 ± 0.08	9.36 ± 0.15
Crude Fiber	0.68 ± 0.03	5.56 ± 0.33	4.70 ± 0.02	4.00 ± 0.16	5.21 ± 0.16
Ash	11.78 ± 0.07	11.03 ±0 .04	12.10 ± 0.02	13.11 ± 0.05	10.78 ± 0.03
Gross energy (kJ g^{-1})	1917.55	1885.70	1971.70	1767.84	1853.84

[1] TripleNine Fishmeal, Denmark
[2] Tuna oil, commercial oil
[3] Kota Kinabalu Wet Market
[4] Carboxymethyl cellulose (Calbiochem, USA)
[5] Vitamin premix. (mg or IUkg^{-1}): vitamin A, 5000000 IU, vitamin D3, 1000000 IU; vitamin E, 50000 mg; vitamin B1, 15000 mg; vitamin B2, 15000 IU; vitamin B6, 12000 IU; vitamin B12, 25 mg; biotin, 500 mg; vitamin K3, 5000 mg; vitamin c, 300000 mg; calpan, 25000 mg; folic acid, 2500 mg; niacin, 50000 mg; inositol, 125000 mg. Dexchem Industries Sdn. Bhd, Malaysia
[6] Mineral premix (mgkg^{-1}): Iron, 25000 mg; copper, 785 mg; zinc 1894 mg; iodine, 150 mg; manganese, 800 mg; cobalt, 100 mg; sodium, 20 mg; dcp, 723251 mg; sodium chloride, 60000 Mg; anti caking, 1000 Mg; potassium chloride, 50000 mg; magnesium sulphate, 137000 mg. Dexchem Industries Sdn. Bhd, Malaysia
[7,8] Sigma, USA

Malaysia, and acclimatised for one week before the start of the experiment. The fish were fed with commercial marine grouper feed (Leong Hup: Crude protein, 44%; crude lipid, 8%) throughout the acclimatisation period. Fish with a mean initial wet body weight of 12.12 ± 0.08 g (mean ± S.E.M) were randomly distributed into groups of 20 fish in 15 fibreglass tanks (150 L) with a flow-through culture system. The system had continuous aeration (flow rate: 3.0 L min^{-1}). Throughout the feeding trial, fish were fed by hand twice daily (8 am and 4 pm) to apparent satiation. Water quality parameters (dissolved oxygen, temperature, salinity, and pH), the amount of feed consumption, and fish mortality were monitored and recorded daily to calculate feed intake, feed conversion ratio, and survival, respectively. Fish were individually weighed at the start and end of the feeding trial and bulk-weighed fortnightly to measure their growth rates. This study followed the Researcher's Guidelines on Code of Practice for the Care and Use of Animals for Scientific Purposes, Universiti Malaysia Sabah.

Feed Digestibility Trial

After 1-2 h post-feeding, the fresh and intact strands of faeces in each experimental tank were collected by gently siphoning, rinsed with distilled water, dried on filter paper, and immediately stored at -20°C until analysis (Shapawi *et al.,* 2007). Daily faecal samples from each tank were pooled until enough for chemical analysis. Chromic oxide of diets and faeces was determined following Furukawa and Tsukahara (1966) method. ADC values were calculated using the following equations:

Sample Collection and Analysis

At the beginning of the feeding trial, 10 fish were sampled for whole-body proximate analysis. At the end of the feeding trial, the fish were starved for 24 h. Commercial anaesthetic Transmore (NIKA™) was used to anaesthetise the fish before body weight and total length were recorded at the beginning and end of the feeding trial. Samples of viscera, liver and intraperitoneal fat from fish in each treatment were excised and weighed for viscerosomatic index (VSI), hepatosomatic index (HSI), and intraperitoneal index (IPF), respectively. The liver and muscle of fish from each treatment were analysed for glycogen content following the method by Naimo *et al.* (1998). Six fish from each treatment were sampled and stored at -20°C until analysis. The sample of selected ingredients, experimental diets, whole-body fish samples, muscle, and liver were homogenised for the proximate analysis of the dry matter, ash, protein, lipid, and fibre following the established methods described in AOAC International (1997). Physiological fuel values of carbohydrates (17.2 kJ g^{-1}), protein (23.6 kJ g^{-1}), and lipids (39.5 kJg^{-1}) were used to calculate the gross energy content of the diet (Halver & Hardy, 2002). Glycogen analysis of the liver and muscle followed by Naimo *et al*. (1998), involving extraction with potassium hydroxide and precipitation with ethanol before being subjected to spectrophotometer readings at 490 nm wavelength.

Haematological Analysis

Blood samples were collected from the caudal vein of anaesthetised fish using a syringe (23 g).

ADC of dry matter (%) = 100 x [1 – (% dietary chromic oxide/fecal chromic oxide)]

ADC of nutrient (%) =100 x [1 – (% fecal nutrient/% dietary nutrient) x (% dietary chromic oxide /fecal chromic oxide)]

The blood samples were centrifuged for 10 mins at 30,000 rpm to separate serum for analysis. Roche reagent kit was used for the determination of serum biochemistry: Triglyceride (cat. 20767107), cholesterol (cat. 03039773), glucose (cat. 03183734), and total protein (cat. 03183734), and the analysis was performed on a Cobas 6000 analyser series module C501 (Switzerland). While for haematological parameters including haemoglobin, red cell count (RCC), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet count and total white cell count (WCC) were performed on a haematology analyser, model XT-1800i Sysmex (China). The XT-1800i performs analysis with an optical detector based on the flow cytometry and hydrodynamic focusing methods.

Performance Evaluation and Statistical Analysis

Fish performances were calculated using the following formulas:

Body Weight gain (BWG) (%) = [Final body weight – Initial body weight/Initial body weight] x 100

Specific Growth Rate (SGR) (% d^{-1}) = [ln (final wt.) – ln (initial wt.)/days] × 100

Survival (%) = (final fish number - initial fish number)/initial fish number x 100

Feed Intake (FI) (g) = Total feed intake for the whole experiment duration

Feed conversion ratio (FCR) = dry feed consumed (g)/wet weight gain (g)

Protein efficiency ratio (PER) = wet weight gain (g)/total protein intake (g)

Net protein utilisation (NPU) = 100 × (final – initial fish body protein)/total protein intake

Condition factor (CF) = [fish weight (g)/(total length)^3] × 100

Hepatosomatic index (HSI) = [liver weight (g)/fish weight (g)] × 100

Viscerosomatic index (VSI) = [viscera weight (g)/fish weight (g)] × 100

Intraperitoneal fat (IPF) = [intraperitoneal fat weight (g)/fish weight (g)] × 100

All the data obtained were subjected to one-way ANOVA to determine the significance due to the effects of diets on weight gain, survival, feed utilisation, body indices, whole-body proximate composition, biochemical, haematology parameters, and apparent digestibility coefficient. Data in percentage form were arcsine transformed before this analysis. Homogeneity of variance was tested with Levene's test, and multiple comparisons among treatments were performed with a Duncan posthoc test. The significant level was set at $P < 0.05$. The statistical package SPSS V22.0 was used for all statistical analyses. Results were presented as mean ± standard error of the mean (S.E.M).

Results

Growth Performance and Feed Utilisation

The effects of dietary protein and starch levels on growth and feed utilisation are shown in Table 2. The dietary treatments did not affect growth performance, FCR, and SGR ($P > 0.05$). Fish grew from 12.0 to 81.52–87.43 g by the end of the feeding trial. BWG, SGR, and FCR were between 576.62–618.91%, 3.29–3.40% day-1, and 1.48–1.59, respectively with no significant differences observed among diets. The dietary protein and carbohydrate levels somehow affected total feed intake, with no particular trend observed. PER and NPU values of the control diet were lower than the values in other dietary treatments.

Body Indices

The body indices including VSI, HSI, IPF and CF, are shown in Table 3. The VSI significantly affected the dietary treatments, which ranged from 8.73% (Diet 50P20S) to 10.71% (Diet 45P25S). The HSI, IPF and CF of fish were not significantly different from each other, ranging from 0.97 to 1.09%, 1.76 to 2.11% and 1.89 to 1.94%, respectively.

Whole-Body, Liver and Muscle Composition

The whole-body moisture content ranged from 71.48% to 72.63% and was not influenced by the different levels of dietary protein and carbohydrates. Significant differences were observed in whole-body protein, lipid, and ash contents of fish fed varying protein and carbohydrate levels. Fish-fed Diet 40P25S yielded a significantly higher whole-body protein (16.93%) and lipid deposition (5.79%) than other diets. The ash contents for fish-fed Diets 50P20S (5.09%), 40P25S (5.13%), 40P30S (5.18%) were significantly higher than those fed Diets 45P25S (4.79%) and 45P30S (4.78%).

For liver composition, the moisture ranged from 63.49 to 66.96%. The liver protein was the highest in fish-fed Diet 45P30S (13.04%),

Table 2: Growth performance and feed utilisation of hybrid grouper after 57 days of feeding trial

	Experimental Diets				
	50P20S	**45P25S**	**45P30S**	**40P25S**	**40P30S**
FBW (g)	87.43 ± 1.23^{a}	81.52 ± 3.45^{a}	85.96 ± 2.94^{a}	84.29 ± 4.40^{a}	82.74±0.79^{a}
BWG (%)	618.91 ± 9.13^{a}	576.62 ± 28.94^{a}	609.07 ± 24.74^{a}	594.76 ± 38.29^{a}	579.94±7.07^{a}
SGR (% day^{-1})	3.40 ± 0.02^{a}	3.29 ± 0.08^{a}	3.38 ± 0.06^{a}	3.34 ± 0.10^{a}	3.30±0.02^{a}
Total FI (fish^{-1})	117.91 ± 2.2^{b}	108.85 ± 1.50^{a}	108.74 ± 1.13^{a}	114.24 ± 3.61^{ab}	110.25±1.54^{a}
FCR	1.57 ± 0.04^{a}	1.58 ± 0.10^{a}	1.48 ± 0.07^{a}	1.59 ± 0.09^{a}	1.56±0.03^{a}
PER	1.20 ± 0.04^{a}	1.33 ± 0.08^{ab}	1.49 ± 0.07^{b}	1.45±0.08^{b}	1.48±0.03^{b}
NPU	20.13 ± 0.74^{a}	22.14 ± 1.30^{ab}	24.37 ± 1.16^{b}	24.85 ± 1.4^{b}	24.28±0.66^{b}
Survival (%)	98.33 ± 1.67^{a}	96.67 ± 1.67^{a}	98.33 ± 1.67^{a}	93.33 ± 1.67^{a}	98.33±1.67^{a}

Values with different superscripts within a row are significantly different ($P < 0.05$); mean ± S.E.

Table 3: Body indices of hybrid grouper fed different dietary treatments

	Experimental Diets				
	50P20S	**45P25S**	**45P30S**	**40P25S**	**40P30S**
VSI	8.73 ± 0.20^{a}	10.71 ± 0.28^{b}	9.88 ± 0.64^{ab}	9.07 ± 0.53^{a}	10.0 ± 0.16^{ab}
HSI	1.09 ± 0.09^{a}	1.08 ± 0.07^{a}	1.09 ± 0.17^{a}	0.97 ± 0.07^{a}	1.05 ± 0.04^{a}
IPF	1.76 ± 0.17^{a}	2.11 ± 0.03^{a}	2.05 ± 0.19^{a}	1.88 ± 0.44^{a}	2.08 ± 0.10^{a}
CF	1.93 ± 0.02^{a}	1.89 ± 0.01^{a}	1.93 ± 0.01^{a}	1.94 ± 0.08^{a}	1.92 ± 0.01^{a}

Values with different superscripts within a row are significantly different ($P < 0.05$); mean ± S.E.

followed by Diets 45P25S (12.83%), 50P20S (12.62%), 40P30S (12.32%), and 40P25S (11.98%). The liver lipid deposition was significantly higher in fish-fed Diets 45P30S (7.59%) and 45P25S (6.97%) than in other diets (5.21–5.89%). Unlike in the muscle, the glycogen content in the liver was somehow affected by the dietary treatments. The dietary treatments also affected the moisture, protein, and lipid contents of muscle. The Diet 50P20S produced fish with significantly lower muscle moisture (78.37%) and significantly higher muscle protein (18.65%) than other experimental diets. Significantly lower muscle lipid was observed in fish-fed Diets 50P20S (1.04%) and 45P30S (1.13%) than in other diets (Table 4).

Biochemical Parameters

Table 5 shows that cholesterol and glucose were affected, but triglyceride and total protein were not by different protein and carbohydrate levels. Diets with lower protein content (40% and 45%) resulted in lower cholesterol content when compared with the control diet containing 50% protein. The cholesterol content was significantly higher in Diet 50P20S (6.73 mmol L^{-1}), followed by Diets 45P30S (5.37 mmol L^{-1}), 40P25S (5.10 mmol L^{-1}), 45P25S (4.93 mmol L^{-1}), and 40P30S (4.50 mmol L^{-1}). Irrespective of protein level, glucose content decreased with the increase in carbohydrate level. Diet 45P25S yielded the highest blood glucose (1.17 mmol L^{-1}) and the lowest in Diet 40P30S (0.77 mmol L^{-1}). Triglyceride and total protein ranged between 1.87-2.20 mmol L^{-1} and 34.67-36.67 mmol L^{-1}, respectively.

Haematological Parameters

Mean corpuscular haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and

Table 4: Proximate compositions of whole-body, liver, muscle and glycogen contents in liver and muscle of hybrid grouper (% of wet weight)

	Experimental Diets				
	50P20S	**45P25S**	**45P30S**	**40P25S**	**40P30S**
Whole-body composition					
Moisture	72.63 ± 0.23^{a}	72.50 ± 0.74^{a}	72.55 ± 0.25^{a}	71.48 ± 0.78^{a}	72.09 ± 0.61^{a}
Protein	16.63 ± 0.11^{bc}	16.50 ± 0.11^{ab}	16.26 ± 0.09^{a}	16.93 ± 0.10^{c}	16.31 ± 0.09^{ab}
Lipid	5.39 ± 0.18^{a}	5.63 ± 0.09^{ab}	5.53 ± 0.06^{ab}	5.79 ± 0.07^{b}	5.47 ± 0.06^{ab}
Ash	5.09 ± 0.08^{b}	4.79 ± 0.04^{a}	4.78 ± 0.0^{a}	5.13 ± 0.09^{b}	5.18 ± 0.06^{b}
Liver composition					
Moisture	63.49 ± 0.23^{a}	63.53 ± 1.28^{a}	64.30 ± 1.93^{a}	66.96 ± 0.80^{a}	64.70 ± 1.42^{a}
Protein	12.62 ± 0.13^{c}	12.83 ± 0.02^{cd}	13.04 ± 0.08^{d}	11.98 ± 0.08^{a}	12.32 ± 0.06^{b}
Lipid	5.69 ± 0.26^{a}	6.97 ± 0.16^{b}	7.59 ± 0.27^{b}	5.21 ± 0.33^{a}	5.89 ± 0.18^{a}
Glycogen	0.28 ± 0.03^{ab}	0.28 ± 0.03^{ab}	0.32 ± 0.02^{b}	0.25 ± 0.04^{ab}	0.22 ± 0.02^{a}
Muscle composition					
Moisture	78.37 ± 0.15^{a}	79.84 ± 0.14^{b}	80.16 ± 0.40^{b}	79.34 ± 0.28^{b}	79.45 ± 0.27^{b}
Protein	18.65 ± 0.07^{d}	17.48 ± 0.13^{b}	17.04 ± 0.01^{a}	17.77 ± 0.09^{c}	17.81 ± 0.07^{c}
Lipid	1.04 ± 0.12^{a}	1.58 ± 0.10^{b}	1.13 ± 0.06^{a}	1.55 ± 0.06^{b}	1.67 ± 0.03^{b}
Glycogen	0.06 ± 0.02^{a}	0.06 ± 0.01^{a}	0.07 ± 0.02^{a}	0.06 ± 0.01^{a}	0.05 ± 0.01^{a}

Table 5: Biochemical parameters of hybrid grouper fed different experimental diets

	Experimental Diets				
	50P20S	**45P25S**	**45P30S**	**40P25S**	**40P30S**
Cholestrol (mmol L^{-1})	$6.73 \pm 0 .09^b$	4.93 ± 0.19^a	5.37 ± 0.60^a	5.10 ± 0.20^a	4.50 ± 0.62^a
Triglyceride (mmol L^{-1})	2.17 ± 0.20^a	1.87 ± 0.09^a	2.20 ± 0.06^a	2.03 ± 0.12^a	2.00 ± 0.10^a
Glucose (mmol L^{-1})	1.03 ± 0.09^{ab}	1.17 ± 0.07^b	0.83 ± 0.12^a	0.90 ± 0.06^{ab}	0.77 ± 0.09^a
Total Protein (mmol L^{-1})	34.67 ± 0.33^a	35.67 ± 0.88^a	35.0 ± 1.15^a	36.67 ± 0.33^a	35.33 ± 1.20^a

Values with different superscripts within a row are significantly different ($P < 0.05$); mean ± S.E.

Table 6: Haematological parameters of hybrid grouper at the end of feeding trial

	Experimental Diets				
	50P20S	**45P25S**	**45P30S**	**40P25S**	**40P30S**
Haemoglobin (%)	4.97 ± 0.23^a	5.80 ± 0.25^b	5.67 ± 0.19^b	5.20 ± 0.00^{ab}	4.93 ± 0.15^a
RBC ($X10^{12}$ L^{-1})	1.07 ± 0.27^a	1.40 ± 0.30^a	1.53 ± 0.24^a	1.37 ± 0.19^a	1.40 ± 0.12^a
PCV	0.20 ± 0.05^a	0.26 ± 0.06^a	0.28 ± 0.03^a	3.34 ± 0.10^a	0.27 ± 0.02^a
MCH (pg)	59.50 ± 2.60^b	35.00 ± 2.52^a	38.67 ± 4.33^a	0.25 ± 0.04^a	35.00 ± 2.08^a
MCV (fl)	178.50 ± 6.64^a	183.33 ± 4.98^a	182.67 ± 9.21^a	36.00 ± 2.08^a	192.0 ± 3.06^a
MCHC (g L^{-1})	344.0 ± 7.51^c	176.33 ± 1.86^a	229.5 ± 9.53^b	177.5 ± 2.60^a	190.5 ± 3.75^a
Platelet count ($X10^9$ L^{-1})	13.67 ± 1.45^a	13.33 ± 2.73^a	14.67 ± 3.71^a	9.0 ± 2.31^a	16.0 ± 2.31^a
Total WBC[6] ($X10^9$ L^{-1})	520.75 ± 6.78^b	555.45 ± 6.44^c	606.60 ± 8.26^d	562.4 ± 9.01^c	489.90 ± 4.95^a

Values with different superscripts within a row are significantly different ($P < 0.05$); mean ± S.E.

total white blood cell (WBC) were affected by the different protein and carbohydrate levels (Table 6). No significant difference among the haemoglobin values in Diets 45P25S (5.80%), 45P30S (5.67%), and 40P25S (5.20%) but haemoglobin was significantly lower in Diets 50P20S (4.97%) and 40P30S (4.93%). The Diet 50P20S has significantly higher MCH (59.50 pg) than other tested diets (35.00–38.67 pg). The significantly higher MCHC was observed in Diet 50P20S (344.0 gL^{-1}), followed by Diets 45P30S (229.5 gL^{-1}), 40P30S (190.5 gL^{-1}), 40P25S (177.5 gL^{-1}), and 45P25S (176.33 gL^{-1}). The total WBC was significantly higher in Diet 45P30S (606.6) and significantly lower in Diet 40P30S (489.9). Meanwhile, red blood cell (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), and platelet counts were independent of different protein and carbohydrate levels in the diet.

Digestibility of Diets

The different dietary protein and carbohydrate levels influenced the apparent digestibility coefficients (ADC) of dry matter, protein, and lipid. ADC for dry matter, protein, and lipid ranged from 39.85–65.96%, 88.12–91.85%, and 95.51–97.89%, respectively. Diet 40P25S yielded the lowest values of ADC for dry matter, protein, and lipid (Table 7).

Discussion

Generally, the growth of experimental fish was not significantly affected when the protein level was reduced from 50–40% and the carbohydrate level was increased from 20–30%. Previous studies also reported similar findings on grouper species. For example, diets with 7–28% corn starch inclusion fed to the fish were not affecting the fish growth and it was suggested that starch inclusion could reduce protein input (Luo *et al.*, 2016). In a study using Malabar grouper (*E. malabaricus*, Bloch Schneider, 1801), protein level was reduced from 46–42% by increasing starch level from 19.5–24.6% with findings of no influence on weight gain and feed efficiency, which also suggested a protein sparing effect (Shiau & Lin, 2001). The ability of carbohydrates to spare protein was also reported in other fish species such as Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758) (Shiau & Peng, 1993; Wang *et al.,* 2017) and blunt snout sea bream (*Megalobrama amblycephala*, Yih, 1955) (Li *et al.*, 2015). In this study, hybrid grouper showed a better protein efficiency ratio (PER) at the low protein level with an increasing level of carbohydrates. Irrespective of protein level, PER tends to increase with the increase in carbohydrate level from 25–30%. Protein-sparing effect of carbohydrates was also observed in a study conducted by Ye *et al.* (2009) at the same protein level, and the PER increased as the carbohydrate level increased. In other species such as yellow catfish (*Pelteobagrus fulvidraco*, Richardson, 1846) and silver barb (*Puntius gonionotus*, Bleeker, 1849), high PER was observed when fish fed with a low protein diet, indicating that protein was efficiently utilised for fish growth (Mohanta *et al*., 2007; Ye *et al.,* 2009). The growth promotion and protein-sparing of carbohydrates in fish may be related to the fact that glucose is the preferred oxidative substrate for nervous tissue and blood cells, and carbohydrates in fish diets can depress gluconeogenic activity, thus diverting amino acids away from oxidative pathways (Sánchez-Muros *et al*., 1996; Hemre *et al.,* 2002; Stone, 2003).

Whole-body protein and lipid of hybrid grouper decreased as the carbohydrate level increased regardless of the protein level of the diets. A similar effect was also observed in muscle lipids. When dietary lipid was set at a constant level, an increase in whole-body lipid deposition was related to an increase in carbohydrate level and indicated that lipid was synthesised from carbohydrates (Erfanullah & Jafri, 1995; Amin *et al.,* 2014). However, the opposite effect observed in the present study denoted that an increase in carbohydrate level did not produce undesirable lipid accumulation because of the *de novo* synthesis of fatty acids (Zhou *et al.,* 2015). The reduced lipid deposition because of increased carbohydrates in the diets was also following findings in other species such

Table 7: Apparent coefficient digestibility (ADC) of dry matter, protein, and lipid of hybrid grouper

Treatments	ADC Dry Matter (%)	ADC Protein (%)	ADC Lipid (%)
50P20S	65.96 ± 2.39^{c}	91.19 ±0.57^{b}	97.0 ± 0.55^{bc}
45P25S	59.56 ± 1.75^{bc}	91.85 ± 0.09^{b}	96.47 ± 0.22^{ab}
45P30S	55.46 ± 3.20^{b}	92.19 ± 1.11^{b}	96.10 ± 0.42^{ab}
40P25S	39.85 ± 3.37^{a}	88.12 ± 0.91^{a}	95.51 ± 0.49^{a}
40P30S	51.02 ± 3.21^{b}	91.49 ± 0.56^{b}	97.89 ± 0.07^{c}

Values with different superscripts within a row are significantly different ($P < 0.05$); mean ± S.E.

as turbot (*Scophthalmus maximus*, Linnaeus, 1758*)* and grass carp *(Ctenopharyngodon idella*, Valenciennes, 1844*)* (Nijhof & Bult, 1994; Gao *et al.,* 2010).

In the present study, liver protein and lipid increased with the increase of carbohydrate levels at the same protein level. Similarly, Ye *et al.* (2009) also reported that the liver lipid of yellow catfish, *P. fulvidraco* increased as the carbohydrate level was increased at the same protein level. This trend of glycogen content showed that the absorbed carbohydrate that was not used as energy was deposited in the liver as glycogen after being converted (Wu *et al*., 2007; Moreira *et al.,* 2008).

Blood serum glucose was reduced when the carbohydrate level was increased to 30% in the present study. This is in contradiction with previous studies on other fish species, including Atlantic salmon (*Salmo salar*, Linnaeus, 1758) and rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792*),* which suggested that blood glucose increased with an increased level of starch in the diets (Hemre *et al.,* 1995; Hemre & Krogdahl, 1996). In Atlantic salmon *(S. salar)*, the increase in plasma glucose without any glycosylation of haemoglobin indicated no strict regulation and limited transfer of glucose from plasma to cell utilisation (Hemre *et al.,* 1995). However, in a similar study on European whitefish (*Coregonus lavaretus*, Linnaeus, 1758), no differences in plasma glucose between fish fed a carbohydrate-free diet and a diet with 33% corn starch after rapid water cooling-induced stress (Vielma *et al.,* 2003). Meanwhile, dietary starch in Atlantic salmon and *S. salar* did not correlate with serum glucose (Waagbø, 1994). Irrespective of protein level, the cholesterol increased with the increase in carbohydrate level in the present study. According to Hemre *et al.* (1995), high dietary carbohydrate levels did not influence serum cholesterol, whilst serum protein was negatively correlated to dietary carbohydrate (positively correlated with dietary protein level) in adult salmon. An increase in plasma cholesterol with dietary carbohydrate levels suggested more active lipid synthesis and transport due to high carbohydrate levels (Chen *et al.*, 2012; Zhou *et al.,* 2015).

The haematological parameters of fish are known to be affected by a range of factors, including species, size, age, physiological status, environmental conditions, and dietary regime (Houston, 1997; Lim *et al.*, 2000; Sowunmi, 2003; Gabriel *et al.,* 2007). The RBC, MCV, MCH, and MCHC are haematological indices that indicate the erythrocyte status and oxygen-carrying capability in fish (Houston, 1997). The fish tissue received a certain amount of oxygen depending on the maturity of the erythrocytes and their haemoglobin content which helps detect any changes in fish health conditions (Goda, 2008). In the present study, the dietary starch has some influence on the haematological parameters of hybrid grouper, even though no clear trend was observed.

Atlantic salmon *(S. salar)* fed moist diets containing increasing wheat starch from 0 – 30% was negatively correlated with increasing wheat starch level and decreased blood haemoglobin concentration (Waagbø *et al.,* 1994). The significant reduction in haemoglobin, MCV, and MCH with increasing starch levels in the fish diet may be due to the increased ‘left-over’ starch in the fish intestine as dietary starch increases, which may have disturbed the absorption of iron (Hemre *et al.,* 1995*)*. Although the reference value for a haematological profile of fish species has not been fully defined and the changes in the parameter tested were not well-characterised, the haematological analysis may prove to be an important tool to monitor the physiological status in response to dietary treatments, environmental changes, and stress (Shalaby *et al.,* 2006).

The ADC of protein and lipids are known to be associated with the composition of amino acids and fatty acids (Carter *et al*., 2001; Suryaningrum *et al*., 2017). In this study, high values of ADC of protein (88.12–92.19%) and lipid (95.51–97.89%) showed the ability of hybrid grouper to digest fishmeal, which is consistent with their carnivorous nature.

High carbohydrate levels in diets reduced the protein and lipid digestibility in juvenile cobia *(Rachycentron canadum,* Linnaeus, 1766*)*, white seabream (*Diplodus sargus*, Linnaeus, 1758), and European sea bass *(Dicentrarchus labrax*, Linnaeus, 1758) (Enes *et al*., 2006; Sá *et al.*, 2007; Ren *et al*., 2011) which was not observed in the present study. Meanwhile, lower ADC of dry matter (39.85%) in Diet 40P25S (which contained higher ash than other diets) was observed compared to other tested diets in the present study. A previous study reported that the high ash content of diet ingredients could reduce ADC of dry matter (Bureau *et al*., 1999). The reduction in ADC of dry matter is possibly due to the presence of fibres, sugars or non-starch polysaccharides, which can affect the gastrointestinal transit time of feed (Lupatsch *et al*., 1997; Storebakken *et al*., 1999). It can also be affected by the quantity and chemical composition of the carbohydrate used, nutritional composition, presence of minerals and the percentage in feed formulation also contributed to low ADC of dry matter (Lee, 2002; Suryaningrum *et al*., 2017).

Conclusion

Reducing protein from 50 to 40% and increasing carbohydrates from 20% to 30% did not significantly affect the growth of the fish, but the digestibility of diets was compromised. Hybrid grouper shows some protein-sparing ability, especially when protein was reduced from 50% to 45% and carbohydrate was increased from 20% to 30%. The blood profile of hybrid grouper obtained in the present study can contribute some baseline data for reference in the future. In general, dietary treatments have some effects on haemoglobin, MCH, MCHC and total WBC of hybrid grouper. The present study has also provided valuable knowledge for the aquaculture feed industry to reduce feed cost and environmental impact by reducing the protein component in the diet dependent on the expensive fish meal.

Acknowledgements

This research work was funded by a research grant awarded by the Ministry of Higher Education, Malaysia, under the Niche Research Grant Scheme (NRGS 0004).

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