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Crocodile meat meal as a fishmeal substitute in juvenile dusky kob (*Argyrosomus japonicus*) diets: Feed utilization, growth performance, blood parameters, and tissue nutrient composition

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ABSTRACT

Overreliance on fishmeal (FM) as an aquafeed ingredient has become economically and ecologically unsustainable because wild stocks of forage fish are declining causing disruptions in aquatic food webs. On the other hand, the crocodile skin business generates substantial quantities of crocodile meat whose demand for human consumption is extremely low. The potential value of crocodile meat meal (CMM) as a FM alternative in fish diets is unknown. Therefore, this short-term, preliminary study investigated the effect of replacing FM with raw or cooked CMM on feed utilization, growth performance, haemato-biochemical parameters, and tissue nutrient composition in juvenile dusky kob (*Argyrosomus japonicus*, Temminck and Schlegel, 1843). Diets were formulated by replacing FM in a commercial diet (control) with 1. cooked CMM at 50 % (CCR50), 2. raw CMM at 50 % (RCR50), 3. raw CMM at 100 % (RCR100), and 4. cooked CMM at 100 % (CCR100). Fingerlings (7.55 ± 0.87 g) were offered diets at 2.8 % body weight, twice daily, in a recirculating aquaculture system (20 tanks; 110 fish/tank) for 5 weeks. Weight was measured weekly while blood and fillet samples were collected in week 5. Complete replacement of FM with CMM significantly reduced feed intake, weight gain, specific growth rate (SGR), and protein efficiency ratio (PER) while increasing FCR over the 5-week period. The RCR100 and CCR100 diets also resulted in higher levels of urea, alkaline phosphatase, and lower triglycerides in the serum of fish. Complete replacement of FM with CMM increased palmitoleic and oleic acids in fish muscle compared to the control. Regardless of level of FM substitution, CMM had an adverse impact on linoleic acid, linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid concentration of the dusky kob fillet. A supplementation strategy using oils rich in *n*-3 fatty acids could mitigate the negative impact of dietary CMM on feed utilization, growth performance, and polyunsaturated fatty acid levels in dusky kob fillet without raising economic and ecological costs.

1. Introduction

Fishmeal (FM) and fish oil are major ingredients in diets of many farmed fish (Yones and Metwalli, 2015). In South Africa, these valuable aquafeed components are produced from captured wild forage fish (anchovies, and pilchards) whose other role is to sustain a productive marine ecosystem as a principal food source for bigger fish, seabirds, and marine mammals (Engelhard et al., 2014). However, wild fish stocks have started to decline over the last few years due to climatic changes (Tourre et al., 2007) and overfishing driven by rising demand for FM and fish oil (Olsen and Hasan, 2012). Overfishing depletes targeted and non-targeted fish species, which negatively modifies the structure and function of marine ecosystems (Myers and Worm, 2005). To compound matters, FM is also used as a feed ingredient for other food animals,

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which increases its demand and drives market prices. High financial and ecological costs of FM have driven the search for alternative protein sources that could improve the sustainability of farmed fish. Indeed, terrestrial protein sources such as insect meals (Belforti et al., 2015; Belghit et al., 2018) and poultry by-products (Karapanagiotidis et al., 2018) have been evaluated as FM alternatives in carnivorous fish diets with mixed results. Crocodile meat meal (CMM), a co-product of the lucrative crocodile skin business, is another novel terrestrial protein that could be used as an alternative to FM in aquafeeds.

Globally, crocodiles are predominantly farmed for their skin that is used to make fashion accessories as well for their tail meat that is preferred for human consumption (Ashton, 2010). Global statistics on the production of crocodile meat are often understated and unreliable, however, an estimated 1 000 tons of crocodile meat were officially traded internationally in 2015 (Caldwell, 2017). This figure represents meat that is only used for human consumption, which is usually the tender tail fillet that constitutes 33 % of the empty carcass weight (Hoffman et al., 2000). This suggests that 2000 tons (67 %) of crocodile meat remains on farms worldwide and could be converted into CCM for use in carnivorous fish diets. In South Africa, local demand for crocodile meat for human consumption is very limited resulting in farmers discarding large quantities of this co-product (Personal communication: Pit Süssmann, Managing Director, Izintaba Farm Crocodile (Pty) Ltd, 25 November 2011). The meat is a good source of proteins, lipids, minerals, and vitamins (Cernikova et al., 2015) and could be used to replace FM in farmed fish diets. Apart from a comparative analysis of the proximate composition of Nile crocodile meat and FM by Luthada-Raswiswi et al. (2019), we have not found any published studies that evaluate crocodile meat as a novel dietary ingredient for fish. Using crocodile meat as an alternative source of protein for carnivorous fish such as the dusky kob could be an ingenious strategy that adds value to the crocodile farming enterprise while ensuring economically and environmentally sustainable dusky kob aquaculture. However, in farmed fish, the nutrient composition of fillet is highly dependent on diet composition, among other factors such as species, temperature, and water salinity (Xu et al., 2020). Fish are an irreplaceable source of amino acids, fats, vitamins, and minerals for human nutrition (Bogard et al., 2015). In particular, fish of marine origin have higher levels of *n*-3 long chain polyunsaturated fatty acids (PUFA) compared to terrestrial animal products (Tacon and Metian, 2013). Long chain *n*-3 PUFA such as docosahexaenoic acid (DHA, 22:6*n*-3) and eicosapentaenoic acid (EPA, 20:5*n*-3) have a well-documented role in the prevention of metabolic disorders, cancer, and cardiovascular diseases in humans (Gill et al., 2012). Replacing FM with CMM has the potential to alter the nutrient profile of fish fillet and thus affect the nutrition and health of fish consumers. Therefore, this study was designed to determine the effect of complete or partial (50 %) replacement of dietary FM with raw or cooked CMM on feed utilization, growth performance, haematology, serum biochemistry, and tissue nutrient composition in juvenile dusky kob (*Argyrosomus japonicus*, Temminck and Schlegel, 1843), an economically important aquaculture fish in South Africa. The study tested the hypothesis that replacing FM with CMM would not compromise feed utilization, growth performance, haematology, serum biochemistry, and tissue nutrient composition in juvenile dusky kob.

2. Material and methods

2.1. Ethical statement

This study was conducted in accordance with South African Animals Protection Act, 1962 (Act 71 of 1962) and was approved (FANS17) by the Animal Research Ethics Committee of the University of Mpumalanga.

2.2. Preparation of crocodile meat meal

The handling of live Nile crocodiles was conducted in compliance with the South African Animals Protection Act, 1962 (Act 71 of 1962). Nile crocodiles reared on a chicken-based diet at Lalele Crocodile Farm (Mookgopong, South Africa) were slaughtered at 4 years of age (12 ± 3 kg liveweight) to harvest skins and tails (primary products). The animals were exposed to a low voltage electrical shock to immobilize them. Once immobile, a 0.22 subsonic velocity cartridge fired from a silenced rifle at point blank was used to displace the brain through the back of the cranial platform to prevent any movement and suffering of the animal in accordance with the South African National Standard, SANS 631:2009 (SABS, 2009). With the aid of a sharp knife, left and right veins of the neck and the spinal cord were severed, and the crocodiles were left to bleed out for 10 min. Meat from the torso, legs, and neck was deboned, minced, mixed, and used to prepare CMM. To prepare cooked CMM, half of the minced raw crocodile meat was vacuum packed in a polyethylene plastic bags before being placed in a 75 °C water bath and left to cook for 55 min as described by Luthada-Raswiswi et al. (2019). Both the cooked and raw crocodile meat was spread evenly on plastic sheets and exposed to a crossflow air current using household electric floor fans in a ventilated room at 25 °C and dried to a constant mass. The dried CMM was homogenized into a powder using a blender before being incorporated into experimental diets.

2.3. Experimental diets

Dietary treatments were hand prepared at the Marine Research Aquarium, Cape Town, South Africa. Except for CMM, all ingredients (FM, corn starch, cellulose, and premix) were purchased from SA Feed (Pty) Ltd, Western Cape, South Africa. Five diets were prepared (Table 1) as follows: 1. dusky kob commercial diet in which only FM was the main protein source (control), 2. dusky kob diet in which all FM was replaced with raw CMM (RCR100), 3. dusky kob diet in which all FM was replaced with cooked CMM (CCR100), 4. dusky kob diet in which 50 % FM was replaced with cooked CMM (CCR50), and 5. dusky kob diet in which 50 % of FM was replaced with raw CMM (RCR50). The mixture of ingredients was moistened with water and kneaded to produce a dough that was rolled on a plastic sheet into a thin layer. The thin layer of paste was dried off to constant mass and the dried paste was flaked using a maize kernel hand grinder (Tafelberg, Cape Town) with and adjustable pressure disc. The five diets were then sampled for analysis of proximate composition, amino acids, and fatty acids profiles. The methods used for these analyses are described in Section 2.7 below. The formulations, proximate composition and amino acid profile of the control and experimental diets are shown in Table 1. The fatty acid composition of the diets is presented in Table 2.

2.4. Facilities and feeding trial

The experimental system used was a recirculating aquaculture system (RAS) consisting of twenty 465 L black high density polyethylene tanks, whose components and function have been described by Madibana and Mlambo (2019). Water temperature, dissolved oxygen concentration, and salinity were monitored using a YSI multiparameter instrument (YSI Incorporated, USA) and maintained at 25 °C, 6 mg/L, and 34 ppt, respectively. Ammonia concentration was monitored twice weekly using a Sera ammonium/ammonia test kit (North Rhine-Westphalia, Germany) and was consistently below 0.05 mg/L throughout the study period. A total of 2200 dusky kob fingerlings with an average mass of 7.55 ± 0.87 g were sourced from Kingfish Enterprises PTY LTD, East London, South Africa. They were transported in 950 L of sea water with 28 ppt salinity and a temperature range of 25.3–26.6 °C. Pure oxygen was injected at a rate of about 11 mg/L and the water was recirculated through two degassing towers. Upon arrival the fish were acclimatized to diets and experimental tanks for a week before the

Table 1
Formulation, proximate composition, and amino acid composition of control and crocodile meat meal-containing treatment diets.

	Diets[1]				
	Control	RCR50	CCR50	RCR100	CCR100
Ingredients (g/kg)					
Fishmeal	545	272.5	272.5	0	0
Raw crocodile meat	0	272.5	0	545	0
Cooked crocodile meat	0	0	272.5	0	545
Corn starch	280	280	280	280	280
Cellulose	95	95	95	95	95
Premix[2]	80	80	80	80	80
Proximate composition					
Dry matter (g/kg)	939.9	958.1	961.0	976.3	982.2
Ash (g/kg)	86.3	55.7	55.3	25.0	20.3
Crude protein (g/kg)	476.6	478.9	483.8	481.2	481.0
Gross energy (Kcal/kg)	1986.0	2002.8	2005.5	2019.5	2025.0
Crude fat (g/kg)	252.4	259.0	248.6	265.6	244.7
Essential amino acids (g/100 g DM)					
Arginine	3.54	4.38	4.08	5.21	4.62
Threonine	1.66	2.02	1.97	2.38	2.27
Methionine	0.71	1.03	1.02	1.35	1.33
Valine	2.47	2.86	2.76	3.25	3.04
Phenylalanine	1.93	2.12	2.05	2.30	2.17
Isoleucine	1.92	2.52	2.44	3.05	2.90
Leucine	3.42	3.73	3.73	4.03	4.03
Histidine	1.34	1.23	1.79	1.11	2.24
Lysine	2.92	4.42	4.16	5.91	5.40
Tryptophan	5.40	5.75	5.70	6.10	5.99
Non-essential amino acids (g/100 g DM)					
Alanine	2.43	2.67	2.49	2.99	2.63
Tyrosine	1.22	1.60	1.70	1.98	2.17
Proline	2.45	2.57	2.35	2.68	2.25
Serine	1.85	2.05	1.99	2.24	2.12
Aspartic acid	3.36	4.09	3.97	4.81	4.57
Glutamic acid	6.17	7.04	6.91	7.91	7.65
Glycine	2.68	3.43	2.83	4.18	2.98

[1] Diets: Formulated by replacing FM in a commercial diet (control) with cooked CMM at 50 % (CCR50); raw CMM at 50 % (RCR50); raw CMM at 100 % (RCR100); and cooked CMM at 100 % (CCR100).

[2] Premix: Vitamins/minerals mix composed of procaine HCI (15 mg) methylsulphonylmethane (MSM) (300 mg) lecithin (300 mg) alpha-tocopherol (vitamin E) (30 mg) thiamine HCI (vitamin B1, 10 mg) riboflavin (vitamin B2, 3 mg) pyridoxine HCI (vitamin B6, 3 mg), nicotinamide (10 mg), calcium pantothenate (10 mg), choline (40 mg), magnesium (100 µg), chromium (25 µg), zinc amino acid chelate (10 mg), inositol (30 mg), manganese (75 µg) and iron (5 mg).

Table 2
Saturated, mono-unsaturated, and poly-unsaturated fatty acid content (%) of fishmeal control and crocodile meat meal-containing treatment diets.

Fatty acids (%)	Diets[1]				
	Control	RCR50	CCR50	RCR100	CCR100
Saturated fatty acids					
Myristic acid	0.26	0.19	0.22	0.11	0.17
Palmitic acid	2.65	4.76	5.69	6.87	8.72
Stearic acid	0.62	1.17	1.40	1.72	2.17
Arachidic acid	0.00	0.02	0.03	0.04	0.05
Heneicosanoic acid	0.00	0.00	0.07	0.00	0.14
Tricosanoic acid	0.00	0.00	0.04	0.00	0.08
Mono-unsaturated fatty acids					
Palmitoleic acid	0.39	1.41	1.72	2.43	3.05
Oleic acid	4.80	9.65	11.85	14.50	18.89
Poly-unsaturated fatty acids					
Linoleic acid	2.81	3.41	4.06	4.01	5.30
Linolenic acid	0.32	0.27	0.31	0.21	0.30
cis-Eicosadienoic acid	0.00	0.03	0.04	0.05	0.08
Eicosatrienoic acid	0.00	0.12	0.15	0.23	0.30
Eicosapentaenoic acid	0.33	0.17	0.17	0.00	0.00
Docosahexaenoic acid	0.38	0.19	0.19	0.00	0.00

[1] Diets: Formulated by replacing FM in a commercial diet (control) with cooked CMM at 50 % (CCR50); raw CMM at 50 % (RCR50); raw CMM at 100 % (RCR100); and cooked CMM at 100 % (CCR100).

commencement of measurements. No artificial lighting was provided to prevent cannibalism. Each diet was randomly allocated to four replicate tanks (experimental units), each containing 110 fingerlings. Fish were hand-fed twice daily at 08:00 am and 15:00 pm at 2.8 % of their body weight, which has been previously determined as the optimum feeding intensity where juvenile dusky kob do not leave any feed refusals (Madibana and Mlambo, 2019). In each of the twenty tanks, fish were subjected to weekly measurements of body mass to determine the feed conversion ratio, weight gain and specific growth rate. No mortalities were recorded after the commencement of the feeding trial.

2.5. Calculations

Specific growth rate (SGR, % body weight gain/day) was calculated as follows:

$$SGR = \frac{In(final\ weight\ (week\ 5)) - In(Initial\ weight\ (week\ 1))}{Time\ intervals\ (days)} \times 100$$

Feed conversion ratio (FCR) was calculated as:

$$\mathrm{FCR} = \frac{Feed\ consumed\ (g)}{Fish\ weight\ gain\ (g)}$$

Protein efficiency ratio (PER) was calculated as:

$$\mathrm{PER} = \frac{Weight\ gain\ (g)}{Protein\ intake\ (g)}$$

2.6. Haematology and serum biochemical analyses

At the end of the feeding trial, blood was collected under anaesthesia from five fish, randomly selected from each tank. Fish were bled through the ablation of the caudal fin and blood separately collected into two types of tubes. Haematological analyses were performed using blood samples containing EDTA anticoagulant. Manual blood count (light microscope; [Olympus, Tokyo, Japan] under oil immersion at ×100 magnification) was performed for thrombocytes, lymphocytes, monocytes, neutrophils, basophils, and eosinophils. Haematocrit values were determined using the microhematocrit method from freshly sampled blood processed in microhematocrit centrifuge (NI 1807 Nova Instruments, Berkshire, United Kingdom) for 5 min at 10,000 rpm. Serum biochemical parameters were performed using blood samples that had been left to clot at room temperature and centrifuged for 15 min at 3500 rpm. Serum was pipetted into clear sterilized bottles for analysis. Alkaline phosphatase (ALP) activity was measured using the modified method of Wright et al. (1972), while aspartate aminotransferase (AST) and alanine transaminase (ALT) activities were determined according to the methods described by Reitman and Frankel (1957). Total protein (TP) was analyzed according to the Biuret method standardized for the RA-1000 (Technicon method no. SM4-0147K82, 1982). Albumin and creatinine were determined according to the Technicon method numbers SM4-0131K82 (1982) and SM4-0141K82 (1982), respectively. Total globulin fraction was determined by subtracting the albumin from the TP. A standard RA-1000 enzymatic method was applied for the analysis of triglycerides using the Boehringer Mannheim GPO-PAP kit. The monocholesterol (CHOD-PAP) method was used to determine cholesterol content. Urea content was estimated by following the Crest Biosystems Modified Berthelot method (Fawcett and Scott, 1960).

2.7. Analyses of diet and fish samples

At the end of the feeding trial, ten fish were randomly sampled from each tank to provide fillet for proximate, fatty acids, and amino acids analyses. The fillets were vacuum-packed in plastic bags and stored in a freezer pending tissue nutrient composition analysis. Experimental diets and fish fillets were analysed for dry matter, crude protein, crude fat, and ash according to standard AOAC (2005) methods. Dry matter (method no. 930.15) was determined by oven-drying the samples at 105 °C for 12 h and ash content (method no. 942.05) was determined after incineration at 550 °C for 12 h. Nitrogen (N) content was determined using the Kjeldahl method (method no. 976.05) and crude protein (CP) content was calculated as N × 6.25. Gross energy was determined using an adiabatic oxygen bomb calorimeter (Parr Instruments, Moline, IL, USA) calibrated with benzoic acid. Amino acid analysis was done after acid hydrolysis, pre-column derivatisation, separation by HPLC, and detection using a fluorescence detector based on a method originally described by Einarsson et al. (1983). Concentration of amino acids was expressed in g/100 g of sample. Lipids were extracted from the samples based on a method by Folch et al. (1957) and then methylated to fatty acid methyl esters (FAMEs) using BF_3 in methanol. FAMEs were quantitatively measured by capillary gas chromatography against C11:0. Pyrogallic acid was added to minimize oxidative degradation of fatty acids during analysis. Total fat was calculated as the sum of individual fatty acids expressed as triglyceride equivalents.

2.8. *Statistical analysis*

All the data collected were tested for normality using the NORMAL option in the Procedure Univariate statement before being subjected to statistical analysis. Data were also analysed for homogeneity of variance using Levene's test. Dietary effects on feed utilization, growth performance, blood parameters, and tissue nutrient composition were analysed using the general linear model procedure of SAS (2010). The probability of difference option was used to compare least squares means and significance level was set at $P \le 0.05$.

3. Results

3.1. *Dietary composition*

The control and experimental diets were similar in terms of dry matter, crude protein, crude fat, and gross energy content (Table 1). Dietary replacement of FM with CMM resulted in numerically higher concentration of all assayed essential amino acids except for histidine. Diets containing raw CMM had numerically lower histidine while those containing cooked CMM had higher histidine concentration than the control diet. Except for proline, CMM-containing diets had higher non-essential amino acid content than the control. All the diets had nearly identical proline concentration. Increasing dietary inclusion of CMM led to increases in palmitic, stearic, and arachidic acids (saturated fatty acids); palmitoleic acid and oleic acid (mono-unsaturated fatty acids); and linoleic, cis-eicosadienoic, and eicosatrienoic acids (poly-unsaturated fatty acids) in experimental diets (Table 2). Diets containing cooked CMM had higher levels of heneicasonic and tricosanoic acids (saturated fatty acids) compared to the control and raw CMM-containing diets, where these saturated fatty acids were not detected. The CMM-containing diets had lower eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids compared to the control diet. EPA and DHA were not even detected in diets where FM had been completely replaced with CMM. Partial replacement of FM with CMM resulted in diets with about half of EPA and DHA concentration as in the control diet.

3.2. *Feed utilization and growth performance*

No mortality was recorded in the five-week feeding period. Feed intake in juvenile dusky kob declined as the level of CMM inclusion increased (Table 3). Fish reared on the control diet had the highest ($P < 0.05$) feed intake followed by fish fed with CCR50. The lowest feed intake was observed in RCR50, RCR100, and CCR100 fish groups. As expected, a similar trend was observed for protein intake. Juvenile dusky kob reared on the control diet exhibited the highest ($P < 0.05$) weight gain followed by those fed on diets in which FM had been partially (50 %) replaced with either raw or cooked CMM. Fish fed on diets where FM had been completely replaced with raw or cooked CMM, exhibited the lowest weight gain. The SGR, FCR, and PER of fish were similar ($P > 0.05$) in fish reared on control, RCR50, and CCR50 diets, but these values were higher than in fish reared on RCR100 and CCR100 diets. No differences in feed utilization and growth performance parameters of juvenile dusky kob were observed between diets containing cooked and raw CMM, regardless of replacement levels (50 and 100 %).

Table 3

Effect of partial or total substitution of dietary fishmeal with raw or cooked crocodile meat meal on feed utilization and growth performance in juvenile dusky kob.

Diets[1]						
Parameters	Control	CCR50	RCR50	CCR100	RCR100	SEM[2]
Feed intake (g/fish)	21.14^{c}	18.20^{b}	17.52^{ab}	16.79^{a}	16.57^{a}	0.34
Protein intake (g/fish)	10.08^{c}	8.81^{b}	8.39^{ab}	8.24^{ab}	7.98^{a}	0.16
Weight gain (g)	25.75^{c}	20.02^{b}	20.24^{b}	13.47^{a}	13.63^{a}	0.66
SGR[3] (%)	3.38^{b}	3.08^{b}	3.10^{b}	2.36^{a}	2.36^{a}	0.09
FCR[4]	0.83^{a}	0.91^{a}	0.87^{a}	1.19^{b}	1.22^{b}	0.04
PER[5]	2.55^{b}	2.28^{b}	2.42^{b}	1.71^{a}	1.71^{a}	0.08

^{a,b,c}In a row, means with the same superscript do not differ ($P > 0.05$).

[1] Diets: Formulated by replacing FM in a commercial diet (control) with cooked CMM at 50 % (CCR50); raw CMM at 50 % (RCR50); raw CMM at 100 % (RCR100); and cooked CMM at 100 % (CCR100).

[2] SEM = Standard error of the mean.

[3] Specific growth rate = [ln (Final weight) −ln (Initial weight)] / time intervals (days)×100.

[4] Feed conversion ratio = feed intake (g) /fish weight gain (g).

[5] Protein efficiency ratio = fish weight gain (g) /protein intake (g).

3.3. *Haematology and serum biochemistry*

Diets had no effect ($P > 0.05$) on all the measured haematological parameters except for haematocrit (Table 4). Fish reared on the control diet had statistically similar ($P > 0.05$) haematocrit values as those on all the CMM-containing diets. The diet with a higher inclusion level of raw CMM (RCR100) promoted higher haematocrit compared to the one with lower inclusion level of cooked CMM (CCR50). Experimental diets did not affect ($P > 0.05$) creatinine, albumin/globulin ratio, and aspartate aminotransferase (AST) but affected ($P < 0.05$) urea, total protein, albumin, globulin, alanine aminotransferase (ALT), alkaline phosphatase, cholesterol, and triglyceride levels in the serum of fish (Table 4). Urea and alkaline phosphatase (ALKP) levels were highest in fish reared on RCR100 and CCR100 and lowest in control and RCR50 fish. The opposite was true for triglycerides levels. Diets in which FM had been completely replaced (RCR100 and CCR100) promoted higher ($P < 0.05$) serum urea and ALKP compared to those in which FM had been partially replaced (RCR50 and CCR50). With regards to albumin and total protein, all diets promoted statistically similar ($P < 0.05$) serum levels except for RCR100, which promoted higher ($P < 0.05$) levels of these metabolites. Control fish had statistically similar levels of serum globulin ($P > 0.05$) as all the other dietary groups except for CCR50 fish that had lower ($P < 0.05$) levels of the metabolite. No differences ($P > 0.05$) were observed for alanine aminotransferase (ALT) in fish reared on CCR100 and CCR50 diets. Similarly, fish reared on RCR100 and RCR50 had the same ($P > 0.05$) levels of ALT.

3.4. *Proximate composition and amino acid content of fish fillet*

Complete replacement of FM with CMM resulted in lower fillet ash content compared to the control (Table 5). Fish reared on RCR100 and CCR100 diets produced fillets with higher crude protein content compared to those fed on control, RCR50, and CCR50 diets. The control,

Table 4

Effect of partial or total substitution of dietary fishmeal with raw or cooked crocodile meat meal on some blood parameters of juvenile dusky kob.

Haematology	Diets[1]					
	Control	RCR50	CCR50	RCR100	CCR100	SEM[2]
Haematocrit (%)	38.95^{ab}	36.00^{ab}	32.93^{a}	42.90^{b}	40.71^{ab}	2.04
Thrombocytes (mm^3)	4.48	2.30	1.73	0.85	2.53	1.26
Lymphocytes (%)	77.0	81.5	87.5	83.5	84.0	4.41
Monocytes (%)	5.75	3.00	3.25	7.00	6.00	1.04
Neutrophils (%)	0.50	1.25	0.50	1.25	1.25	0.49
Basophils (%)	3.00	4.00	2.25	3.25	4.00	0.89
Eosinophils (10^3 cells/μL)	3.75	3.25	0.75	1.50	2.75	0.80
Serum biochemistry						
Urea (mmol/L)	1.50^{a}	2.05^{ab}	2.68^{b}	3.83^{c}	3.80^{c}	0.22
Creatinine (umol/L)	18.5	13.8	16.5	31.3	23.3	5.53
Total protein (g/L)	43.5^{a}	40.0^{a}	38.8^{a}	48.8^{b}	43.5^{a}	1.31
Albumin (g/L)	14.8^{a}	14.3^{a}	14.3^{a}	16.8^{b}	15.5^{ab}	0.48
Globulin (g/L)	28.8^{b}	25.8^{ab}	24.5^{a}	32.0^{b}	28.0^{ab}	1.02
Albumin:Globulin	0.58	0.58	0.58	0.50	0.58	0.03
Alanine aminotransferase (U/L)	74.8^{b}	41.3^{ab}	25.0^{a}	99.5^{b}	32.3^{a}	9.62
Aspartate aminotransferase (U/L)	144.3	91.5	101.8	208.0	108.0	31.19
Alkaline phosphatase (U/L)	39.8^{a}	62.3^{ab}	77.0^{b}	100^{bc}	112.5^{c}	6.94
Cholesterol (mmo/L)	3.35^{b}	2.66^{ab}	2.11^{a}	2.70^{ab}	2.71^{ab}	0.23
Triglycerides (mmol/L)	15.4^{b}	10.6^{ab}	7.06^{a}	9.57^{a}	7.33^{a}	1.26

[a,b] In a row, means with similar superscripts do not differ ($P > 0.05$).

[1] Diets: Formulated by replacing FM in a commercial diet (control) with cooked CMM at 50 % (CCR50); raw CMM at 50 % (RCR50); raw CMM at 100 % (RCR100); and cooked CMM at 100 % (CCR100).

[2] SEM = Standard error of the mean.

Table 5

Effect of partial or total substitution of dietary fishmeal with raw or cooked crocodile meat meal on the proximate composition (g/kg DM, unless stated otherwise) of dusky kob fillet.

Proximate composition	Diets[1]					
	Control	RCR50	CCR50	RCR100	CCR100	SEM[2]
Dry matter (g/kg)	967.2	974.4	964.4	972.4	966.4	4.89
Ash	137.3^{b}	115.8^{a}	136.9^{b}	100.9^{a}	103.0^{a}	4.6
Crude protein	477.5^{a}	517.3^{a}	528.5^{a}	555.3^{b}	535.8^{b}	14.3
Crude fat	267.3	266.4	249.6	259.3	255.0	4.5
Gross energy (kJ/100 g)	1948.25^{a}	1984.75^{a}	1920.50^{a}	2027.00^{b}	1972.00^{a}	17.88

[a,b,c] In a row, means with similar superscripts do not differ ($P > 0.05$).

[1] Diets: Formulated by replacing FM in a commercial diet (control) with cooked CMM at 50 % (CCR50); raw CMM at 50 % (RCR50); raw CMM at 100 % (RCR100); and cooked CMM at 100 % (CCR100).

[2] SEM = Standard error of the mean.

RCR50, and CCR50 fillets had statistically similar ($P > 0.05$) crude protein levels. Fillet from fish reared on RCR100 had higher gross energy content compared to fillet from the rest of the diets. The control, CCR50, RCR50, and CCR100 fillets had statistically similar ($P > 0.05$) gross energy content.

Fillet from fish fed diets containing 50 % CMM (RCR50 and CCR50) had lower ($P < 0.05$) arginine levels compared to those fed on diets containing 100 % CMM (CCR100 and RCR100) (Table 6). Threonine levels were higher in RCR100 and CCR100 fish compared to control and RCR50 fish. Fish reared on the control diet had lower ($P < 0.05$) methionine content compared to CCR50 and CCR100 fish. However, methionine content of control diet fillet was statistically similar ($P > 0.05$) to that in RCR50 and RCR100 fish. RCR100 diets promoted higher ($P < 0.05$) phenylalanine levels in fish compared to the control, RCR50, CCR50 and CCR100 diets, which promoted similar ($P > 0.05$) phenylalanine levels. Control fish had higher ($P < 0.05$) isoleucine compared to all CMM-containing diets, while the opposite was true for histidine. Complete replacement of FM with CMM (RCR100 and CCR100) produced fillets with lower ($P < 0.05$) tryptophan levels compared to the control. RCR100 diet promoted higher ($P < 0.05$) proline and alanine levels in fish compared to the control, RCR50, CCR50, and CCR100 diets while the opposite was true for tyrosine. Fish reared on RCR50 and CCR50 had lower ($P < 0.05$) serine levels compared to those reared on CCR100 and RCR100. The control diet promoted lower ($P < 0.05$) glutamic acid in fish compared to CCR50 and RCR100. Fillet from fish reared on RCR100 and CCR100 had higher glycine levels compared to fillets from control and RCR50 fish. Higher alanine levels were observed in fish reared on RCR100 compared to all the other diets. The other four diets promoted statistically similar alanine levels.

3.5. *Fatty acids*

Fish fed the control diet had higher ($P < 0.05$) myristic acid content compared to those fed on CCR50, RCR100, and CCR100 (Table 7). When included at 100 % level, both raw and cooked crocodile meat meal (RCR100 and CCR100) promoted higher palmitic acid and stearic acid compared to all other diets. No dietary influence was observed ($P > 0.05$) for arachidic acid content of fish. Control and RCR50 fish had statistically similar ($P > 0.05$) heneicosanoic acid and tricosanoic acid levels. Fish reared on control diet had lower palmitoleic, eicosatrienoic, and oleic acids content compared to fish reared on all CMM-containing diets. In contrast, control fish had the highest ($P < 0.05$) linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids followed by RCR50 and CCR50 fish while RCR100 and CCR100 fish had the lowest ($P < 0.05$) values.

Table 6
Amino acid content (g/100 g dry weight) of fillet from juvenile dusky kob reared on fishmeal and crocodile meat meal-containing diets.

	Diets[1]					
	Control	RCR50	CCR50	RCR100	CCR100	SEM[2]
Essential amino acids						
Arginine	3.37^{ab}	3.12^{a}	3.06^{a}	3.94^{b}	3.62^{b}	0.12
Threonine	1.77^{a}	1.74^{a}	1.85^{ab}	2.16^{b}	1.99^{b}	0.05
Methionine	1.18^{a}	1.26^{ab}	1.44^{b}	1.22^{ab}	1.54^{b}	0.08
Valine	2.60^{a}	2.67^{a}	2.75^{ab}	2.95^{b}	2.73^{ab}	0.06
Phenylalanine	1.82^{a}	1.91^{a}	1.86^{a}	2.11^{b}	1.94^{a}	0.04
Isoleucine	3.02^{b}	2.24^{a}	2.27^{a}	2.35^{a}	2.28^{a}	0.10
Leucine	3.15	3.00	3.06	3.26	3.15	0.08
Histidine	2.16^{a}	2.56^{b}	2.60^{b}	2.97^{c}	4.75^{d}	0.08
Lysine	3.75	3.54	3.67	3.45	3.94	0.13
Tryptophan	6.20^{b}	6.11^{ab}	5.93^{ab}	5.86^{a}	5.88^{a}	0.07
Non-essential amino acids						
Alanine	2.95^{a}	2.99^{a}	2.97^{a}	3.38^{b}	3.05^{a}	0.06
Tyrosine	1.51^{b}	1.66^{bc}	1.62^{bc}	1.11^{a}	1.72^{c}	0.04
Proline	2.46^{ab}	2.20^{a}	2.35^{ab}	2.87^{c}	2.51^{b}	0.07
Serine	1.78^{a}	1.70^{a}	1.77^{a}	2.17^{b}	2.42^{c}	0.04
Aspartic acid	13.10	3.77	3.91	4.19	4.08	4.16
Glutamic acid	6.10^{a}	6.19^{ab}	6.69^{b}	6.87^{b}	6.48^{ab}	0.15
Glycine	4.44^{a}	4.41^{a}	4.80^{ab}	5.33^{b}	4.98^{b}	0.11

^{a,b,c,d}In a row, means with similar superscripts do not differ ($P > 0.05$).

[1] Diets: Formulated by replacing FM in a commercial diet (control) with cooked CMM at 50 % (CCR50); raw CMM at 50 % (RCR50); raw CMM at 100 % (RCR100); and cooked CMM at 100 % (CCR100).

[2] SEM = Standard error of the mean.

4. Discussion

4.1. Feed utilization and growth performance

This study provides the first report on the effects of partial or complete replacement of FM with CMM on feed utilization efficiency, growth performance, blood parameters, and tissue nutrient content of farmed dusky kob. A related preliminary study only compared the proximate composition of CMM to that of FM (Luthada-Raswiswi et al., 2019). Results from the current study show that complete replacement of FM with either raw or cooked CMM significantly reduced feed intake and weight gain of fish. However, partial replacement of FM with either raw or cooked CMM did not compromise SGR, PER, and feed conversion efficiency while the opposite was true when FM was completely replaced with CMM. These findings suggest that, unlike FM, CMM was unable to meet the nutrient requirements of juvenile dusky kob. This could be attributed to lower feed intake, lower concentration of some essential nutrients, and/or low digestibility of the diets of CMM-containing diets. It is important to note that the concentration of essential and nonessential amino acids in CMM-containing diets was either similar or greater than that of the control diet suggesting that essential amino acids were not limiting.

Crocodile meat meal-containing diets had higher levels of saturated fatty acids (except for myristic acid), higher levels of mono-unsaturated fatty acids, and lower levels of eicosapentanoic (EPA) and docosahexanoic (DHA) acids. Indeed, of the three long chain polyunsaturated fatty acids (PUFA) (arachidic acid, EPA and DHA) required by fish for their growth and development (March, 1992), none were found in higher concentration in CMM-containing diets compared to the control. These three PUFAs are critical for the structural and functional integrity of cell membranes as well as in the biosynthesis of the highly biologically active paracrine hormones called eicosanoids in fish (Sargent et al., 1999). Thus, the low levels of EPA and DHA could be the reason why feeding CMM-containing diets resulted in poor feed conversion and growth performance of fish in this study. Supplemental sources of EPA and DHA can be evaluated as a strategy to enhance the value of CMM as a FM substitute in the dusky kob. On the other hand, the concentration of linoleic acid, linolenic acid and arachidic acid, also considered to be essential fatty acids for fish (March, 1992), in CMM-containing diets were similar (linolenic acid) or higher (linoleic and arachidic acids) than in the control diet. Cooking CMM was also of interest in the current study because heat-treatment often alters feed intake and nutrient utilization in animals (Li-Chan et al., 1985). However, cooking did not influence intake, weight gain, SGR, FCR, and PER in juvenile dusky kob, suggesting that heating did not sufficiently change the protein structure to affect feed utilization and growth performance. Heating has been reported to cause protein denaturation and modification of protein functional properties (Marin et al., 1992) but we found no effect of heating on the performance of dusky kob.

4.2. Blood parameters

Blood parameters are an effective tool for monitoring the nutritional, toxicity, and health status as well as other physiological problems in intensively farmed fish (Madibana et al., 2017). In the current study, experimental diets did not induce any significant variation in

Table 7
Saturated, mono-unsaturated, and poly-unsaturated fatty acid content (%) of fillet from juvenile dusky kob reared on crocodile meat meal-containing diets.

Fatty acids (%)	Diets[1]					
	Control	RCR50	CCR50	RCR100	CCR100	SEM[2]
Saturated fatty acids						
Myristic acid	0.31^{b}	0.23^{ab}	0.16^{a}	0.20^{a}	0.16^{a}	0.02
Palmitic acid	4.30^{a}	4.12^{a}	4.39^{a}	4.46^{b}	4.81^{b}	0.09
Stearic acid	1.37^{a}	1.47^{b}	1.59^{c}	1.66^{c}	1.75^{d}	0.02
Arachidic acid	0.01^{a}	0.01^{a}	0.00^{a}	0.02^{a}	0.04^{a}	0.01
Heneicosanoic acid	0.27^{a}	0.39^{b}	0.30^{a}	0.31^{ab}	0.27^{a}	0.02
Tricosanoic acid	0.03^{a}	0.09^{b}	0.06^{ab}	0.08^{b}	0.05^{ab}	0.01
Mono-unsaturated fatty acids						
Palmitoleic acid	0.97^{a}	1.84^{c}	1.68^{b}	2.18^{d}	2.24^{d}	0.04
Oleic acid	8.77^{a}	10.78^{bc}	10.19^{b}	10.77^{bc}	11.49^{c}	0.19
Poly-unsaturated fatty acids						
Linoleic acid	4.35^{c}	3.62^{b}	3.34^{b}	2.83^{a}	3.06^{ab}	0.08
Linolenic acid	0.42^{d}	0.24^{c}	0.20^{b}	0.14^{a}	0.15^{a}	0.01
cis-Eicosadienoic acid	0.04^{a}	0.09^{b}	0.04^{a}	0.05^{a}	0.05^{a}	0.004
Eicosatrienoic acid	0.06^{a}	0.17^{b}	0.17^{b}	0.21^{c}	0.21^{c}	0.01
Eicosapentaenoic acid	0.49^{c}	0.33^{b}	0.38^{b}	0.24^{a}	0.22^{a}	0.01
Docosahexaenoic acid	0.61^{c}	0.42^{b}	0.45^{b}	0.25^{a}	0.23^{a}	0.02

^{a,b,c,d}In a row, means with similar superscripts do not differ ($P > 0.05$).

[1] Diets: Formulated by replacing FM in a commercial diet (control) with cooked CMM at 50 % (CCR50); raw CMM at 50 % (RCR50); raw CMM at 100 % (RCR100); and cooked CMM at 100 % (CCR100).

[2] SEM = Standard error of the mean.

thrombocytes, lymphocytes, monocytes, neutrophils, basophils, and eosinophils counts but influenced the haematocrit level. Haematocrit levels, which measure the volume of red blood cells, were numerically higher in fish reared on diets where FM had been completely replaced with CMM compared to those on diets where FM had been partially replaced. The superiority of CMM-containing diets to the control in terms essential and non-essential amino acids could explain their observed positive influence on haematocrit levels in juvenile dusky kob. Indeed, the positive influence of protein nutrition on erythropoiesis has long been demonstrated in fish (Witeska, 2013). However, Karapanagiotidis et al. (2018) reported that feeding poultry by-product meal did not influence the haematocrit level of juvenile gilthead seabream (*Sparus aurata*). The haematological parameters reported in the current study fell within the normal range for dusky kob as reported by Madibana and Mlambo (2019).

Serum biochemical parameters have the potential to be used to diagnose any changes in the normal physiological conditions of fish due to disease (Patriche et al., 2011), toxicity or suboptimal nutritional status. In the current study, experimental diets had no significant effect on creatinine, albumin:globulin ratio, and aspartate aminotransferase (AST) levels in the serum. These findings were different from those of Abou-Daoud et al. (2014), who reported that AST activity increased in marbled spinefoot (*Siganus rivulatus*) as dietary protein decreased while alanine aminotransferase (ALT) activity was not affected by changes in dietary protein. However, the concentration of urea was higher in fish reared on diets containing the higher inclusion level of cooked or raw CMM (RCR100 and CCR100). Since the experiment was conducted in a recirculating aquaculture system equipped with biofilters, ammonia accumulation in tanks can be ruled out as the cause of the increased in blood urea. The essential and non-essential amino acid content of the CMM-containing diets tended to be much higher than that of the control diet, which could have resulted in excess circulating amino acids and blood urea levels in juvenile dusky kob. Excess amino acids are known to increase nitrogenous waste, impose an extra energy cost and retard growth in *Cyprinus carpio* Var. *Specularis* (Ahmed and Maqbool, 2017).

Results from this study show that total serum protein and albumin in fish reared on the control diet was similar to that in groups reared on diets in which FM had been partially replaced with CMM. Complete replacement of FM with CMM tended to increase both total protein and albumin levels in juvenile dusky kob, possibly because of higher dietary essential and non-essential amino acids in CMM-based diets. Indeed, total serum protein and albumin are known to vary with dietary protein in fish (Hrubec et al., 2001). However, these findings contradict those of Yones and Metwalli (2015) who reported no changes in serum total protein and albumin levels of Nile tilapia fed poultry by product meal. Cholesterol and triglycerides are important indices for health status of fish (Mohammed et al., 2019). An increase in cholesterol levels indicates disorders associated with lipoprotein and lipid metabolism that include liver disease (Zhou et al., 2009). In the current study, both cholesterol and triglycerides tended to be lower in fish reared on CMM-containing diets than the control group. In contrast, Zhu et al. (2011) observed that 50 % replacement of FM with poultry by-product meal had no effect on cholesterol but increased triglyceride levels of *Acipenser baerii*.

Evaluation of the two liver-bound enzymes, ALT and ALKP in fish is more complicated than in mammals. For instance, levels of ammonia may increase due to high levels of transaminase activities or vice versa (Azeez and Mohammed, 2017). In the current study, the two liver-bound enzymes responded differently to the presence of CMM diets. The control diet promoted higher levels of ALT compared to cooked CMM diets (CCR50 and CCR100) while ALKP was higher in fish reared on diets containing CMM regardless of inclusion level or heat treatment. Alkaline phosphatase levels reflect the integrity of the plasma membrane because it is a marker enzyme for plasma membrane (Adeyemi and Muhammad, 2010). Elevated levels of this enzyme indicate possible damage to the external plasma membrane (Madibana et al., 2020). Thus, an increase of ALKP in dusky kob fed CMM-containing diets may be an indication of compromised integrity of the plasma membranes. This is not surprising given that RCR50 and CCR50 diets had half the level of EPA and DHA as the control diet while RCR100 and CCR100 had no EPA and DHA at all. These PUFAs are responsible for the structural and functional integrity of cell membranes in fish (Sargent et al., 1999) hence their deficiency in CMM-containing diets could have compromised the integrity of plasma membranes.

4.3. Fillet proximate and amino acid composition

Fish is an irreplaceable animal protein source providing essential and often limiting nutrients of high bioavailability in human diets. These essential nutrients include protein, fat, mineral and vitamins (Desta et al., 2019). In the present study, replacing FM with CMM did not change crude fat content but influenced crude protein, ash, and gross energy levels in fish fillet. Fillets from fish fed on FM-free diets (CCR100 and RCR100) contained higher CP compared to those fed the control diet (Table 5). Fish fillet with higher crude protein content is desirable for humans because it is richer in peptides and essential amino acids, which are often limiting in plant-based human diets (Tacon and Metian, 2013). Complete FM replacement with CMM resulted in fillets with lower ash content compared to control fillets. This could have because the control diet had three times the ash content of CCR100 and RCR100 diets (Table 1). Fillet from juvenile kob fed on RCR100 had higher gross energy content compared to fillet from fish on all the other diets, a result that was unexpected.

Fishmeal is the preferred dietary protein source for fish due to its high protein content, balanced essential amino acids (EAA, Barroso et al., 2014) and high digestibility of EAA (Nguyen et al., 2009). However, amino acid availability does not only depend on the nature of the protein source but also on how it was processed prior to feeding (LjØkjel et al., 2000). Cooking, among other processing methods, can impact on the amount of digestible protein (Nunes et al., 2014). Histidine content in the fish fillet was high in CCR100 compared to the control and other CMM-containing diets. However, the findings were expected since the CCR100 diet had higher histidine content. Arginine content in the fillet was higher when fish were fed CCR100 and RCR100 diets compared to CCR50 and RCR50, also a reflection of the dietary levels of this amino acid. However, the higher dietary arginine levels had no effect on the growth performance of fish fed on CCR100 and RCR100 diets in concordance with findings reported by Renna et al. (2017) when feeding *Hermetia illucens L.* larvae meals to rainbow trout. Threonine, which was found to be higher in RCR100 and CCR100 diets, is an important EAA for fish growth and fillet yield (Michelato et al., 2015) because it is used in the de novo synthesis of non-essential amino acids such as serine and glycine (Michelato et al., 2015). In the current study, feeding RCR100 and CCR100 diets resulted in fillets with higher threonine and glycine content compared to feeding control and RCR50 diets. Fish fillet threonine content has been established to range between 5–18 g/kg for species such as rainbow trout and Atlantic salmon (Bondin et al., 2008); and catfish (Wilson et al., 1978), which is in line with the findings of the current study.

Like other fish species, the kob has requirements for total sulphur amino acids, which can be met by methionine (Fagbenro et al., 1999). In the current study, dusky kob fillets from fish reared on raw CMM-containing diets had similar methionine content as control fillets. However, fillet from fish fed cooked CMM-containing diets had higher methionine content compared to those from fish reared on the control diet. This could be explained by the higher level of methionine in cooked CMM-containing diets compared to the FM control diet. This shows that cooked CMM positively influenced methionine content of fish fillet compared to raw CMM. Valine cannot be synthesized by fish, so it must be ingested through the diet and it is often limiting (Bae et al., 2012). In the current study, fillet from the control diet had low valine content compared to RCR100, a reflection of the higher valine levels in CMM compared to FM. However, the valine content in the fillet was within the normal range for dusky kob (Adesola et al., 2017). The control diet promoted higher tryptophan content in the fillet compared to RCR100 and CCR100 diets, which reflects the high content of this amino acid in the control diet.

Although non-essential amino acids do not need to be part of fish diets (Belghit et al., 2018), they enhance fish performances and health when available in the right concentrations (Wu, 2013). Serine content in the fillet was higher when fish were fed CCR100 and RCR100 diets compared to CCR50 and RCR50, which is simply a reflection of dietary levels of this amino acid (Table 1). Similarly, fish reared on RCR100 diets produced fillets with lower levels of tyrosine compared to those reared on the FM control. However, the opposite was true for proline levels in fish fillet. The lower levels of tyrosine in fish fed RCR50 and RCR100 were not expected since the dietary tyrosine was higher in the raw CMM-containing diets compared to the control diet.

4.4. Fatty acids

All other factors constant, the fatty acid (FA) profile of fish fillets usually mirrors that of the diets consumed (Renna et al., 2017). The fillet from the control fish had higher myristic acid content compared to fillet from all the other diets, which can be explained by the control diet having twice as much myristic acid as FM-free diets. The current study findings contradict those of Renna et al. (2017), where a rise in lauric acid and myristic acid was observed when a trout was fed a terrestrial protein sources, black soldier fly, in place of FM. Terrestrial protein sources have been reported to increase the total saturated fatty acid content of fish fillet (Renna et al., 2017). Human epidemiological studies have shown that myristic acid is positively correlated with higher cholesterol levels in plasma, which could increase the risk of cardiovascular disease (Briggs et al., 2017). Therefore, the lower amount of myristic acid in the dusky kob fillet reared on CMM-containing diets could be beneficial to human health (Siddik et al., 2019). However, palmitic acid and stearic acid levels were higher in RCR100 and CCR100 compared to RCR50 and CCR50 fillets. As expected, the saturated fatty acids, heneicosanoic and tricosanoic acid, were lower in control compared to RCR50 fillet. Mono-unsaturated fatty acids (MUFA), palmitoleic and oleic acids, were higher in fish reared on CMM-containing diets compared to the FM control, a reflection of the MUFA fatty acid dietary levels, where CMM contained nearly five times as much palmitoleic and oleic acids as the FM.

Regarding polyunsaturated fatty acids (PUFA), fish reared on the control diet had higher levels of linoleic, linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) compared to fish fed CMM-containing diets. These findings were expected since the FM-based control diet had higher levels of linolenic, EPA, and DHA compared to CMM-containing experimental diets (Table 7). However, the FM-based control diet had lower linoleic acid content compared to CMM-containing diets even though it promoted higher linoleic acid content in the fillet. In addition, lipid-protein interactions might have negatively affected the tissue concentration of PUFA in fish fed CMM-diets. Likewise, inferior protein quality reduces the metabolism of EPA (Bouzaine et al., 1994), and low protein quality reduces plasma lipoprotein formation and lipid transport (Demonty et al., 2002). Sabbagh et al. (2019) also reported a decrease in EPA and DHA in fish muscle fed diets in which FM had been replaced with poultry by product meal. The observation in the current study that partial or complete replacement of FM with CMM resulted in fish with lower EPA and DHA levels corroborates Belforti et al. (2015), who reported significant reduction in EPA and DHA levels in fillet of trout fed *Tenebrio molitor* meal, a terrestrial protein source, in place of FM. The EPA and DHA are *n*-3 PUFA with well-known beneficial effects on human health through the prevention of metabolic disorders, cancer, and cardiovascular diseases (Gill et al., 2012). Indeed, fish is an important food source that seems to reduce heart disease incidences in humans due to its high levels of PUFA (Hossain, 2011). Unfortunately, complete replacement of FM with CMM resulted in ~50 % reduction in the levels of EPA and DHA in fish (Table 7), which is concerning considering the value of fish as a major dietary source of these fatty acids for humans. Partial replacement (50 %) of FM with CMM resulted in ~30 % reduction in EPA and DHA levels in juvenile kob fillet suggesting that lower inclusion levels of CMM could be the way forward. Supplementing CMM-based diets with PUFA-rich oils such as microalgae oil, could mitigate the observed adverse effects of CMM and should be explored in future studies.

5. Conclusion

This study provides new information on the potential use of CMM as a novel protein and lipid source in juvenile dusky kob diets. Dietary CMM reduced feed intake and growth performance, particularly when included as a complete replacement of FM. Lower EPA and DHA levels in CMM-containing diets could explain the suboptimal growth performance of juvenile dusky kob reared on these diets. Replacement of FM with CMM did not affect hematological and biochemical parameters but had an adverse effect on linolenic acid, EPA, and DHA concentration in juvenile dusky kob. The PUFA composition is one of the most significant attributes of fish for human nutrition, hence this finding is concerning. The use of supplemental PUFA sources such as microalgae oil, could mitigate the negative impact of feeding CMM on feed utilization, growth performance, and *n*-3 long chain PUFA concentration in the kob. Short- or long-term finishing of CMM-fed dusky kob with n-3-rich oils could be considered as a sustainable way of improving feed utilization, growth performance, and fillet nutrient composition in juvenile dusky kob reared on CMM-based diets.

Author contributions

Conceptualization, R.M.M., V.M., G.O., M.M. and M.J.M.; methodology, V.M., M.J.M., M.M.; software, V.M., M.J.M.; validation, V.M., R.M.M. and M.J.M.; formal analysis, V.M., R.M.M.; investigation, M.J.M., R.M.M., M.M.; resources, M.J.M.; data curation, R.M.M., M.J.M., V.M.; writing—original draft preparation, R.M.M., V.M.; writing—review and editing, R.M.M., M.J.M., M.M., V.M., G.O.; visualization, V.M., R.M.M.; supervision, V.M., M.J.M., G.O.; project administration, M.J.M., M.M.; funding acquisition, M.J.M. All authors have read and agreed to the published version of the manuscript.

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The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Animal Research Ethics Committee of the University of Mpumalanga (FANS17; 21 May 2020).

Informed consent statement

Not applicable.

Declaration of Competing Interest

The authors declare no conflict of interest.

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