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Use of Poultry By-product and Plant Protein Sources in Diets of Redclaw Crayfish (*Cherax quadricarinatus*)

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Abstract

A total of 300 juvenile crayfish (13.0±0.03 g) were randomly distributed among 5 dietary groups (n=60, 3 replicates) held within 15×500 L^{-1} fiberglass tanks connected to a recirculation system (RAS), at 20 crayfish per tank. Each group was fed for 12 weeks one of five experimental diets where the main protein sources were: 1) control, fish-meal-based diet (FM, 48% of the diet); 2) 10% FM + 52.5% poultry by-product meal (PoM); 3) 34.5% soybean meal + 34.5% corn gluten meal (Pmix); 4) 34.5% PoM + 32.soybean/corn gluten meal mix (PoM/Pmix); and 5) 10% FM + 27.5% soybean + 27.5% corn gluten meal (FM/Pmix). The results demonstrated that there were no significant differences among diets in terms of growth and feed utilization efficiency. Muscle amino acid profile of redclaw crayfish fed the FM diet had the highest level of total essential amino acids, followed by FM/PMix, Pmix, PoM/Pmix, and PoM diets. Particularly, in all experimental groups, the highest essential amino acids (EAA) were lysine, arginine, and leucine. Based on these findings, we conclude that redclaw can perform well with FM-free vegetable diets and PoM-based diets although more research is needed into the total composition of EAA and FA in muscle.

Introduction

The freshwater crustacean redclaw crayfish, *Cherax quadricarinatus,* is characterized by a range of positive biological, ecological and commercial attributes for aquaculture (Jones, 1995; Saoud et.al., 2012; Saoud *et al*., 2013). These attributes have contributed to the expansion of its aquaculture across the world beyond its native origin in North-Eastern Australia (Zeng *et al*., 2019). Given that redclaw crayfish is a highly sought after food resource for human consumption, being the second most globally cultured crayfish species after red swamp crayfish, *Procambarus clarkii* (FAO, 2020) The research has long been devoted to develop practical diets enhancing a successful culture of such crayfish species in pond based production.

Since the cost of feed for crustacean production accounts for 70% of the total cost of production, it is important to identify options for low-cost feeds for commercial-scale production without sacrificing essential dietary nutrients (Thompson *et al.,* 2005; Pavasovic, 2008). For decades, nutritionists have endeavoured to develop aquafeed formulations that support or enhance the growth of cultured redclaw crayfish while controlling costs (Pavasovic, 2008). Numerous researchers have collectively made great strides in addressing the many constraints associated with optimal feed formulation in crustaceans (Webster

et al., 2004; Thompson *et al.,* 2005; Thompson *et al.,* 2006; Saoud *et al.,* 2008, Venero *et al.,* 2008; Garza *et al.,* 2012; Saoud *et al.,* 2012). While the cultivation of omnivorous aquatic species easily switched to feeds containing minimal amounts of fish meal or oil (Turchini *et al.,* 2019). Until recently, replacing or minimizing the inclusion of fishmeal in commercial red swamp crayfish diets examined only the use of vegetable proteins (Tan *et al.,* 2018). However, more recently potential alternatives to fish meal including the by-products of animal-based product chains such as poultry, have been considered potentially useful sources of animal protein (Pavasovic *et al.,* 2007; Saoud *et al.,* 2008; Garza de Yta *et al.,* 2012).

To date, the substitution of fish meal with plant or animal by-product proteins has been mostly reported to produce cost-effective diet formulations without any detrimental effects on the production performance of crustaceans, particularly penaeids (Muzinic *et al.,* 2004; Thompson *et al.,* 2005; 2006; Saoud *et al.,* 2008; Fuertes *et al.,* 2012; 2013; 2014; Glencross *et al.,* 2014; Glencross *et al.,* 2018; Tan *et al.,* 2018). However, regarding crayfish, the efforts have been partially limited, compared to other commercially cultivated penaeid shrimps (Saoud *et al.,* 2008; Lunda Roy *et al.,* 2020).

Most recently, Qian *et al.* (2021) examined four plant protein sources (cottonseed, soybean, peanut and rapeseed meal) incorporated at 30% inclusion level in commercial diets for redclaw. However, there are limited available data for the growth performance in redclaw crayfish fed with 100% plant and/or animal by-product meal sources under intensive culture conditions such as in closed recirculating aquaculture systems. Rodríguez-Canto *et al.,* 2002) and García-Ulloa *et al*. (2012) examined the growth performance of redclaw crayfish fed with a commercial diet containing 35% protein at high stocking density. However, in the light of the previous findings, there is still a need to elucidate the plant and/or animal-based protein sources for optimum growth of redclaw crayfish cultured in intensive culture conditions. Given the importance of endogenous productivity to the nutrition of crustaceans farmed in pond systems (Gamboa-Delgado, 2014), we hypothesised that their nutrition would be subject to additional sensitivities under intensive culture conditions.

Several studies have attempted to assess the growth performance of the crayfish in either indoor or outdoor systems. In particular, distinct differences in growth performance were observed in these studies when the protein sources and level of the nutrients in the experimental diets were altered (Rodríguez-Canto *et al.,* 2002; García-Ulloa *et al.,* 2012; Saoud *et al.,* 2012). As carbon, nitrogen and phosphorous outputs are generally a greater concern in high stocking density in crayfish culture systems feed is the ultimate source of those elements a significant effort has been devoted to reducing their losses (Van Rijn, 2013; Schneider *et al.,* 2004). Despite these significant findings, no studies have been recently evaluated the suitability of various plant and animal by-product-based ingredients and their combination as potential substitutes for fish meal for redclaw feed formulation. Therefore, aim of this study was to to develop economic and sustainable diets for the production of redclaw crayfish. The impacts of dietary modifications on growth performances, proximate body composition, muscle percentage of essential amino acids and fatty acids as well as nutrient retentions are reported.

Materials and Methods

Crayfish Rearing System and Experimental Diets

The juvenile redclaw crayfish were produced at the Research and Development Center in the Faculty of Fisheries, Cukurova University, Adana, Turkey. The juveniles were collected and placed in an indoor nursery tank system for three weeks before the experiment. Individually selected and weighed crayfish were stocked into one of 15 cylindrical fiberglass tanks (500 L, 0.5 m^{-2} bottom area) connected to the RAS. Within the RAS system, water was re-circulated through a sand filter and a biofilter aerated by air stones distributed along with the experimental tanks. The recycling rate of water in the system was maintained at 3-4 L min^{-1} throughout the experiment. At the beginning of the experiment, 300 acclimated crayfish juveniles (13.0±0.03 g) were randomly distributed into five treatment groups with triplicate fiberglass tanks, at 20 crayfish per tank (40 crayfish m^{-2}). Each tank contained two pieces of 15 × 15 cm hollow bricks used for refuge of individuals and one air stone for aeration and water mixing. During the experimental period, water conditions were measured weekly with mean ± S.D. as follows: temperature 25.47±1.97°C, pH 7.85±0.41 (Hanna pH-meter), dissolved oxygen content 6.5-7.0 mg L^{-1} (YSI Oxygen meter, USA), and total ammonia nitrogen content <0.05 mg L^{-1}, nitrite from 0 to 0.5 mg L^{-1}, nitrate from 0.25 to 100 mg L^{-1}. Nitrite, nitrate, ammonium, and phosphate concentrations were measured using Aquamerck® (Darmstadt, Germany) reagent kits. All water quality parameters were considered within optimum levels for redclaw crayfish.

Each tank of crayfish was fed for 12 weeks with one of five isolipidic (crude lipit, %=9.1±0.18) and isonitrogenous (crude protein, %=38.0±0.36) experimental diets. The protein contents of each diet were based on (Tables 1 and 2):

1) Fish meal only (FM, 48% of the diet);
2) 10% FM + 52.5% poultry by-product meal (PoM);
3) 34.5% soybean meal + 34.5% corn gluten meal (Pmix);
4) 10% FM + 27.5% soybean + 27.5% corn gluten meal (FM/Pmix);
5) 34.5% PoM + 32.4 SBM/CGM mix (PoM/Pmix).

All ingredients used as protein sources were ground to pass through a 100 – 150 µ size screen and were thoroughly homogenized. Other components, such as mineral and vitamin premixes, were mixed and added into the blended ingredients. Fish oil and water (150 ml) were added to the dry ingredients and completely mixed to form a homogeneous mixture. Experimental diets were pelleted with a diameter of 1.9 mm using a pellet machine (Pasfil Machinery, İstanbul, Turkey), then kept at 110°C and 0.5 bar for 30 min in a pressure vessel. This process increases the water stability of the experimental diets according to Kumlu (2018). All pellets were air-dried to approximately 10% moisture and stored at -20°C until used.

All groups of crayfish were fed three times daily at 09:00, 13:00 and 18:00 at 12% body weight per day in the first week and then gradually reduced to 3-5% body weight day^{-1} by the end of the experiment. Uneaten feed and faecal waste were removed daily by siphoning before the first feeding. Collected uneaten feed was used to calculate feed intake after drying at 40°C for 6 hours. The experimental tanks were visually checked daily for the presence of exuvia as described by Barki *et al.* (1997) and the average number of the exuvia was recorded.

Sampling and Analysis

At the commencement of the trial, five individuals from a common pool crayfish were sampled randomly and stored at -20°C for subsequent determination of initial proximate composition. At the end of the trial, three crayfish per tank (n=3, N=9 per treatment) were sacrificed and frozen until analysis. Proximate body composition analysis was performed according to AOAC (1995) and the measurement of the fatty acid profile of the experimental diets and the whole body was carried out following the total lipid extraction (Folch *et al.,* 1957). The amino acid analyses of the experimental diets and muscle were determined using the Ultra-Fast Liquid Chromatography system with a UV detector (Gheshlaghi *et al.,* Douglas, 2008).

Calculations and Statistical Analysis

The growth and feed utilization parameters were calculated as follows: specific growth rate (SGR %day^{-1})=100 (Ln final weight – Ln initial weight) t^{-1}, weight gain (WG, %)=(final weight-initial weight)/initial weight x 100, feed conversion ratio (FCR)=dry feed intake (g)/weight gain g).

Lipid, protein and energy retention (deposition) were calculated as follows: nutrient gain (g kg ABW day^{-1})=(final body nutrient content (g) – initial body nutrient content (g))/kg ABW)/days, nutrient gain (g kg ABW day^{-1})=(final body nutrient content (g) − initial body nutrient content (g))/kg ABW)/days, nutrient retention (%)=100 × (nutrient gain/nutrient intake). Average body weight (ABW)=(final weight (g) + initial weight (g))/2 (Akpınar *et al.,* 2012). Gross energy was calculated using conversion factors of 39.5, 23.6, and 17.2 kJ g^{-1} for lipid, protein, and carbohydrate, respectively (NRC, 2011).

Essential amino acid index (EAAI), based on A/E ratios, was calculated according to Peñaflorida (1989) and Gunasekera *et al*., (2002). According to EAAI derivation, the following equations were applied:

A/E ratio=(essential amino acid/total essential amino acids)×100

aa/AA=A/E ratio in feed/A/E ratio in crayfish

$$EAAI=\sqrt[n]{\frac{aa1}{AA1}} \times \frac{aa2}{AA2} \times \ldots \frac{aan}{AAn}$$

The numerical values of aa1/AA1, aa2/AA2… were set to 0.01 minimum and an EAAI closer to 1 is indicative of the degree of conformity of the EAA profiles of the ingredient used in the experimental crayfish diets and therefore, the potential of the ingredient to meet the essential amino acid requirements of the species.

Data were analyzed by one-way Analysis of Variance (ANOVA) at a significance level of 0.05%, after confirmation of normality and homogeneity of variance. After all variables were homogenous and normally distributed, significant differences were detected. Then the data were subjected to a Tukey-Kramer post hoc test for identifying homogeneous subsets. All statistical analyses were performed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). The significance level was set at $P<0.05$. The results are presented as means ± standard deviations (n=3), except amino acid results (n=2).

Results

The experimental diets were observed to be reasonably water-stable and acceptable by the crayfish. The results of dietary amino acid profiles (Table 2) revealed that each diet was rich in one or more essential

Table 1. Proximate composition (%) of the test ingredients used in the trial

	Protein	Lipids	Dry matter	Ash	Carbohydrate*
Fish meal	64.9	9.7	91.2	12.1	4.5
Soybean meal	40.1	11.7	91.9	7.6	32.2
Corn gluten meal	56.5	3.4	89.3	4.5	25
Poultry by-product meal	55.5	12.5	89.8	10.7	9.5
Wheat meal	10.8	1.4	88.5	0.5	75.8

*Carbohydrate = Dry matter – (protein+lipid+ash)

Table 2. Formulation and proximate composition of the experimental diets

Ingredients	FM	PoM	Pmix	PoM/Pmix	FM/Pmix
Fish meal (Anchovy meal)[1]	48.0	10.0	-	-	10.0
Soybean meal[1]	-	-	34.5	16.2	27.5
Corn gluten meal[1]	-	-	34.5	16.2	27.5
Poultry by-product meal[2]	-	52.5	-	34.5	-
Wheat meal[1]	42.5	24.3	16.1	18.5	21.5
Fish oil[1]	2.4	2.7	2.4	2.5	2.5
Soy lecithin (70%)[3]	2.0	2.0	2.0	2.0	2.0
Gluten (wheat) [4]	3.1	3.1	3.1	3.1	3.1
Dicalcium phosphate[4]	-	1.1	1.8	1.8	1.0
CaCO3[5]	-	2.1	2.9	2.8	2.5
DL-methionine[1]	-	0.1	0.1	0.1	0.1
L-Lysine[1]	-	0.1	0.6	0.3	0.3
Vitamin premix [†,1]	0.8	0.8	0.8	0.8	0.8
Mineral premix [‡,1]	0.2	0.2	0.2	0.2	0.2
Vitamin C (Stay C)[1]	0.1	0.1	0.1	0.1	0.1
Composition (% DM unless otherwise stated)					
Protein	38.1±0.2	40.7±0.3	38.2±0.1	39.4±.02	38.0±0.4
Lipids	9.1±0.28	11.3±0.16	9.2±0.27	10.9±0.14	9.3±0.28
Dry matter	89.6±0.2	89.6±0.3	90.1±0.4	89.8±0.2	90.0±0.3
Ash	6.6±0.2	11.1±0.4	9.0±0.3	10.8±0.3	8.3±0.2
Energy (kJ g^{-1})	18.7±0.9	18.6±0.2	18.6±0.4	18.5±0.6	18.5±0.4
EAA (g/100 g)					
Arginine	2.0±0.00	1.9±0.01	1.1±0.00	1.1±0.00	1.0±0.00
Histidine	1.0±0.03	1.0±0.02	0.8±0.01	0.8±0.01	1.0±0.01
Isoleucine	1.8±0.01	2.1±0.01	1.7±0.01	2.4±0.01	1.8±0.01
Leucine	3.4±0.01	3.5±0.01	5.1±0.04	5.0±0.02	5.1±0.01
Lysine	3.1±0.01	2.8±0.04	2.7±0.01	2.0±0.01	4.0±0.02
Methionine	0.9±0.01	0.8±0.00	0.6±0.00	0.8±0.00	0.9±0.01
Phenylalanine	1.9±0.01	2.2±0.01	2.5±0.01	3.2±0.01	2.7±0.00
Threonine	1.3±0.00	2.0±0.01	1.7±0.00	2.0±0.01	1.4±0.00
Valine	2.2±0.01	3.0±0.02	1.8±0.01	3.3±0.01	2.1±0.01
NEAA (g/100 g)					
Alanine	2.4±0.01	2.9±0.01	2.6±0.01	3.2±0.01	2.7±0.01
Tyrosine	1.4±0.03	1.7±0.01	1.8±0.01	1.9±0.00	1.9±0.01
Aspartate	2.5±0.01	1.5±0.00	1.1±0.01	0.8±0.01	1.2±0.01
Glutamate	6.7±0.03	5.1±0.02	7.2±0.02	3.3±0.04	6.8±0.03
Glycine	2.3±0.01	4.1±0.01	1.9±0.01	3.3±0.01	2.0±0.01
Serine	1.3±0.01	2.6±0.02	2.0±0.01	1.7±0.01	1.9±0.01
CEAA (g/100g)					
Proline	2.4±	4.8±	4.5±	7.4±	4.1±
Cystine	*vest*	*vest*	*vest*	*ND*	*vest*
Hydroxyproline	*vest*	*vest*	*ND*	*ND*	*vest*
ΣAA	*36.6±*	*42.0±*	*39.1±*	*42.2±*	*40.6±*
ΣEAA	*16.4±*	*19.3±*	*18±*	*20.6±*	*20.0±*
Fatty acid (mole%)					
Total SFA	30.1±0.40	24.9±0.09	19.5±0.01	23.4±0.46	23.4±0.22
Total MUFA	25.0±0.20	32.1±0.12	38.2±0.25	30.7±0.85	27.2±0.16
Total PUFA	43.8±0.59	42.3±0.18	41.9±0.05	45.4±0.38	48.7±0.41
n-6 LC PUFA	1.1±0.01	0.8±0.01	1.3±0.01	0.8±0.00	0.9±0.01
n-6 PUFA	18.2±0.03	31.4±0.06	33.3±0.04	37.4±0.65	35.9±0.34
n-3 LC PUFA	25.0±0.29	10.4±0.27	7.90±0.06	7.67±0.32	12.5±0.47
n-3 PUFA	25.6±0.59	10.9±0.23	8.53±0.02	8.08±0.31	12.8±0.74

[1]Sibal Corporation, Sinop, Turkey (Anchovy meal-crude protein 70%).

[2]Egehan Feed, (Poultry meal rendering and bone meal rendering-pet food grade) Turkey.

[3] Tito Soy lecithin, Germany, Smart Chemical Trading and Consultancy Company Limited, Turkey.

[4] Kimetsan Ltd., Turkey.

[5] Sigma Aldrich Co Ltd, St. Louis, USA.

[†] Vitamin Premix (mg/kg): retinyl acetate 2.58 mg, DL-cholecalciferol 0.037 mg, DL-α tocopheryl acetate 30 mg, menadione sodium bisulphite 2.5 mg, 4hiamine 7.5 mg, riboflavin 15 mg, pyridoxine 7.5 mg, nicotinic acid 87.5 mg, folic acid 2.5 mg, calcium pantothenate 2.5 mg, vitamin B12 0.025 mg, ascorbic acid 250 mg, inositol 500 mg, biotin 1.25 mg and choline chloride 500 mg.

[‡] Mineral Premix (mg/kg): calcium carbonate (40% Ca) 2.15 g, magnesium hydroxide (60% Mg) 1.24 g, potassium chloride 0.9 g, ferric citrate 0.2 g, potassium iodine 4 mg, sodium chloride 0.4 g, calcium hydrogen phosphate 50 g, copper sulphate 0.3 g, zinc sulphate 40 g, cobalt sulphate 2 g, manganese sulphate 30 g, sodium selenite 0.3 g.

EAA, essential amino acids; CEAA, conditionally essential amino acids; NEAA, nonessential amino acids; vest, vestigial amount of amino acid (< 0.01 mg g^{-1}); ND, not determined.

amino acids. The dietary total EAA was similar among the experimental diets. Of the EAA, leucine and lysine were the most abundant (ranged from 3.4 to 5.1 g/100 g and 2 to 4 g/100 g, respectively), while methionine was the least abundant (0.6-0.9 g/100 g) (Table 2). The FM diet was characterized by a high content of saturated fatty acid (SFA), n-3 polyunsaturated fatty acids (n-3 PUFA), n-3 long-chain polyunsaturated fatty acids (n-3 LC PUFA), including eicosapentaenoic acid (EPA, 20:5n- 3) and docosahexaenoic acid (DHA, 22:6n-3). As expected, the Pmix was higher in monounsaturated fatty acids (MUFA) and n-6 long-chain polyunsaturated fatty acids (n-6 LC-PUFA) while the PoM/Pmix was higher in n-6 polyunsaturated fatty acids (n-6 PUFA) (Table 2).

Growth performance parameters, survival and molt cycle data of each treatment are presented in Table 3. The results indicated that there were no significant differences among diets in terms of growth performance, SGR, WG, FCR, survival, and exuviation number ($P>0.05$). Although not significant, the molt cycle was longer in crayfish fed PoM/Pmix while survival was higher in crayfish fed FM.

The moisture content ranged between the highest significant value of 79.7% (PoM) and the lowest significant value of 77.7% (FM). The protein content ranged between the highest significant value of 22.1% (FM/Pmix) and the lowest significant value of 17.5 (PoM) ($P<0.05$). The lipid content ranged between the highest significant value of 1.07% (PoM/Pmix) and the lowest significant value of 0.96% (PoM) ($P>0.05$). Ash content ranged between the highest significant value of 1.47 (FM) and the lowest significant value 1.27 (FM/Pmix) FM group was the lowest in moisture and the highest in ash. PoM group was the highest in moisture and the lowest in protein and lipids; Pmix group showed an average content of moisture, protein, lipids and ash; FM/Pmix group was as high in moisture as FM, the highest in protein and the lowest in ash ($P<0.05$) (Table 3).

While the muscle AA concentrations were identical (ranged from 79.8 to 84.2%); EAA, as proportion of the total AA, was ranged from 47.9 to 51.6% among the experimental diets, without any significant different ($P>0.05$) (Table 4). However, some specific amino acids were dominant across the experimental diets. In all groups, the highest EAA was lysine, followed by arginine, leucine (except than in PoM where leucine was higher than arginine), valine, isoleucine, phenylalanine, threonine, methionine (except than in PoM and PoM/Pmix where threonine was higher than methionine) and finally histidine ($P<0.05$). Crayfish fed FM were superior in arginine, lysine, methionine and total EAA contents. Crayfish fed PoM showed higher content of histidine, threonine, methionine and valine ($P<0.05$). The Pmix enhanced the isoleucine, phenylalanine and threonine contents in crayfish. Lysine and threonine were greater in crayfish fed PoM/Pmix. Overall, a correlation between the essential amino acid results of each experimental diet and the respective EAAI was 1.02 to 1.06 (Table 5).

Whole-body fatty acids composition is reported in Table 6. The SFAs were higher in PoM (23.4 mole %) and lower in FM (21.9 mole %) but there were no significant differences among Pmix, PoM/Pmix and FM/Pmix groups (22.1, 23.1 and 22.0 mole %, respectively). MUFA showed no significant differences among groups; however, the poultry meal-based diets (PoM and PoM/Mix) were lower in MUFA (26.6 and 26.8 mole %) than FM (28.0 mole %) and vegetable protein-based (Pmix and FM/Pmix, 28.2 and 27.0 mole %) groups. PoM/Pmix group showed the highest percentage of linoleic acid (18:2n–6, 16.8 mole %) and n-6 PUFA (19.4 mole %). The lowest levels of linoleic and n-6 PUFA were observed in the FM group (8.7 and 10.8 mole %, respectively). There were no differences in levels of linoleic and n-6 PUFA among PoM, Pmix and FM/Pmix groups ($P>0.05$). There were no significant differences among groups in n-6 LC-PUFA. Levels of n-3 LC PUFA and n-3 PUFA were higher in those crayfish fed the FM diet with respect to the other groups (38.6 and 39.1 mole %)

Table 3. Growth performance parameters and muscle compositions of redclaw crayfish, *Cherax quadricarinatus,* fed the five experimental diets for 12-weeks[‡]

	Dietary treatments				
	FM	PoM	Pmix	PoM/Pmix	FM/Pmix
Initial W, g	13.0±2.2^{a}	12.8±0.0^{a}	13.2±0.0^{a}	13.0±0.0^{a}	12.9±0.0^{a}
Final W, g	20.7±0.42^{a}	20.2±0.98^{a}	19.6±0.99^{a}	20.2±0.95^{a}	20.8±0.99^{a}
SGR	0.50±0.024^{a}	0.54±0.058^{a}	0.47±0.09^{a}	0.52±0.04^{a}	0.56±0.05^{a}
WGR, %	59.3±3.21^{a}	57.8±7.70^{a}	48.5±4.89^{a}	55.4±7.37^{a}	61.±10.9^{a}
Feed intake, g	10.1±0.89^{a}	11.1±1.5^{a}	9.42±1.1^{a}	10.7±0.86^{a}	10.0±0.79^{a}
FCR	1.31±0.08^{a}	1.55±0.36^{a}	1.53±0.35^{a}	1.52±0.19^{a}	1.28±0.30^{a}
Survival %	86.7±10.4^{a}	76.7±11.5^{a}	81.7±2.89^{a}	75.0±2.89^{a}	81.7±0.0^{a}
Exuviation number	7.0±3.0^{a}	5.3±1.5^{a}	7.3±2.3^{a}	8.7±1.2^{a}	5.7±3.1^{a}
Proximate composition (%)					
Dry matter	22.3±0.49^{a}	20.3±1.25^{a}	21.0±0.63^{a}	21.8±1.01^{a}	20.4±1.04^{a}
Protein	20.1±0.35^{b}	17.5±0.20^{d}	18.6±0.60^{c}	19.9±0.00^{b}	22.1±0.35^{a}
Lipids	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.07±0.06^{a}	1.00±0.10^{a}
Ash	1.47±0.11^{a}	1.37±0.06^{ab}	1.33±0.6^{ab}	1.43±0.06^{ab}	1.27±0.06^{b}
Energy (kJ g^{-1})	5.20±0.05	4.63±0.16	4.86±0.07	5.12±0.02	5.60±0.09

[‡]Data are expressed as mean ± SD. Values in the same row with different superscripts are significantly different by the Tukey post-hoc test ($P<0.05$).

Table 4. Essential amino acids in the muscle tissue (g/100 g) of redclaw crayfish fed the five experimental diets‡

	Dietary treatments				
	FM	PoM	Pmix	PoM/Pmix	FM/Pmix
EAA					
Arginine	7.37±0.27^{a}	6.31±0.00^{d}	6.61±0.07^{cd}	6.98±0.01^{b}	6.70±0.02^{bc}
Histidine	1.69±0.01^{b}	1.98±0.01^{a}	1.37±0.07^{c}	1.71±0.00^{b}	1.29±0.00^{c}
Isoleucine	3.77±0.02^{b}	3.81±0.02^{ab}	3.82±0.02^{ab}	3.46±0.02^{c}	3.85±0.00^{a}
Leucine	6.33±0.01^{c}	6.45±0.00^{b}	6.46±0.01^{b}	6.06±0.01^{d}	6.52±0.04^{a}
Lysine	12.21±0.29^{a}	9.22±0.09^{c}	11.48±0.15^{b}	11.86±0.05^{ab}	11.57±0.06^{b}
Methionine	1.77±0.02^{ab}	1.85±0.01^{a}	1.75±0.06^{bc}	1.68±0.01^{c}	1.84±0.02^{a}
Phenylalanine	3.53±0.01^{b}	3.49±0.01^{c}	3.54±0.02^{ab}	3.33±0.00^{d}	3.57±0.01^{a}
Threonine	2.25±0.13^{b}	2.46±0.00^{a}	2.31±0.08^{ab}	2.29±0.00^{ab}	2.23±0.00^{b}
Valine	4.23±0.03^{b}	4.30±0.01^{a}	4.17±0.03^{bc}	3.80±0.01^{d}	4.15±0.01^{c}
NEAA					
Alanine	4.53±0.06^{b}	5.10±0.00^{a}	4.60±0.00^{b}	4.20±0.00^{c}	4.60±0.00^{b}
Tyrosine	9.23±0.06^{a}	8.67±0.06^{c}	8.90±0.10^{b}	8.40±0.10^{d}	8.87±.06^{bc}
Aspartate	3.20±0.00^{b}	3.30±0.00^{a}	3.17±0.06^{b}	2.87±0.06^{c}	3.20±0.00^{b}
Glutamate	4.50±0.00^{c}	5.20±0.00^{a}	4.67±0.06^{b}	4.53±0.06^{c}	4.77±0.06^{b}
Glycine	2.60±0.00^{ab}	2.70±0.00^{a}	2.60±0.10^{ab}	2.63±0.06^{ab}	2.50±0.00^{b}
Serine	13.67±0.15^{b}	15.20±0.10^{a}	13.27±0.25^{c}	12.93±0.06^{c}	13.00±0.00^{c}
CEAA					
Proline	3.30±0.00^{a}	3.27±0.06^{ab}	3.23±0.06^{ab}	3.10±0.00^{c}	3.20±0.08^{b}
Cystine	*vest*	*vest*	*ND*	*ND*	*ND*
Hydroxyproline	*vest*	*vest*	*ND*	*ND*	*vest*
ΣAA	84.2	83.2	81.9	79.8	81.9
ΣEAA	43.1	39.9	41.5	41.2	41.7
ΣAA/ ΣAA (%)	51.2	47.9	50.7	51.6	51.0

‡ Data are expressed as mean ± SD. Values in the same row with different superscripts are significantly different by the Tukey post-hoc test (P<0.05). EAA, essential amino acids; CEAA, conditionally essential amino acids; NEAA, nonessential amino acids; vest, vestigial amount of amino acid (<0.01 mg g−1); ND, not determined.

Table 5. Muscle essential amino acid indices (EAAI†) of the redclaw fed experimental diets‡

	Dietary treatments				
	FM	PoM	Pmix	PoM/Pmix	FM/Pmix
Arginine	0.75±0.01^{b}	0.78±0.00^{a}	0.62±0.00^{c}	0.55±0.00^{d}	0.56±0.00^{d}
Histidine	1.11±0.01^{c}	1.04±0.01^{d}	1.15±0.03^{b}	0.97±0.00^{e}	1.26±0.01^{a}
Isoleucine	1.01±0.00^{c}	1.06±0.00^{b}	1.01±0.00^{c}	1.19±0.01^{a}	1.00±0.00^{c}
Leucine	1.06±0.00^{c}	1.06±0.00^{c}	1.35±0.01^{a}	1.29±0.00^{b}	1.28±0.00^{b}
Lysine	1.02±0.01^{a}	0.79±0.00^{c}	0.74±0.03^{d}	0.58±0.00^{e}	0.85±0.00^{b}
Methionine	1.03±0.00^{a}	0.95±0.00^{c}	0.92±0.01^{d}	0.98±0.00^{b}	0.98±0.00^{b}
Phenylalanine	1.07±0.00^{e}	1.14±0.00^{d}	1.27±0.01^{b}	1.38±0.00^{a}	1.25±0.00^{c}
Threonine	1.12±0.03^{b}	1.29±0.00^{a}	1.29±0.04^{a}	1.31±0.00^{a}	1.16±0.00^{b}
Valine	1.03±0.00^{c}	1.20±0.00^{b}	1.01±0.02^{d}	1.32±0.01^{a}	1.02±0.00^{cd}
Average EAAI	1.02±0.01	1.04±0.00	1.04±0.02	1.04±0.00	1.06±0.01

† EAAI=√aa/AA= √(A/E ratio in feed)/ (A/E ratio in crayfish)

‡ Data are expressed as mean ± SD. Values in the same row with different superscripts are significantly different by the Tukey post-hoc test (P<0.05).

among which there were no significant differences (PoM, Pmix, PoM/Pmix and FM/Pmix, with 31.1, 30.8, 29.7, 32.8 mole % and 31.8, 31.8, 30.6 and 33.7 mole % for n-3 LC PUFA and n-3 PUFA, respectively (P>0.05). Alpha-linolenic acid (*18:3n-3*) content was significantly higher in PM/Pmix group (0.98 mole %) and lower in the FM (0.16 mole %) group. EPA (20:5-n3) content was higher in the FM group (18.3 mole %) and lower in the PoM (15.3 mole %) and PoM/Pmix (14.3 mole %) groups, while there were no significant differences between FM/Pmix (15.9 mole %) and Pmix (16.7 mole %) groups and between FM and Pmix groups. DHA (22:6-n3) was higher in FM/Pmix group (7.9 mole %) and lower in PoM/Pmix group (5.8 mole %), while there were no differences among FM, PoM, and Pmix groups (6.6, 6.0, and 6.4 mole %, respectively).

Following the 12-week growth trial different lipid, protein and energy retention (deposition) responses were observed by the redclaw crayfish (Figure 1). Lipid retention (ranged from 10.1% to 13.1%) was similar across the diets (P>0.05). In contrast, nitrogen retention efficiency varied among treatments and was highest with the FM/Pmix diet (69.7%) and lowest with the PoM diet (34.4%) (P<0.05). In the present study, energy retention was significantly higher in the FM/Pmix, FM and PoM/Pmix diets compared with the PoM and Pmix diets that were comparable to each other (P>0.05).

Table 6. Muscle fatty acids composition (mole%) of redclaw fed the experimental diets‡

	Dietary treatments				
	FM	PoM	Pmix	PoM/Pmix	FM/Pmix
14:0	0.36±0.01^{a}	0.26±0.01 ^{b}	0.26±0.00^{b}	0.27±0.03^{b}	0.26±0.02^{b}
16:0	13.3±0.52^{ab}	13.6±0.16^{ab}	12.9±0.15^{b}	13.8±0.37^{a}	13.0±0.29^{ab}
18:0	8.0±0.25^{c}	9.4±0.05^{a}	8.8±0.05^{b}	8.9±0.26^{ab}	8.6±0.33^{b}
24:0	0.2±0.09^{a}	0.15±0.01^{ab}	0.13±0.01^{b}	0.14±0.01^{b}	0.15±0.01^{ab}
Total SFA[1]	21.9±0.67^{b}	23.4±0.24^{a}	22.1±0.21^{ab}	23.1±0.62^{ab}	22.0±0.62^{ab}
15:1	0.2±0.01^{a}	0.14±0.17^{b}	0.15±0.0^{b}	0.14±0.01^{b}	0.14±0.02^{b}
16:1n-7	1.7±0.05^{a}	0.77±0.02^{c}	0.89±0.07^{b}	0.74±0.03^{c}	0.98±0.10^{b}
17:1n-7	0.3±0.01^{a}	0.10±0.00^{c}	0.14±0.02^{b}	0.11±0.02^{bc}	0.13±0.00^{bc}
18:1n-7	2.4±0.11^{ab}	2.2±0.08^{c}	2.5±0.03^{ab}	2.3±0.07^{bc}	2.5±0.07^{a}
18:1n-9	22.6±1.24	22.9±0.36	23.7±0.06	22.8±0.59	22.3±0.66
20:1n-11	0.8±0.01^{a}	0.63±0.01^{b}	0.87±0.04^{a}	0.68±0.03^{b}	0.89±0.065^{a}
Total MUFA[2]	28.0±1.43	26.6 ±0.42	28.2±0.14	26.8±0.73	27.0±0.78
18:2n-6	8.7±0.30^{c}	15.6±0.31^{b}	15.0±0.04^{b}	16.8±0.59^{a}	14.9±0.37^{b}
18:3n-6	0.2±0.01^{b}	0.16±0.01^{c}	0.18±0.00^{a}	0.16±0.01^{c}	0.17±0.01^{b}
20:2n-6	1.78±0.06	2.06±0.02	2.4±0.06	2.2±0.05	1.7±1.4
20:3n-6	0.09±0.01^{d}	0.16±0.01^{a}	0.12±0.01^{c}	0.15±0.01^{b}	0.11±0.0^{c}
20:4n-6	0.08±0.01	0.1±0.09	0.09±0.01	0.07±0.01	0.06±0.01
Total n-6 LC PUFA[3]	1.9±0.07	2.3±0.11	2.6±0.11	2.5±0.06	1.9±1.43
Total n-6 PUFA[4]	10.8±0.37^{c}	18.0±0.42^{ab}	17.7±0.07^{ab}	19.4±0.64^{a}	17.0±1.20^{b}
18:3n-3	0.16±0.02^{d}	0.80±0.01^{c}	0.96±0.04^{ab}	0.98±0.04^{a}	0.89±0.03^{b}
20:3n-3	3.2±0.26^{c}	5.1±0.15^{a}	2.7±0.09^{c}	4.24±0.30^{b}	2.7±0.33^{c}
20:5n-3	18.3±1.07^{a}	15.3±0.24^{c}	16.7±0.16^{ab}	14.3±0.06^{c}	15.9±0.83^{b}
22:5n-3	10.5±4.02^{a}	4.6±1.14^{b}	5.0±0.17^{ab}	5.3±1.87^{ab}	6.2±1.00^{ab}
22:6n-3	6.6±0.23^{b}	6.0±0.34^{b}	6.4±0.15^{b}	5.8±0.58^{b}	7.9±0.56^{a}
Total n-3 LC PUFA[5]	38.6±2.49^{a}	31.1±1.12^{b}	30.8±0.16^{b}	29.7±2.04^{b}	32.8±0.49^{b}
Total n-3 PUFA[6]	39.1±2.46^{a}	31.8±1.11^{b}	31.8±0.14^{b}	30.6±2.01^{b}	33.7±0.52^{b}
Total PUFA	49.9±2.10	49.8±0.69	49.5±0.20	50.0±1.37	50.7±1.40

‡ Data are expressed as mean ± SD. Values in the same row with different superscripts are significantly different by the Tukey-Kramer test ($P < 0.05$).
[1]SFA (saturated fatty acid) the sum of all fatty acids without double bounds; includes 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 24:0.
[2]MUFA (monounsaturated fatty acid) the sum of all fatty acids with a single double bounds; includes 14:1, 15:1, 16:1n-7, 17:1n-7, 18:1n-7, 18:1n-9, 20:1n-11, 22:1n-9 and 24:1n-9
[3]n-6 LC PUFA; n-6 fatty acids with chain lenght ≥20 carbon atoms and ≥ 2double bounds
[4]n-6 PUFA; Sum of all n-6 fatty acids
[5]n-3 LC PUFA; n-3 fatty acids with chain lenght ≥20 carbon atoms and ≥2 double bounds
[6]n-3 PUFA; Sum of all n-3 fatty acids

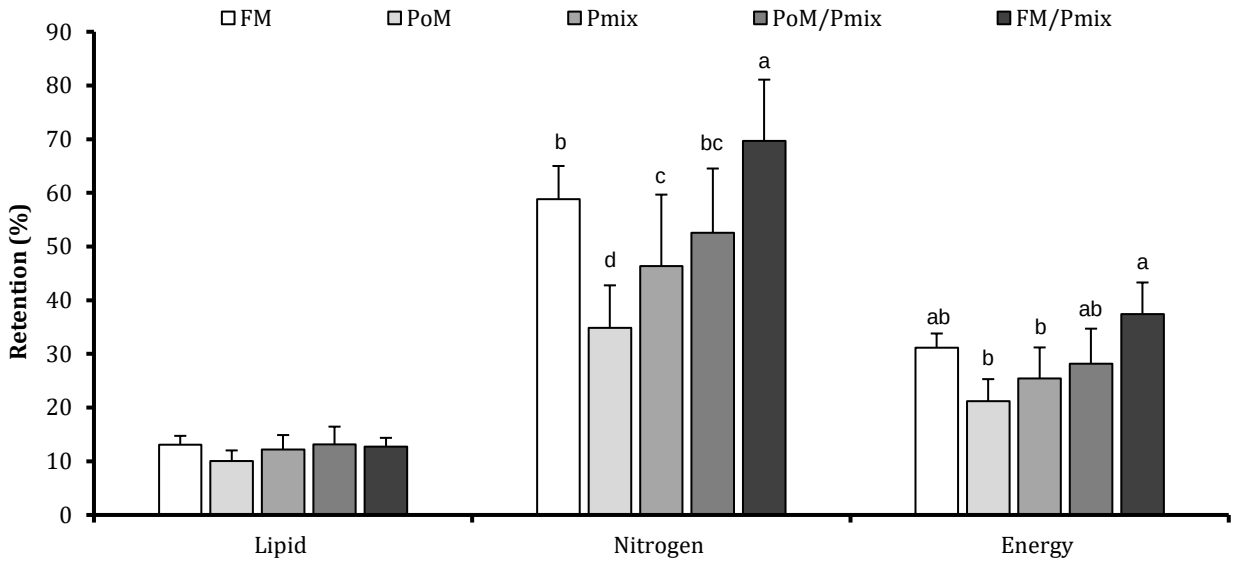

Figure 1. Lipid, nitrogen and energy retention (%) in crayfish fed with experimental diets.

Discussion

In this trial, redclaw crayfish were fed a range of diets formulated from commercially available local ingredients in Turkey to investigate the potential for increasing the intensive production of crayfish. The crayfish's apparent growth and survival were similar among diets. Levels of moulting were not similar to previous studies on crayfish (4 times in 3 months; Jones, De Silva, & Mitchell, 1996). However, the growth rate of our crayfish in the RAS system was not correspondent to those of other studies based in pond or flow through systems. Noteworthy, our crayfish increased approximately 7 g within 84 days; a value lower than that observed in previous studies. Redclaw crayfish can reach 40-60 g from 12.8 g after six months of cultivation (Masser & Rouse, 1997; Jones & Ruscoe, 2001). García-Guerrero *et al.,* (2013) stocked 10 redclaw crayfish (0.8 g) into 60 L plastic tanks and recorded a final weight of 6.68 g after 90 days of feeding on a 35% CP diet. Crayfish appear to grow faster in open ponds systems implicating a pond-derived factor in their performance. For instance, Naranjo-Páramo *et al.,* (2004) recorded a body weight of 25 g after 80 days feeding trial in natural ponds, with an initial weight of 1.3 g at a stocking density of 11 crayfish m^{-2}, with once-daily feeding of a 35% CP diet. Crayfish (8 g initial weight) reared in earthen ponds at a very low stocking density of 1.2-2.4 m^{-2} achieved a final body weight of 58 g after 70 days of feeding on a commercial marine shrimp diets containing 35% CP (Webster *et al.,* 2004). Garza de Yta *et al.,* (2012) observed that crayfish (0.125 g initial weight) stocked at 12.5 m^{-2} and fed for 8 weeks diets with 35% CP containing SBM (~58% of diet) together with 10% of poultry by-product meal, fish meal, ground pea meal or distillers dried grains attained a final body weight of about 4.98 g. We hypothesize that the comparatively low growth rate observed in our study, relative to some of the literature data, is attributable to the higher stocking density that was 40 crayfish m^{-2} and the higher initial weight that was 12-13 g. The specific growth rate of our crayfish was similar to that observed in the marron (*Cherax cainii*) fed for two months a 30% CP poultry by-product (~39% of diet) based-diet (Foysal *et al.,* 2019). Although our study demonstrates the potential of poultry by-product meal as a suitable replacer for fish meal in crayfish diets, it is important to note that other factors can affect the crayfish growth, such as the aggressive behaviour, temperature, farming system, the ingredient composition of the diets and photoperiod along with the stocking density. Previous studies report no adverse effects of replacing dietary fish meal with poultry by-product meals on crayfish growth performance (Saoud *et al.,* 2008, 2012; Garza de Yta *et al.,* 2012; Fuertes *et al.,* 2013; Foysal *et al.,* 2019), so much that poultry by-product meal has been routinely added up to 21% (Saoud *et al.,* 2008), 31.3% (Fuertes *et al.,* 2013), 39.0% (Foysal *et al.,* 2019) in crayfish diets. One possible explanation can reside in the fact that the use of animal protein sources has not been shown to have an advantage over plant protein sources for crayfish's apparent growth and survival. Indeed, redclaw crayfish demonstrated higher digestibility for plant proteins than animal protein sources (Pavasovic *et al.,* 2007). Redclaw crayfish performed comparably with fish meal-free vegetable diets with 28-35% CP or 30% CP diet containing 15% anchovy FM (Thompson *et al.,* 2005; 2006). With a mainly omnivorous feeding habit, redclaw crayfish can grow well on plant proteins-based diets (Lawrence & Jones, 2002; Saoud *et al.,* 2012) most probably as it produces various digestive enzymes including cellulases (Xue *et al.,* 1999; Figueiredo *et al.,* 2001; Saoud *et al.,* 2012).

In the present study, all diets containing plant and animal resources were equally accepted by crayfish with concomitant comparable growth performance, suggesting a general acceptance of both dietary plant and animal proteins by this species. It has been reported that the feeding preferences and growth potential of this species are depended on the activity of digestive enzymes (Lu *et al.,* 2020; Xu *et al.,* 2021). Moreover, oversupply of dietary protein can mask the deficiencies of various protein sources when fed to fish (Glencross *et al.,* 2008). Previous studies propose that crayfish require relatively low levels of dietary protein (22-28%) (Thompson *et al.,* 2005; 2006). It has been reported that 26-33% CP and 8% lipid are optimum for redclaw crayfish growth and survival during juvenile and pre-adult stages (Cortés-Jacinto *et al.,* 2004; Cortés-Jacinto *et al.,* 2005), whilst a dietary protein level of 32% is optimal for reproductive performance (Rodríguez-González *et al.,* 2006; Rodríguez-González *et al.,* 2009). Diets with 30% CP were reported to be ideal for crayfish (Jones *et al.,* 1996; Foysal *et al.,* 2019). The relatively higher CP level in our diets compared with those reported in the literature may not be as valuable for redclaw crayfish in terms of increasing protein use for energy expenditure. With this respect, the highest nitrogen retention was in the FM/Pmix which, in turn, a remarkable decrease in solid and dissolved nitrogenous waste. This could be a result of the better availability of FM/Pmix protein and amino acid profiles, which could be favoured in high stocking density systems ie. RAS. Our findings are in accordance with numerous studies (Steffens, 1999; McGoogan & Gatlin, 2000). Furthermore, Cho & Bureau (2001) noted that diets with unbalanced amino acid profiles in relation to species requirements increase nitrogenous losses and increase protein use for energy production. However, it appears that high protein (as nitrogen) retention, in the present study, can be attributed through improving dietary amino acid balance, specifically when lysine was sufficient in the diet which is also evident in other studies (Fynn-Aikins *et al.,* 1995; Gaylord & Barrows, 2009). Likewise, the FM/Pmix group, and particularly the PoM/Pmix group, preserve nitrogen and energy for maximizing the feed efficiency and growth in the present study. In this context, our nitrogen retention

was high when compare to the normal ranged reported for fish (Peres & Oliva-Teres, 1999; Gaylord & Barrows, 2009; Meiler *et al.,* 2021). This was probably due to different biological response on protein retention efficiency (or the efficiency of retention of synthesised protein) in invertebrates, which has lower protein turnover than in fish and mammals (Mente Coutteau *et al.,* 2002; Carter & Mente, 2014). Further research, thus, is suggested on the nutritional requirement of crayfish reared under various stocking density and feeding practices with the consideration of ammonia production and energy utilization.

There was no consistent trend in the response of the muscle AA profile of crayfish to the experimental diet. Nevertheless, in the present study, the major part of the variation among the EAA is interpreted in concentrations of single amino acid rather that uniformly high or low concentrations in all amino acid pool (Carter *et al.,* 2000). Each diet induced an increase of one or more of EAA content in crayfish except lysine and arginine contents that were consistently higher in crayfish muscle than their respective values supplied in experimental diets. Irrespective of the dietary groups; arginine, leucine and lysine respectively accounted for 8.3, 7.7 and 13.7 of the total EAA even though EAA, as proportion of the total AA, was not significantly different across dietary groups. There is no clear explanation for this response since data on EAA digestibility are missing in our study. Overall, there was no great difference among diets in the EAA profile of crayfish in terms of absolute values. This is consistent with previous studies stating that a mix of plant protein ingredients or animal-plant protein mix could be satisfactory for optimal growth and performance of crayfish (Saoud *et al.,* 2012). Dietary protein quality based on EAAI is considered good when the index is above 0.90, sufficient when it is around 0.8 and inadequate for values below 0.70 (Peñaflorida, 1989). Considering these reference values, our EAAI data was in the range of 1.02 to 1.06 which suggested that overall EAAI was sufficient for the redclaw crayfish. The arginine EAAI data, in the present study, exhibited inadequate value (0.55 to 0.78) across the experimental diets. However, it is important to note that fulfilment of arginine requirement of crayfish calculated as FCR multiplied by arginine content of the experimental diets (as suggested by Lunda *et al.* (2020) showed redclaw crayfish in FM (2.6), PoM (2.9) and Pmix (1.8) group had sufficient arginine in these diets. Moreover, methionine and lysine are usually considered the most critical EAA for marine and crayfish (NRC, 2011; Lunda et al., 2020). In the present study, these two important EAA were at or above standards of NRC (2011), which cover well for most of the EAA requirement in crayfish (Lunda *et al.,* 2020).

There is limited information about the fatty acid requirements of crayfish (Thomson *et al.,* 2010; Saoud *et al.,* 2012). Redclaw crayfish grew equally well when fed diets with various lipid sources including menhaden oil, linseed oil, canola oil, corn oil and beef tallow, suggesting that crayfish may have an ability to synthesize long-chain fatty acids (20:5n-3, 22:6n-3 and 20:4n-6) from their 18 carbon precursors (18:3n-3 and 18:2n-6) (Thomson *et al.,* 2010), which was verified to a certain degree by Wu *et at.* (2018). In the present study, fish oil and soy lecithin were used as lipid and phospholipid sources in all diets but the incorporation of various protein sources created remarkable changes in dietary fatty acid profiles which resulted in comparable growth performance. However, the fatty acid profile of crayfish on the poultry by product-based diets showed a lower level of LC-PUFA (particularly EPA) compared to other treatments, which could be a reflection of the high lipid content of poultry by-product meal (19.9%) and its high level of inclusion in the diets at 35 and 52%. In terms of health and growth performance, previous studies reported no adverse effects of replacing fish meal with poultry by-product meals in crayfish diets up to 21% (Saoud *et al.,* 2008), 31.3% (Fuertes *et al.,* 2013), and 39% (Foysal *et al.,* 2019). However, further research is needed regarding the effect of poultry by-product meal/oil on the fatty acid profile of LC-PUFA in crayfish.

In conclusion, our study shows that redclaw crayfish can perform well with low-level and fish meal-free vegetable diets (Pmix) and poultry by-product meal-based diets (PoM and PoM/Pmix). Furthermore, better nitrogen and energy retentions by crayfish fed the FM/Pmix diet (10% FM + 27.5% SBM + 27.5% CGM) compared with the 48% FM diet were observed. Increasing dietary terrestrial plant and animal protein sources while more closely considering diet amino acid profiles may be a strategy for improving the growth performance and nitrogen losses of redclaw crayfish reared under high stocking density. Thus, future studies should consider a more stringent focus on the EAA requirements and development of diets containing different plant, animal by-products and their mixtures in redclaw crayfish reared in RAS.

Ethical Statement

All fish handling procedures complied with Turkish Ethical guidelines for animal care (No. 28141) set by the Ministry of Food, Agriculture and Livestock. Tests were conducted according to the guidance of ethical systems of Cukurova University (CU). The care and use of experimental animal were permitted by the Ethics Committee of CU.

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Author Contribution

O.T.E. and M.E. Conceived and designed the research; H.E., M.K., E.K., E.E., H.A.Y. design the feed formulation and conducted a feding trial anaylses data; B.G. and M.P. interpreted the results, O.T.E. and M.E. contirbutre to the interpreted and presentation of the experimental full data set. All authors wrote, read and contributed to and approved the manuscript.

Conflict of Interest

No conflict of interest exists in the submission of this manuscript. All authors have seen the manuscript and approve it to submit to your journal. It is not being submitted to any other journal.

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