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Research Article
South Eastern Nigerian Seafood Diets have Desirable Effects on Biochemical Indices of Experimental Rabbits

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Abstract
Objective: The objective of this study was to evaluate the effect of some seafood diets on some important biochemical parameters with a view to recommending them for inclusion in the daily diets of resource-constrained persons who are hapless victims of the deadly collision of malnutrition and a flotilla of communicable diseases prevalent in a tropical country like Nigeria. **Methodology:** Adult rabbits were placed into six dietary groups with the positive control fed normal commercial rabbit diet while the diets of the five treated groups were supplemented with *Cambarellus diminutus* (crayfish) *Cardisoma armatum* (crab), *Tympanotonus fuscatus* (periwinkle), *Ergeria radiata* (clam) and a combination of the four, respectively. Serum lipids, proteins and electrolytes of the rabbits were estimated after 28 days of feeding. **Results:** The results showed that supplementation of normal feed with seafoods had positive health outcomes based on the biochemical parameters assesed. **Conclusion:** Seafoods could be utilized in dietary modifications to improve the nutritional need of the human and animals and to lower plasma cholesterol concentration.

Key words: Seafoods, serum lipids, serum, electrolytes, Nigeria

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Seafood consists of sea animals or plants which are taken or consumed as food by humans. Included in seafood are seawater animals, such as fish and shellfish (molluscs and crustaceans). The term shellfish comprises of crustaceans (shrimps, crabs, lobsters, crayfish etc) and mollusks (bivalves, univalves, squids, snails etc) which possess single (univalve) or double (bivalve) shells for a covering. Studies have shown that regular consumption of seafoods could lower prevalence of coronary heart disease (CHD). Seafood consumption also confers immunity against invasion pathogens. Even in pregnant women, consumption of seafoods during pregnancy prevents delivery of premature babies while babies breastfed by mothers who ate seafoods develop better eyesight than others whose mothers did not. Besides, seafoods are quite useful in the defense against dreadful human pathogens because of some antimicrobial peptides present in them[1]. Most seafoods contain reduced amount of saturated fat and more of omega-3 and omega-9 fatty acids. Omega-6 and omega-9 fatty acids are most abundant in terrestrial food sources while omega-3 fatty acids are most abundant in marine food sources. Long chain omega-3 fatty acids occur in terrestrial animals only at very low levels. In addition, cholesterol levels are higher in terrestrial animals than in marine animals. Seafood consumption could therefore result in lower amounts of cholesterol and triacylglycerols (TAG) in the blood of consumers. Omega-3 fatty acids which are major constituents of seafoods have several physiological effects, such as protecting against coronary heart disease. They also reduce platelet aggregation and so have anti-thrombotic effects[2,3].

Seafoods are also good sources of proteins and vitamins-B which are also beneficial to human health. In addition, seafoods have good amounts of calcium especially shellfish making their consumption beneficial. Seafood proteins are said to prevent insulin resistance. They also improve glucose-insulin metabolism, reverse dyslipidemia[1,4] and diminish development of high blood pressure and hypercholesterolemia[5]. The most essential nutritional feature of seafoods is their advantageous fatty acid profile resulting from their high content of essential polyunsaturated fatty acids (omega-3 fatty acids) such as eicosapentaenoic acid (C20:5 n-3) also called EPA or 5,8,11,14,17-Eicosapentaenoic acid and docosahexaenoic acid (C22:6 n-3) also called DHA or 4,7,10,13,16,19-Docosahexaenoic[1,6]. Common examples of seafoods in Nigeria include crayfish, crabs, clams and periwinkles.

Crayfish, *Cambarellus diminutus*, sometime called craw fish, is a fresh water crustacean resembling small lobsters to which they are related. Crayfish breathe through feather-like gills and are found in bodies of water that do not freeze to the bottom. Crayfish is a cheap source of protein compared to the much expensive shrimp and lobster and since it has a relatively short life span and fact that they lose accumulated heavy metals and other hazardous chemicals from their environment when they moult. Crayfish are also important indicators of water quality and environmental health, flourishing in clean water and perishing in polluted water[7]. Crayfish of the genus *Cherax* are considered detritivorous (feeding on dead plants and animals) and omnivorous, consuming a range of plant, animal, algal and detrital (decaying animal or plant) material. Their digestive enzymes are capable of hydrolyzing the wide variety of substrates that they encounter in their natural diet reflecting their capacity to adapt to varied diets[8].

Crabs belong to the order *Decapoda* which includes some well known crustaceans like prawns, shrimps, lobsters, crayfishes and hermit crabs. Two common genera *Cardisoma armatum* and *Callinectes sapidus* are found in the riverine areas of Nigeria. Both are true crabs and belong to the sub-order *Reptantia*. This sub-order consists of crawlers while the other *Decapoda* sub-order *Natantia* consists of swimmers like shrimps, prawns and lobsters. Crabs are good sources of cheap animal protein and they are usually eaten as snacks or as part of main meals by people of Southern Nigeria[9].

Periwinkles are invertebrates belonging to the phylum *Mollusca* and class *Gastropoda*. Periwinkles are known marine mollusks that are represented in mangrove swamps, lagoons and estuaries by two genera *Tympanotonus* the brackish water species and *Pachymelania* the fresh water periwinkle in Nigeria. Each periwinkle has gills and an operculum. *Tympanotonus fuscatus,* the common periwinkles in Nigeria, are shellfish dominantly found in brackish waters of the riverine areas of Nigeria, where they are highly prolific. This prolific feature had made them a cheap source of protein in many homes when compared to other conventional protein sources. They are also transported to many non-riverine towns and cities, where they are used to prepare various palatable dishes in hotels and restaurants, across the country, Nigeria[10].

Clams are heterogenous class of freshwater mussels and other freshwater bivalves, as well as marine bivalves. In the United States, "Clam" can be used in several different ways: One, as a general term covering all bivalve molluscs. The word can also be used in a more limited sense, to mean bivalves that burrow in sediment, as opposed to ones that attach themselves to the substrate (for example oysters and mussels), or ones that can swim and are migratory, like scallops. In the United Kingdom, "Clam" is one of the common names of various species of marine bivalve mollusc, but it is not used as a general term to cover edible clams that burrow and it is not used as a general term for all bivalves (www.wikipedia.org). Numerous edible marine bivalve species live buried in sand or mud and respire by means of siphons, which reach to the surface. They survive over very long periods and like other bivalves they are useful tools in environmental and pollution studies[11]. Enjoyed as a food source since prehistoric times, there are over 2,000 varieties of clams. There are two main types of clam: Hard-shell (*Mercenaria mercenaria*) and soft-shell (*Mya arenaria*). Hard-shell clams generally live in deeper waters, whereas the soft-shell resides in tide flats. Soft-shells are generally not eaten raw. The siphon neck protrudes from soft-shells, so they cannot completely close their shell. It is reported to be very rich in vitamin B12[12].

One of the most pressing nutritional problems facing Nigeria and several other third world countries is how to be self-sufficient in food production. Citing several references of Bassey and his co-workers summarised the food crises and the attendant negative consequences in a work stressing that studies on Nigerian foods show inadequacy in terms of quality and quantity of the foods as examplified by widespread malnutrition and that most affected are the pre-school age children, pregnant and lactating mothers. Furthermore, protein deficiency is a major nutritional problem leading to the syndromes of protein energy malnutrition. They therefore suggest ways of improving the nutritional status of Nigerians through mechanized agriculture, increasing available protein foods through conventional animal and plant foods, increasing conventional oil seed proteins, single cell proteins and fish protein concentrates. Other suggested approaches include use of green leaves and leaf protein concentrates, use of lesser-known proteins and fortification of local infant weaning foods[13].

Malnutrition is now widespread in Nigeria affecting all age groups in varying degrees with clinical symptoms of tissue wasting, growth failure, body weakness and weakened immune competence. The use of lesser-known proteins by peasants in the face of economic downturn affecting developing countries needs to be encouraged[13]. Our team has been involved in various food security strategies aimed at finding cheap and readily available sources of micro-and macro-nutrients to combat the menace of malnutrition and deficiency-related diseases[14,15]. This present effort is aimed at studying the possible beneficial health outcomes of readily available seafoods in South Eastern Nigeria, some of which have been studied elsewhere. To the best of our knowledge, such studies are scanty in South Eastern Nigeria, hence this study. It is expected that the report of the study will help policy makers in contemporary food security strategies especially in this resource-constrained area where malnutrition is a major problem among children and pregnant women.

MATERIALS AND METHODS

Collection and preparation of seafood samples: Fresh clams were purchased from a major market in Owerri, Imo State, Nigeria. A sterile stainless steel knife was used to dislodge and remove the soft tissue of each clam from the shell[16]. The clam flesh was then washed to remove ingested organic and inorganic particles and drained of water in a tray. Fresh crabs were also decapitated, the legs and other remaining parts of the body washed and drained of water. Mature fresh periwinkles of sizes ranging from 40-45 mm in length and 4.50-5.20 g in weight from brackish water in Rivers State of Nigeria were purchased from a major market in Owerri, Imo State, Nigeria. The pointed ends of the periwinkles were cut off before boiling them under laboratory conditions at 100°C for 10 min. The flesh was extracted from the shell with the aid of a thick metal loop. The extracted flesh was also washed and drained of water. Dry crayfish samples were sorted to remove stones and other particles. All samples were then dried overnight in an oven at 60°C to a constant weight. The oven-dried samples were ground to a coarse powder. Finally each processed sample was put in a labelled airtight container and stored in a refrigerator until required[14].

Grouping and treatment of experimental animals: This study was carried out with 30 male, white New Zealand Buck rabbits of 2 months old obtained from the Animal House of Biochemistry and Pharmacology Departments, University of Port Harcourt, Rivers State Nigeria. The animals were acclimatized in laboratory cages at room temperature (25 ± 4°C), in a natural dark and light cycle at the Federal University of Technology, Owerri, for 2 weeks and maintained on standard Vitafeed[(a)] pellet diet (Bendel Feed and Flour Mill Ltd, Nigeria). The animals were grouped and fed for 28 days as shown in Table 1. Water and feed were provided *ad libitum* through out the study.

Collection and preparation of serum: After 28 days of test feeding the animals were anaesthetized in a chloroform-saturated chamber as permitted by University Ethical Committee. The animals were sacrificed by cutting the jugular vein with a surgical blade before collecting blood from there into anti-shock test tubes. Whole blood collected was allowed to clot for 2 h and then centrifuged at 3,000 rpm for 30 min using Wisperfuge Model 1384 centrifuge (Tamson, Holland). The resulting supernatant (serum) was used to determine lipid profile, electrolytes and total protein as in our previous work[17].

Estimation of serum lipids: Serum total cholesterol concentration was estimated by the method of Allain *et al.*[18], high-density lipoprotein (HDL) cholesterol by the method of Warnick *et al.*[19] and triacylglycerol by the method of Fossati and Prencipe[20].

Estimation of serum electrolytes and total protein concentration: Serum levels of cation electrolytes were estimated by flame photometry while the serum levels of anion electrolytes (chloride and bicarbonate) were estimated by titration procedures. Total protein was determined in serum according to the method described by Tietz[21].

Statistical analysis: The results were analyzed for statistical significance by one way ANOVA using the SPSS statistical program and post hoc test (LSD) between groups using MS Excel program. All data were expressed as Mean±SEM. Differences at $p \leq 0.05$ were considered significant.

Table 1: Grouping and treatment of experimental animals

Groups	Diets
I (NC)	Commercial feed only
II	20 g of commercial feed+5 g of crayfish
III	20 g of commercial feed+5 g of crab
IV	20 g of commercial feed+5 g of periwinkle
V (TFC)	15 g of feed+2.5 g of each test seafood
VI	20 g of commercial feed+5 g of clam

NC: Normal Control, TFC: Test Feed Control

RESULTS

The results of the lipid profile (Table 2) showed that the value of the cholesterol concentrations ranged from 71.26±1.18 in the rabbits fed with clam to 111.56±1.13 in the rabbits fed with crab. The result showed that the rabbits fed with clam, periwinkle and the combination of clam, periwinkle, crab and crayfish had lower cholesterol concentrations when compared with the normal control while the rabbits fed with crayfish and crab had higher cholesterol concentrations than the positive control. The triacylglycerol values ranged from 111.68±0.71 in the periwinkle group to 159.56±1.45 in the normal control. The triacylglycerol values showed that the entire seafood-treated groups had lower triacylglycerol levels relative to the normal control. The results also showed that the normal control group had the highest value of low density lipoprotein-cholesterol concentration when compared to the treated groups while the group that fed on the combined diet had the lowest LDL-cholesterol level. Also the values of the high density lipoprotein-cholesterol showed marginal variation in all the groups. The results of serum electrolyte estimation are shown in Table 3. Potassium levels ranged from 6.14±0.30-6.14±0.30, chloride levels from 83.14±0.55-96.08±1.81, bicarbonate levels from 24.42±1.30-32.92±1.68 and sodium levels from 189.30±1.15 to 217.58±0.63. The result of the serum total protein (Table 4) showed marginal change in the concentration of the total protein in the treated groups when compared with the control. Total protein concentration ranged from 6.32±0.12 in the periwinkle group to 7.38±0.22 in the control.

Table 2: Results of lipid profile of experimental animals (mg dL^{-1})

Diets	Cholesterol	Triacylglycerol	LDL-cholesterol	HDL-cholesterol
Crayfish	107.68 ± 1.29^{c}	119.74 ± 2.63^{a}	82.56 ± 2.25^{a}	87.88 ± 0.87^{a}
Crab	111.56 ± 1.13^{d}	122.96 ± 2.10^{a}	63.34 ± 2.10^{a}	88.12 ± 0.60^{a}
Normal control	79.24 ± 0.68^{a}	159.56 ± 1.45^{c}	91.92 ± 2.32^{a}	88.42 ± 0.70^{c}
Periwinkle	78.56 ± 1.29^{a}	111.68 ± 0.71^{b}	52.02 ± 1.54^{a}	88.02 ± 0.62^{a}
Test control	72.98 ± 1.39^{b}	118.84 ± 2.56^{a}	46.56 ± 1.86^{a}	89.86 ± 1.20^{ab}
Clam	71.26 ± 1.18^{b}	112.3 ± 2.73^{b}	71.92 ± 0.96^{a}	90.78 ± 1.51^{b}

Values are mean±standard deviation. Values with different superscripts per column are significantly different ($p<0.05$)

Table 3: Result of serum electrolytes concentrations (mMol L^{-1})

Diets	Potassium	Chloride	Bicarbonate	Sodium
Crayfish	6.14 ± 0.30^{c}	91.00 ± 0.86^{ab}	28.82 ± 0.54^{c}	206.94 ± 1.75^{a}
Crab	7.90 ± 0.16^{a}	83.36 ± 0.92^{c}	33.58 ± 0.49^{a}	215.34 ± 1.32^{b}
Normal control	7.86 ± 0.11^{a}	83.14 ± 0.55^{c}	25.42 ± 0.89^{b}	208.44 ± 0.48^{a}
Periwinkle	6.96 ± 0.11^{b}	91.74 ± 1.51^{a}	32.34 ± 0.64^{a}	217.58 ± 0.63^{c}
Test control	7.32 ± 0.22^{b}	89.00 ± 1.17^{b}	32.92 ± 1.68^{a}	210.66 ± 0.50^{d}
Clam	8.70 ± 0.26^{c}	96.08 ± 1.81^{d}	24.42 ± 1.30^{b}	189.30 ± 1.15^{e}

Values are mean±standard deviation. Values with different superscripts per column are significantly different ($p<0.05$)

Table 4: Results of total protein concentrations

Diets	Protein
Crayfish	7.07±0.11[ab]
Crab	6.85±0.09[a]
Normal control	7.39±0.22[c]
Periwinkle	6.34±0.12[d]
Test control	6.30±0.20[d]
Clam	7.16±0.08[bc]

Values are means of 3 determinations±standard deviation. Values with different superscripts per column are significantly different ($p<0.05$)

DISCUSSION

The health benefits of seafood consumption as reported by Sidhu[22] are due to its content of proteins, unsaturated fatty acids and polyunsaturated fatty acids (PUFAs) especially omega-3 PUFAs. Our results showed that the groups fed with crab and crayfish had higher serum cholesterol and lower triacylglycerol (TAG) levels when compared to the normal control. The reduction of TAG levels in all the test groups is a healthy nutritional outcome. However the fact that the groups fed with periwinkle, clam and the combined diet had lower serum cholesterol and TAG levels when compared to the control means that they are better diets since total cholesterol and TAG levels are significant and independent markers of possible coronary heart events. The lower serum TAG could be as a result of increased amount of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in the seafood supplemented diets. MUFA and PUFA promote better clearance of TAG from the blood[23]. It is also observed that the usually stated inverse correlation between levels of triacylglycerol and HDL-cholesterol does not hold true in this instance.

The significant reduction of triacylglycerol levels observed in this study was not associated with a significant increase in HDL-cholesterol. This present pattern is also described by Ojiako in a previous study in rats[17].

The increased serum cholesterol concentration in crayfish and crab supplemented diets is not a major source of concern since other lipid parameters like LDL-cholesterol and TAG levels were significantly lowered in the animals that fed on the diets containing these seafoods. Moreover, the combined diet containing crab and crayfish did not replicate the same cholesterol-promoting effect. The LDL-cholesterol concentration in this study showed that the rabbits on the seafood diets had lower LDL-cholesterol values. The reason for this may be that unsaturated fatty acids in the sea animals may have been substituted for saturated fatty acids in the commercial feed. This is in line with study by Grundy[24] which reported that monounsaturated fatty acids, when substituted for saturated fatty acids in the diet, effectively reduce plasma LDL-cholesterol concentrations. The value of the LDL was lowest in the test control group which contains all studied seafoods indicating a possible synergistic action that may lead to most of the LDL being taken up and metabolized. Diets low in saturated fats and total cholesterol encourage this process, whereas diets high in those lipids can reduce LDL uptake by the liver and so increase its serum levels[25].

The serum protein levels in all the groups were not significantly different. Since low serum protein could result from low protein diet or from liver or kidney diseases, the non-significant differences in serum protein levels from results indicate that the seafoods supplementary diets had no adverse effects on the liver and kidney. It also suggests that the proteins in the diet were available for metabolic activities.

The electrolyte levels also showed no significant differences when compared to the normal control values. These results corroborate the non-alteration of hepatic and renal integrity shown by non-significant differences in the serum protein levels of the different animal groups.

CONCLUSION

Seafoods could be utilized in dietary modifications to improve the nutritional need of the human and animals and to lower plasma cholesterol concentration. This study has also shown seafood as a veritable source of nutrient which is largely untapped especially by the rural population of Nigeria.

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