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Vitamin Levels in Fresh and Smoke-Dried Body Parts of *Clarias Gariepinus* Depicting the Loss of Vitamins during Heat Processing

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Abstract

Water-soluble and fat-soluble vitamin contents were determined on both wet and dry bases in the body parts (head, muscle and liver) of *Clarias gariepinus* fish. Total trend concentrations (mg/100g) were: Fresh liver (FL) (34.5) > Fresh muscle (FM) (23.1) > Fresh head (FH) (18.4) with the dry set as Dry liver (DL) (29.1) > Dry muscle (DM) (21.4) > Dry head (DH) (17.5). Vitamin E was the most concentrated in all the sample parts; vitamin C was least concentrated in the liver; B9 was the least concentrated in muscle and head. Two most varied vitamins in both fresh and dry samples were A and B2; fresh A and B2 had variation values of 173% and 134% respectively; the dry A and B2 had variation values of 173% and 124% respectively. Statistics showed correlation coefficient (rxy) values were significantly different between FL/DL, FM/DM, FH/DH and FT/DT at critical level of r=0.01.

Keywords: *Clarias geriepinus*; Vitamins; Loss; Heat

Chemical compounds

Chemical compounds studied in this article were:

Retinol (PubChem CID: 445354), Cholecalciferol (PubChem CID: 5280795); Cyanocobalamin (Pub Chem CID: 5311498); alpha-Tocopherol (Pub Chem CID: 14985); 3-Hyroxy-vitamin K (Pub Chem CID: 5280540); Niacin (PubChem CID: 938); Riboflavin (Pub Chem CID: 493570); Pyridoxine (Pub Chem CID: 1054); Thiamine (PubChem CID: 1130); Folic acid (PubChem CID: 6037); Pantothenic (Pub Chem CID: 6613); Ascorbic acid (PubChem CID: 54670067).

Introduction

Throughout history, humans have utilized fish as a food source. Historically and today, most fish protein has come by means of catching fish. Fish is a rich source of lysine suitable for supplementing high carbohydrate diet. However, aquaculture or fish farming which has been practiced since about 3,500 BCE in China [1] is becoming increasingly important in many nations. Overall, about one-sixth of the world's protein is estimated to be provided by fish [2]. Fish is good source of thiamine, riboflavin, vitamins A and D, phosphorus. The proportion

of thiamine, riboflavin, vitamins A and D, phosphorus are considerably elevated in some developing nations and regions heavily dependent on the sea [2]. The Fishery sector is estimated to contribute 3.5% of Nigeria's Gross Domestic Product (GDP) and provides direct and indirect employment to over six million people [3].

Catfish are a diverse group of ray finned fish, named for their prominent barbells, which resemble a cat's whiskers. The African catfish is specie of catfish of the family Clarridae (air breathing fishes) and its scientific name is *Clarias gariepinus* [4]. The catfish is named after its type environment, the Gariep river, the Hottentot name of the Orange river, South Africa and the ability of the fish to live for a long time out of water [4].

Hot smoking exposes the foods to smoke and heat in a controlled environment. The item is hung first to develop a pellicle, and then smoked. Although foods that have been hot smoked are often reheated or cooked, they are typically safe to eat without further cooking. Hot smoking occurs within the range of 52˚C to 80˚C (126ºF-176˚F) [5]. Within this temperature range, foods are fully cooked, moist and flavourful. In Nigeria, traditional direct smoking system using traditional kiln, wire gauze on steel drum are almost exclusively utilized.

The purpose of this study was to determine the vitamin contents of African catfish. It is anticipated that the determination of the vitamin contents of this fish will provide necessary information on the nutrient value of this food for both consumers and researchers who work on nutrient Tables and will also guide the farmers who cultivate this specie in terms of the feeding requirements. Because of our national peculiarity (with poor and unstable electricity supply), results were presented on both fresh (wet) and smoked (dry) bases for comparison to see if any loss (and to what level) occurs during hot smoking. Based on the physical state of the sample used, an hypothesis statement could be made that "there is no significant difference between the vitamin concentrations determined on both fresh (wet) and smoked-dried bases in the body parts of *Clarias gariepinus"*.

Materials and Methods

Fish Materials

Five pieces of matured *Clarias gariepinus* were harvested from a local fish vendor who manages the fish ponds located in Basiri quarters of Ado-Ekiti, Ekiti State, Nigeria in the month of December, 2017. After cropping the life fish samples were weighed giving an average of 1.40kg. The fresh samples were sacrificed and dissected to give three parts (head, muscle and liver). The harvested fish samples were later cleaned and transferred into a frozen container for transportation to the laboratory. The parts (head, muscle and liver) required for the analyses were carefully separated and stored in a freezer prior to sample preparation. The parts were later removed from the freezer, rinsed with deionized water, ground by agate mortar and later blended with an Excella Mixer blender.

The fishes were also smoked using African traditional method of smoking called charcoal smoking. The fishes were smoked for about 12 h at moderate to high temperature (80˚C-110˚C) during which turning over was done at interval of 30 minutes to achieve a uniform smoking. The smoked samples were further dried at low temperature overnight over the ember to ensure that the samples were properly dried. The smoking process involved placing a piece of cardboard over the fishes so as to trap the smoke directly on the fish. The fish parts (head, muscle and liver) were separated from the smoked fish and homogenized using a mortar and pestle before being blended with a blender. Determinations were made in duplicate.

The homogenized samples (smoked and fresh) were stored in glass bottles and kept in a refrigerator at less than 4°C for subsequent analyses. Samples were designated as FL, FM, FH, FT, DL, DM, DH, and DT where F = fresh, L = liver, M = muscle, H = head, D = dry and T = total.

Simultaneous Analysis of Fat and Water Soluble Vitamins

The samples were brought out of the less than 4˚C compartment in the laboratory and placed on the bench to allow acclimatizing to the laboratory conditions.

Extraction of Water-Soluble Vitamins

The sample was grinded with the aid of the laboratory mortar and pestle. The accurately weighed 0.100g of ground sample was put into 100ml volumetric flasks and 80ml of deionized water added. After 15min of ultrasonic extraction, the water was added to the volumetric flask mark.

Extraction of Fat-Soluble Vitamins

An accurately weighed 0.125g of ground sample was added into 10ml volumetric flasks and 8ml of CH_3OH-

CH_2Cl_2 (1:1, v/v) was added to the volumetric flask mark. The prepared solution was stored in the dark; and diluted if necessary.

Prior to injection, the solutions were filtered through a 0.2µm filter (Millex-GN).

Optimized Chromatography Conditions

Water and fat soluble vitamins were separated simultaneously under the following optimized chromatography conditions combined with valve switching, double injection, envelope-injection and wavelength switching.

Column

Acclaim PA, 3µm, 120A, 3.0 x 150mm for fat soluble. Acclaim C18, 3µm, 120A, 3.0 x 150mm for water soluble.

Column Temperature

25°C

Mobile phase

For Water-Soluble Vitamin Determination

(A) 25mm phosphate buffer (dissolved ~ 3.4g KH_2PO_4 in 100ml water, and adjusts pH to 3.6 with H_3PO_4).

(B) CH_3CN-Mobile Phase A (7:3, v/v).

For Fat-Soluble Vitamin Determination

(A) CH_3OH-CH_3CN (8:2, v/v).

(B) Methyl tert-butyl ether (MTBE)

Injection Volume

10µl (Dionex, Technical Note 89) (www.dionex.com).

Statistical Evaluation

Data results on the pairwise groups of FL/DL, FM/DM, FH/DH and FT/DT were subjected to statistical correlation coefficient (r_{xy}), regression coefficient (Rxy), coefficient of alienation (C_A), index of forecasting efficiency (IFE), coefficient of determination or variance (r_{xy}^2). Other calculations were grand mean, standard deviation (SD) and coefficient of variation (CV %). The r_{xy} was compared to the critical Table value to see if significant difference existed among each pair comparison results at r = 0.01 [6].

Results and Discussion

Vitamin Levels in Fresh/Dry Weight Basis Compared For Liver, Muscle and Head

The concentration (mg/100g) of vitamins on fresh and dry bases in the liver of *Clarias gariepinus* can be seen in Table 1. In the group of vitamins, both fat-soluble and water-soluble vitamins were determined. For the fat-soluble vitamins we have vitamins A, D, E and K; for the water-soluble ones we have the B-complex which were vitamins B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_5 (pantothenic acid), B_6 (pyridoxine), B_9 (folic acid), B_{12} (cyanocobalamin) and vitamin C (ascorbic acid).

Vitamin	Fresh Liver (FL)	Dry liver (DL)	Value difference (FL minus DL)	(% diff.)	Mean	Standard deviation	Coefficient of Variation (%)
B_3	2.92	2.63	2.92e-1	(9.99)	2.78	2.07e-1	7.44
B_6	1.58e-1	1.41e-1	1.67e-2	(10.6)	1.49e-1	1.18e-2	7.91
C	3.40e-6	2.68e-6	7.14e-7	(21.0)	3.04e-6	5.05e-7	16.6
A	5.22	4.40	8.19e-1	(15.7)	4.81	5.79e-1	12.1
B_1	7.54e-2	5.69e-2	1.85e-2	(24.6)	6.61e-2	1.31e-2	19.8
B_2	3.37e-1	2.47e-1	9.00e-2	(26.7)	2.92e-1	6.36e-2	21.8
D	1.13e-1	8.30e-2	2.96e-2	(26.3)	9.78e-2	2.09e-2	21.4
E	24.8	21.0	3.79	(15.3)	22.9	2.68	11.7
B_9	1.76e-4	1.32e-4	4.39e-5	(25.0)	1.54e-4	3.10e-5	20.2
K	4.36e-1	2.32e-1	2.04e-1	(46.8)	3.34e-1	1.44e-1	43.2
B_5	4.38e-1	3.26e-1	1.12e-1	(25.6)	3.82e-1	7.91e-2	20.7
B_{12}	1.10e-2	8.39e-3	2.65e-3	(24.0)	9.71e-3	1.87e-3	19.3
Totals	34.5	29.1	5.37	(15.6)	31.8	3.80	11.9

Table 1: Concentration (mg/100g) of vitamins on fresh and dry bases in the liver of *Clarias gariepinus*.

Among the fat-soluble vitamins, vitamin E was the most concentrated as 24.8mg/100g (fresh liver, FL) and 21.0mg/100g (dry liver, DL), the difference in the values being 3.79mg/100g or 15.3% due to hot smoking. This was followed by vitamin A values of 5.22mg/100g (FL) to 4.40mg/100g (DL) with a difference of 8.19e-1 or 15.7%

loss. The least concentrated fat-soluble vitamin was vitamin D with values of 1.13e-1(FL) to 8.30e-2(DL), loss of 2.96e-2mg/100g or 26.3%; however the highest loss in this group was K (third concentration) with values of 4.36e-1mg/100g to 2.32e-1mg/100g with a difference (loss) of 2.04e-1mg/100g (46.8%). The coefficient of variation (CV%) values among the fat-soluble vitamins ranged from 11.7-43.2 meaning the vitamin E that had the highest concentration had the least variation of 11.7% whereas vitamin K that exhibited the highest difference also exhibited the highest variation between FL and DL with a value of 43.2%. For the vitamin B-complex group, vitamin B_3 had the highest concentration of 2.92mg/100g (FL) and 2.63mg/100g (DL) with a difference of 2.92e-1mg/100g (9.99%). Other B-complex vitamins in the range of 0.10mg/100g (and slightly above) were B_6, B_2 and B_5 whereas those in the range of 0.10 (and slightly above) were B_1 and B_{12}. Vitamin B_9 had insignificant values of 1.76e-4mg/100g to 1.32e-4mg/100g with a change of 4.39e-5 (25.0%). The CV% values in the B-complex ranged from 7.44-21.8, this meant that heat effect was more drastic in the reduction of vitamin levels in the fat-soluble vitamins than in the water-soluble vitamins since CV% in vitamin C (water-soluble) of 16.6 was within the values of 7.44-21.8. The least concentrated vitamin in the liver was vitamin C with the values of 3.40e-6mg/100g and difference (loss) of 7.14e-7 or 21.0%. Humans, other primates, guinea pigs, and some bats, birds and fish lack a liver enzyme, L-gulono-γ-lactone oxidase, hence such animals cannot synthesize vitamin C in their liver [7]. The total concentration of vitamins in the liver of *Clarias gariepinus* followed these trends, fresh: 34.5 mg/100g > 29.1 mg/100g; loss of 5.37 mg/100g (15.6%) and CV% of 11.9 meaning that the fresh and dry concentration values were close.

In Table 2 we have the concentration values (fresh and dry) of the fish muscle. As in Table 1, vitamin E was the most concentrated vitamin in both fat- and water-soluble groups; however the vitamin E here was much less than in the liver with muscle values of 8.65mg/100g(FM) and 8.10mg/100g(DM) with a change of 5.50e-1mg/100g (6.36% loss). Vitamin D that was least concentrated in the fat-soluble vitamin group in Table 1 came significantly second here among the fat-soluble vitamins with values of 3.59-2.97mg/100g with a difference of 6.20e-1mg/100g (a percentage loss of 17.3 lower than in Table 1 of 26.3%). Vitamin K was third and A was least in the fat-soluble vitamins with respective values of 1.70e-1 to 1.58e-1mg/100g (and loss of 1.19e-2, 7.01%) and 6.78e-3 to 6.39e-3mg/100g (and loss of 3.81e-4, 5.63%). The fat-soluble vitamins had CV% range of 4.10 -13.4 meaning that their values were relatively close. In the B-complex vitamins, these vitamins had values greater than 2.0mg/100g: B_3, B_6 and B_1; within the range of 0.10mg/100g were B_5 and B_{12} whereas B_2 and B_9 were in the range of 3.21e-2 to 5.09e-5. The percentage loss to smoking was generally low at values of 3.18-5.78 and CV% of 2.29-4.21. The vitamin C content here was much more appreciable than in Table 1; here the values were 2.07mg/100g (FM) and 1.86mg/100g (DM) with a change of 2.18e-1mg/100g and second highest overall percentage loss of 10.5 whereas vitamin D had the highest overall percentage loss (17.3) in Table 2. The total concentrations in Table 2 were 23.1mg/100g (fresh muscle) and 21.4mg/100g (dry muscle) with a difference of 1.75mg/100g (7.56% loss) and low CV% of 5.55.

Vitamin	Fresh Muscle (FM)	Dry muscle (DM)	Value difference (FM minus DM)	(% diff.)	Mean	Standard deviation	Coefficient of Variation (%)
B_3	2.15	2.08	6.83e-2	(3.18)	2.11	4.83e-2	2.29
B_6	2.87	2.75	1.23e-1	(4.28)	2.81	8.68e-2	3.09
C	2.07	1.86	2.18e-1	(10.5)	1.97	1.54e-1	7.84
A	6.78e-3	6.39e-3	3.81e-4	(5.63)	6.58e-3	2.70e-4	4.10
B_1	2.98	2.84	1.40e-1	(4.70)	2.91	9.89e-2	3.40
B_2	3.21e-2	3.08e-2	1.37e-3	(4.25)	3.15e-2	9.65e-4	3.07
D	3.59	2.97	6.20e-1	(17.3)	3.28	4.39e-1	13.4
E	8.65	8.10	5.50e-1	(6.36)	8.37	3.89e-1	4.64
B_9	5.09e-5	4.80e-5	2.94e-6	(5.78)	4.95e-5	2.08e-6	4.21
K	1.70e-1	1.58e-1	1.19e-2	(7.01)	1.64e-1	8.44e-3	5.13
B_5	1.37e-1	1.30e-1	6.90e-3	(5.04)	1.34e-1	4.88e-3	3.65
B_{12}	4.55e-1	4.29e-1	2.53e-2	(5.56)	4.42e-1	1.79e-2	4.04
Totals	23.1	21.4	1.75	(7.56)	22.2	1.23	5.55

Table 2: Concentration (mg/100g) of vitamins on fresh and dry bases in the muscle of *Clarias gariepinus.*

Table 3 has the vitamin concentration values of *C. gariepinus* head. As in Tables 1 and 2, vitamin E had the highest concentration values but much lower than in Table 2 but much, much lower than in Table 1. Vitamin E here had values of 6.92mg/100g (FH) and 6.75mg/100g (DH) with a low value change of 1.70e-1mg/100g (2.45% loss) and CV% of 1.76. Vitamin D [2.67(FH)-2.56(DH) mg/100g] was the second most concentrated among the fat-soluble vitamins in the head. Difference in vitamin D was 1.11e-1mg/100g (4.14% loss) and low CV% of 2.99. As in Table 2, vitamin K was third here and A was fourth here with respective values of 1.21e-1 to 8.65e-2mg/100g (3.46e-2mg/100g difference and 28.6%loss) with CV% of 23.6 and 5.34e-3 to 5.14e-3mg/100g (1.95e-4mg/100g difference and 3.65% loss) with low CV% of 2.63. As in Table 2, vitamin B1 was the highest concentrated vitamin among the B-complex vitamins. Here, it has values range of 2.24mg/100g (FH)-1.94mg/100g (DH), change value of 3.00e-1 with a percentage loss of 13.4 and low CV% of 10.2. Vitamins B6 and B1 had concentration values greater than 2.00mg/100g whereas B3 had value greater than 1.00mg/100g with vitamins B2, B9, B5 and B12 being in the concentration bracket of 1.2e-1 to 4.48e-5mg/100g (FH) with corresponding 1.14e-1 to 4.28e-5mg/100g (FH). Vitamin C in Table 3 had values of 1.79mg/100g (FH) and 1.64mg/100g (DH) having a difference of 1.44e-1mg/100g (a loss of 8.08%) and CV% of 5.95. The vitamin C values here were lower than the values in Table 2 but much higher than the values in Table 1. Generally the CV% values were at a range of 1.76-23.6. Total head vitamins were 18.4mg/100g (FH) and 17.5mg/100g (DH), a change of 9.72e-1mg/100g (5.27%) and low CV% of 3.83.

Vitamin	Fresh Muscle (FH)	Dry head (DH)	Value difference (FH minus DH)	(% diff.)	Mean	Standard deviation	Coefficient of Variation (%)
B_3	1.90	1.84	6.12e-2	(3.21)	1.87	4.33e-2	2.31
B_6	2.23	2.10	1.31e-1	(5.85)	2.17	9.23e-2	4.26
C	1.79	1.64	1.44e-1	(8.08)	1.71	1.02e-1	5.95
A	5.34e-3	5.14e-3	1.95e-4	(3.65)	5.24e-3	1.38e-4	2.63
B_1	2.24	1.94	3.00e-1	(13.4)	2.09	1.21e-1	10.2
B_2	2.81e-2	2.70e-2	1.10e-3	(3.92e-2)	2.75e-2	7.78e-4	2.83
D	2.67	2.56	1.11e-1	(4.14)	2.62	7.83e-2	2.99
E	6.92	6.75	1.70e-1	(2.45)	6.84	1.20e-1	1.76
B_9	4.48e-5	4.28e-5	1.94e-6	(4.33)	4.38e-5	1.37e-6	3.13
K	1.21e-1	8.65e-2	3.46e-2	(28.6)	1.04e-1	2.45e-2	23.6
B_5	1.21e-1	1.14e-1	6.91e-3	(5.72)	1.17e-1	4.89e-3	4.16
B_{12}	4.05e-1	3.94e-1	1.17e-2	(2.89)	3.99e-1	8.28e-3	2.07
Totals	18.4	17.5	9.72e-1	(5.27)	18.0	6.87e-1	3.83

Table 3: Concentration (mg/100g) of vitamins on fresh and dry bases in the head of *Clarias gariepinus.*

Vitamin Levels Compared on Fresh Weight Basis

Table 4 compared the vitamin levels from the fresh samples of *Clarias gariepinus* liver, muscle and head. The glaring variations in the fresh samples were conspicuously demonstrated in the CV% values. The two least varied CV% values were recorded in vitamin B_3 (22.9) and total vitamins (32.6). Other CV% values ranged between 69.9 in vitamin K to 173 in vitamin A meaning that fat-soluble vitamins were the most varied overall. On individual vitamin concentrations, the followings were observed (mg/100g fresh weight): E(40.4), D(6.37), B_3(6.97), B_1(5.29), B_6(5.26), A(5.23), C(3.86); these were values of significance. Total vitamins at trace levels were (mg/100g fresh weight): 3.97e-1(B_2), 7.28e-1 (K), 6.96e-1(B_5) and 8.71e-1(B_{12}); ultra-trace value was in B_9 (2.72e-4). In the body of the fish, we can realise a total value of 76.1mg/100g of various vitamins on wet weight basis. However, since the vitamins are in different body parts of the fish, the concentration distribution had a trend of (mg/100g fresh weight): Liver (34.5) > muscle (23.1) >head (18.4) with CV% of 32.6.

Vitamin	Fresh liver (FL)	Fresh muscle (FM)	Fresh head (FH)	Fresh totals (FT)	Mean	Standard deviation	Coefficient of Variation (%)
B_3	2.92	2.15	1.90	6.97	2.32	5.32e-1	22.9
B_6	1.58e-1	2.87	2.23	5.26	1.75	1.42	80.9
C	3.40e-6	2.07	1.79	3.86	1.29	1.12	87.3
A	5.22	6.78e-3	5.34e-3	5.23	1.74	3.01	173
B_1	7.54e-2	2.98	2.24	5.29	1.76	1.51	85.5
B_2	3.37e-1	3.21e-2	2.81e-2	3.97e-1	1.32e-1	1.77e-1	134
D	1.13e-1	3.59	2.67	6.37	2.12	1.80	84.8
E	24.8	8.65	6.92	40.4	13.5	9.87	73.3
B_9	1.76e-4	5.09e-5	4.48e-5	2.72e-4	9.05e-5	7.39e-5	81.7
K	4.36e-1	1.70e-1	1.21e-1	7.28e-1	2.43e-1	1.69e-1	69.9
B_5	4.38e-1	1.37e-1	1.21e-1	6.96e-1	2.32e-1	1.78e-1	76.9
B_{12}	1.10e-2	4.55e-1	4.05e-1	8.71e-1	2.90e-1	2.43e-1	83.7
Totals	34.5	23.1	18.4	76.1	25.4	8.27	32.6

Table 4: Concentration (mg/100g) of vitamins in the fresh liver, fresh muscle and fresh head samples of *Clarias gariepinus* compared.
Fresh liver, fresh muscle and fresh head were used to calculate mean, SD and CV%,

Vitamin Levels Compared on Dry Weight Basis

In Table 5 the counterpart results of the *C. gariepinus* vitamins were shown on dry weight basis. The dry weight results had their CV% values as varied as observed in Table 4. The CV% range was 18.5-173. Least varied again here as in Table 4 were B_3 (18.5%) and total vitamins (26.2%). As in Table 4, vitamin A had CV% of 173. Vitamins of significant concentrations in the dry samples were (mg/100g dry weight): B_3 (6.55), B_6 (4.99), C (3.50), A (4.41), B_1 (4.83), D (5.61), and E (35.9); others at trace levels were B_2 (3.04e-1), K (4.77e-1), B_5 (5.70e-1) and B_{12} (8.31e-1) and ultra-trace was B_9 (2.23e-4). As in Table 4, the most concentrated vitamin was E at 35.9mg/100g (DT). On dry weight basis total vitamin concentration in the *C. gariepinus* was 68.0mg/100g (DT) with distribution of (mg/100g DT): liver (29.1) >muscle (21.4) > head (17.5) and CV% of 26.2.

Vitamin	Dry liver (DL)	Dry muscle (DM)	Dry head (DH)	Dry totals (DT)	Mean	Standard deviation	Coefficient of Variation (%)
B_3	2.63	2.08	1.84	6.55	2.18	4.04e-1	18.5
B_6	1.41e-1	2.75	2.10	4.99	1.66	1.36	81.6
C	2.68e-6	1.86	1.64	3.50	1.17	1.02	87.1
A	4.40	6.39e-3	5.14e-3	4.41	1.47	2.54	173
B_1	5.69e-2	2.84	1.94	4.83	1.61	1.42	88.1
B_2	2.47e-1	3.08e-2	2.70e-2	3.04e-1	1.01e-1	1.26e-1	124
D	8.30e-2	2.97	2.56	5.61	1.87	1.56	83.5
E	21.0	8.10	6.75	35.9	12.0	7.88	65.9
B_9	1.32e-4	4.80e-5	4.28e-5	2.23e-4	7.43e-5	5.00e-5	67.3
K	2.32e-1	1.58e-1	8.56e-2	4.77e-1	1.59e-1	7.28e-2	45.8
B_5	3.26e-1	1.30e-1	1.14e-1	5.70e-1	1.90e-1	1.18e-1	62.0
B_{12}	8.39e-3	4.29e-1	3.94e-1	8.31e-1	2.77e-1	2.33e-1	84.2
Totals	29.1	21.4	17.5	68.0	22.7	5.95	26.2

Table 5: Concentration (mg/100g) of vitamins in the dry liver, dry muscle and dry head samples of of *Clarias gariepinus* compared.
Dry liver, dry muscle and dry head were used to calculate mean, SD and CV%.

Vitamins E and C are considered antioxidants owing to their ability to reduce the stress response in fish [8]. Vitamin C is essential in many metabolic processes including collagen synthesis (tissue repair), protection of cell membranes, metal absorption and detoxication of xenobiotics. In addition, vitamin C is considered an intra- and intercellular reducing agent. It is known that the concentration of ascorbic acid affects the mixed-function oxidases activity that is involved in the metabolism of xenobiotics [9]. Vitamin C content of pike perch, common carp and European catfish was identified to be 1.91mg/100g, 1.14mg/100g and 2.15mg/100g respectively [10]. The vitamin C content determined by Lall & Parazo [11] in white fish muscle had values of 1.0-5.1mg/100g; reports from seafood of Pacific Northwest were 0.3mg/100g [12]. Vitamin C in the muscle of the sample was 2.07mg/100g (fresh) and 1.86mg/100g (dry) which fell within the values above. Total vitamin C contents were 3.86mg/100g (fresh) and 3.50mg/100g (dry). However, these values fell short of the daily intake of 60-100mg.

Vitamin B_{12} (cyanocobalamin) is found mainly in fish, meat, poultry and dairy products. Individuals following vegetarian diets are at risk for developing Cbl deficiency owing to suboptimal intake as vitamin B_{12} (Cbl) is essential for the synthesis of nucleic acids, erythrocytes and in the maintenance of myelin, deficiency may result in a variety of symptoms. Some of these symptoms may be severe whilst others may be irreversible [13]. Human requirements are extremely small, ca/µg daily. In the samples wet weight values ranged from 10µg/100g - 455µg/100g whereas the dry weight values ranged from 8.39µg/100g -429µg/100g; the highest concentration in both wet and dry samples came from the muscle: 455µg/100g (wet) and 429µg/100g (dry). These values far outstripped the daily human requirement of 1µg.

The entry of calcium and phosphorus into the digestive tract is positively enhanced by the presence of vitamin D [14]. That means that vitamin D is the key to getting calcium and phosphorus to enter the blood stream via the gut. Low levels of vitamin D can result in insufficient levels of both calcium and phosphorus. Therefore, it is not surprising that low levels of vitamin D can also be associated with soft, brittle or deformed bones. Maintaining proper levels of vitamin D has been associated with the prevention of softening and weakening of bones in children (known as rickets) as well as softening of the bones (known as osteomalacia) in adults. Vitamin D and calcium also help protect older adults from reduction in bone mass and thinning of the bone which predisposes the bones to break at the slightest movement known as osteoporosis [15]. People avoiding the sun and those who suffer from milk allergies, or adhere to a strict vegan diet may be at risk for vitamin D deficiency. In the present results, vitamin D in fresh fish body parts ranged from 1.13e-1 to 3.59mg/100g giving a total of 6.37mg/100g FT whereas in the dry samples, it ranged from 8.30e-2 to 2.97mg/100g giving a total value of 5.61mg/100gDT. In each physical state, the muscle predominated. The values here were greater (except in the liver) than the reports in commercially important fish species of Lakshadweep Archipelago, India (10 different fish species were involved) having range values of 0.37-0.77mg/100g [16]. The vitamin D requirement for optimum health is 400IU/d, regardless of age. Since 1µg of vitamin D is equivalent to 40IU of vitamin D, it meant that 400IU/d will be equivalent to 10µg of vitamin D per day. From the results above, all the *C. gariepinus* body parts were good sources of vitamin D.

Vitamin B_1 (thiamine) in the fresh samples ranged from 7.54e-2 to 2.98mg/100g and total of 5.29mg/100g FT whereas the dry samples had values of 5.69e-2 to 2.84mg/100g and total of 4.83mg/100g DT. In mammals, including humans, the organs with high thiamine concentration (µg/g of moist tissue) are the heart (2.8-7.9), kidney (2.4-4.8), liver (2.0-7.6), and brain (1.4-4.4); lesser amounts are found in the spleen, lungs, adrenals and muscle. Normal blood contains ca 90ng/ml which may vary considerably [17]. A value below 40ng/ml may be indicative of thiamine deficiency. Further literature values were in (mg/100g): pike perch (0.04), common carp (0.08) and European catfish (0.08) [10]. Souci, et al. [18] reported that vitamin B_1 level for some marine and freshwater fish species were 0.02-0.2 mg/100g. In the 10 fish species of Lakshadweep Sea, vitamin B_1 ranged from 0.39-0.61mg/100g [16].

Vitamin B_2 ranged in the fresh samples as 2.81e-2 to 3.37e-1 and total value of 3.97e-1 with corresponding dry sample results of 2.70e-2 to 2.47e-1mg/100g and total of 3.04e-1mg/100g. Vitamin B_2 in pike perch, common carp and European catfish had values of (mg/100g): 0.01, 0.04 and 0.03 respectively [10]. whereas in the 10 species of Lakshadweep Archipelago India, B_2 varied from 0.13-0.86mg/100g [6]. In the report for some marine and freshwater fish species by Souci, et al. [18], vitamin B_2 in mackerel was 0.36mg/100g, herring (0.22mg/100g), European eel (0.32mg/100g), rainbow trout (0.08mg/100g) and carp (0.053mg/100g). An adult requires ca 1.5 -3.0mg riboflavin (vitamin B_2) daily.

The niacin (vitamin B_3) levels in the samples were 1.90-2.92mg/100g and totals of 6.97mg/100gFT whereas the dry counterparts varied from 1.84 -2.63mg/100g and totals of 6.55mg/100gDT. The term niacin has been used to encompass the active forms of this vitamin, nicotinic acid and nicotinamide; however, estimates of niacin requirements take into account preformed niacin as well as that obtained as niacin equivalent in the body from tryptophan (Trp) metabolism. Hence it was estimated that when 60mg of Trp is consumed by an adult, enough of Trp is oxidised to produce 1.0mg of niacin [19]. The best estimate of the average requirement for an adult is 15-20mg per day. The recommended dietary allowance (RDA) for adults, expressed as vitamin B_3 equivalents is 6.6mg per 4184 kJ (1000kcal, food calories) and not less than 13mg for an intake of less than 8368 kJ (2000kcal, food calories); one (1) NE is equivalent to 1.0mg niacin (or 60mg Trp) [19]. The Trp levels in the present report (not yet published) were (g/100g) fresh: LF (2.12), MF (2.10) and HF (1.70); for dry: LS (2.16), MS (2.02) and HS (1.88). This meant that the samples would be both good sources of vitamin B_3 either directly or indirectly. B_3 values in the 10 Indian fish species had values that ranged from 0.19-0.87mg/100g [16].

The concentrations of vitamin B_6 were in the range of 1.58e-1 to 2.87mg/100g and total of 5.26mg/100g FT whereas the dry values were 1.41e-1 to 2.75mg/100g and 4.99mg/100g DT. Vitamin B_6 is a generic name used for pyridoxine, pyridoxal and pyridoxamine, the co-enzyme forms of which are pyrodixal phosphate and pyridoxamine phosphate [19]. Vitamin B_6 is needed in the synthesis of DNA bases; it is a co-enzyme in the biosynthesis of thymedine. A dietary vitamin B_6 deficiency or an increase in the thymedine requirement at a critical time during cell division could result in initial cell mutations that develop into a tumor [20]. The RDAs were based on a ratio of 0.02mg of vitamnin B_6 per gramme of protein consumed. From this estimate, the vitamin B_6 required to satisfy the protein composition (assuming as being main protein source) from the body parts would be for fresh: 0.02 x 19.5 (=0.39mg) liver, 0.02 x 20.0 (=0.40mg) muscle and 0.02 x 20.5 (=0.41mg) head; for dry : 0.02 x 56.5 (=1.13mg) liver, 0.02 x 60.7 (=1.21mg) muscle and 0.02 x 58.6 (=1.17mg) head. Most human diets provide 3-10mg daily derived from a variety of natural foods. For adult patients on total parenteral nutrition 15mg is an adequate daily dose and 1mg daily is sufficient for infants [21]. Vitamin B_6 values in the fishes of Lakshadweep Sea ranged from 0.11-0.91mg/100g [10].

People need vitamin B_5 to synthesize and metabolize fats, proteins and co-enzyme A. Some of the important functions of B_5 are: converting food into glucose; synthesizing cholesterol; forming sex and stress-related hormones and forming red blood cells. Deficiency of vitamin B_5 is extremely rare in people; however, clinical trials have shown that deficiency may lead to: tiredness, depression, hypoglycaemia and can cause an increased sensitivity to insulin. Recommended daily intake ranged from 1.7mg per day to 7mg per day depending on age status [22]. Vitamin B_5 in the samples ranged from 1.21e-1 to 4.38e-1mg/100g and total of 6.96e-1mg/100g FT; in dry samples we have 1.14e-1 to 3.26e-1mg/100g and total of 5.70e-1mg/100g DT which were generally close to the values of 0.18-0.81mg/100g observed in 10 fish species of Lakshadweep Sea [16].

Vitamin B_9 (folate, folic acid, folacin) has a recommended adult daily intake in the U.S. of 400µg from foods or direct supplements. Folic acid is essential for the body to make DNA, RNA, metabolise amino acids, which are required for cell division. Not consuming enough folate can lead folate deficiency that may result into a type of anaemia in which low numbers of large red blood cells occur. In adults, normal total body folate is between 10 and 30 mg with blood levels of greater than 7nmol/L (3ng/ml) [23]. Low levels of folic acid in early pregnancy are believed to be cause of more than half of babies born with neural tube defects (NTDs), B_9 values in the fish parts were 4.48e-5 to 1.76e-4 mg/100g and total of 2.72e-4mg/100gFT; for dry we have values of 4.28e-5 to 1.32e-4mg/100g and total of 2.23e-4mg/100gDT. These values translated to the following µg/100g levels: for fresh, we have 0.045-0.176µg/100g and total 0.272µg/100g FT; for dry, results would become 0.043-0.132µg/100g and total 0.223µg/100gDT. These values were much lower than in pike perch (12.13µg/100g), common carp (33.23µg/100g) and European catfish (12.23µg/100g) [10]. Lall & Parazo [11] reported that the average folic acid content of fish and shellfish was 0.5-10µg/100g in flesh. In the innards of male and female samples of *Neopetrolisthes maculatus* (a marine shellfish), values of folic acid ranges as 317µg/100g-419µg/100g(dry weight) [24]; in the flesh of the same shellfish, we have folic acid values of 407µg/100g-454µg/100g [25].

Vitamin A value was the second most concentrated vitamin in the liver but present in ultratrace levels in the muscle and the head. Fresh liver had level of 5.22mg/100g and dry was 4.40mg/100g; on the other

hand, fresh muscle/head had values of 5.34e-3 to 6.78e-3 as mg/100g and dry was 5.14e-3 to 6.39e-3 mg/100g; for fresh muscle/head and dry muscle/head, values in µg/100g would be 5.34-6.78µg/100g and 5.14-6.39mg/100g. It has been said that fat-soluble vitamins in the flesh of fish are affected by the level of fat [10]; this assertion is true in the present results. The crude fat content of muscle (10.4g/100g) whereas the head crude fat was 5.50g/100g; consistently vitamins A, D, E and K were higher in the muscle than in the head but all lower than in the liver. The flesh of the lean fish contains 7.5 to 15µg/100g vitamin A, while in the fatty species vitamin A ranges from 30 to about 1350 µg/100g [26]. In common carp we had 23.52µg/100g and 6.30µg/100g in European catfish [10]. The vitamin A contents in the head and muscle could be said to be medium and low but significantly high in the liver: 5220µg/100g (fresh) and 4400µg/100g (dry). In the 10 fish samples from India, vitamin A content varied from 0.19-0.72 mg/100g [16]. The normal daily requirement of vitamin A for adults is about 5000IU (or 1500 RE, retinal equivalent). One (1) IU of vitamin A = 0.3µg of retinal equivalent. Although vitamin A is known to play a vital role in general metabolism, the only biological function in which its action is clearly understood is vision.

The amount of vitamin E present in any sample of food or tissue is quoted in tocopherol equivalents (TEs). Note that

1mg TE = 1mg of α-tocopherol
= 1IU of tocopherol

Vitamin E requirement is linked to that of PUFA. The requirement of vitamin E suggested by Indian Council for Medical Research [21] is 0.8mg/g of essential fatty acids. This roughly works out to 8-10mg tocopherol per day. The fish sample parts were high in vitamin E (α-tocopherol). In the fresh samples we had (mg/100g): FL (24.8) > FM (8.65) > FH (6.92) with total value of 40.4FT; in the dry we have; DL (21.0) > DM (8.10) > DH (6.75) with total value of 35.9DT. The samples were good sources of α-tocopherol. The per capita daily intake of α-tocopherol in the U.S.A. has been estimated from data on total purchases of food to be ca 15mg. The tocopherols are used as dietary supplements and in food technology as antioxidants (qv) to retard the development of rancidity in fatty materials. The vitamin E content in pike perch was 0.94 mg/100g, common carp had 0.46mg/100g and European catfish had 0.80mg/100g [10].

Values of vitamin K in the samples were as follows (mg/100g, µg/100g): FL (4.36e-l, 436), FM (1.70 e-l, 170), FT (1.2le-l, 121), total (7.28e-l, 728); DL (2.32e-l.232), DM (1.58e-l, 158), DH (8.65e-2, 86.5) and total (4.77e-l, 477). In the 10 fish species of Lakshadweep Archipelago in India, their vitamin K contents ranged from 0.19-0.67mg/100g [16]. The Committee on Medical Aspects of Food Policy of the United Kingdom concluded that 1µg/kg/day of vitamin K is both safe and adequate for adults [21]. Hence the fish body parts were good sources of vitamin K.

Statistical Analyses

Data values from Table 1(FL/DL), Table 2 (FM/DM), Table 3 (FH/DH), Tables 4 (FT) and 5 (DT), that is (FT/DT) were all subjected to statistical analyses of Pearson correlation coefficient (r_{xy}), regression coefficient (Rxy), variance or coefficient of determination (r_{xy}^2), coefficient of alienation (C_A) and index of forecasting efficiency (IFE). The r_{xy} was subjected to critical Table value of $r_{= 0.01}$ to find out if significant differences occurred in the paired comparisons. The results of the statistical analyses were depicted in (Table 6) The following observations were made in FL/DL, FM/DM, FH/DH and FT/DT: all the r_{xy} values were positively high and significantly different at $r_{= 0.01}$ since $r_{c\ 0.9829\text{-}0.99998} > r_T$ 0.684; r_{xy}^2 were high at 0.9661-0.99995; Rxy showed that for each left hand value of compared pair increasing by 1.0mg/100g, the right hand value increased by 0.8455-0.9505; all the mean values were low at 2.69-10.5mg/100g; standard deviation (SD) values corresponding higher than the mean with values range of 4.81 - 22.0; all the coefficient of variation (CV%) were high with values ranging from 162-209. In the coefficient of alienation (C_A), all the values were low having values of 0.0069-0.1841 with corresponding high values of index of forecasting efficiency (IFE) of 0.8159-0.9931. The C_A is an opposite of IFE, the lower the C_A the higher is the corresponding IFE. To predict the physiological or biochemical actions of the paired groups (FL/DL, FM/DM, FH/DH and FT/DT), both C_A and IFE are Important. The IFE is a reduction in the error of prediction of relationship between two entities. For example, in FL/DL, C_A was 0.0069 and IFE was 0.9931. The error of prediction (C_A) of relationship between FL/DL was 0.69% whereas the reduction in the error of prediction (IFE) of relationship between FL/DL was 99.31% thereby making the prediction almost error free. In all the comparisons, since. $C_{A\ (0.0069\text{-}0.184)} <$ IFE $_{(0.8159\text{-}0.9931)}$, then prediction was easy in all of them. Hence the activities of one in the group can conveniently and predictably be carried out by the other member of the pair and vice-versa.

Statistics	FL	FL/DL	DL	FM	FM/DM	DM	FH	FH/DH	DH	FT	FT/DT	DT
r_{xy}		0.99998*			0.9829*			0.9999*			0.99996*	
r_{xy}^2		0.99995			0.9661			0.9997			0.99992	
Rxy		0.8455			0.9003			0.9505			0.8926	
Mean	5.31		4.48	3.55		3.58	2.84		2.69	11.7		10.5
SD	11.1		9.38	6.35		5.81	5.06		4.81	22		19.7
CV%	209		209	179		162	174		179	188		188
C_A		0.0069			0.1841			0.0163			0.0087	
IFE		0.9931			0.8159			0.9837			0.9913	

Table 6: Statistical analyses of results from Table 1 (FL/DL), Table 2 (FM/DM), Table 3 (FH/DH), Tables 4 (FT) and 5 (FD), that is (FT/DT).
Remark: n -2 =13-2 = 11 df for each column; $r_{=0.01}$ = 0.684 (critical value). (*) $r_{=0.01,}$ r_{xy} is significant.

Conclusion

Vitamins of significant concentrations in FL/DL were B_3, A, B_2, D, E, K and B_5; in FM/DM as well as FH/DH were B_3, B_6, B_1, C, D, E, B_5 and B_{12}. In the body of the fish we could realise a total value of 76.1mg/100g of various vitamins on wet weight basis with concentration distribution in liver (L) (34.5) > muscle (M) (23.1) > head (H) (18.4) with CV% of 32.6. The fish would serve as good sources of vitamins D, B_{12}, B_3, A and E. Most vitamin sample body variations were observed in vitamins A and B_2 as: A (fresh) = 173%; B_2 (fresh) = 134% and A (dry) = 173%; B_2 (dry) = 124%. The overall results showed that all the vitamins in the samples were heat labile. Conspicuous among the highest losses were: in the liver, vitamin K was the most heat labile in all the vitamins and among the fat-soluble ones having a loss of 46.8% whereas among the water-soluble, B_2 recorded highest loss of 26.7%; in the muscle, vitamin D recorded the highest loss in all the vitamins and fat-soluble ones with a loss of 17.3% but vitamin C lost highest value in the water-soluble group with 10.5% loss; in the head, vitamin K again recorded the highest loss in value both in all the vitamins and fat-soluble ones with a value of 28.6% but vitamin B_1 was the highest heat labile in the water-soluble vitamins with a loss of 13.4%. These results showed that the fat-soluble vitamins were generally more heat labile than the water-soluble vitamins. The null hypothesis had been rejected by the results of these analyses thereby upholding the alternative hypothesis that significant differences existed between the samples in the two different physical states (wet and dry) showing that significant differences occurred between the sample pairs of FL/DL, FM/DM, FH/DH and FT/DT.

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