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Study of long-chain *n*-6 and *n*-3 polyunsaturated fatty acids and other lipids in brains of bull and hen

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ABSTRACT

Lipid composition of the brain oils of bull and hen found in Nigeria was determined by gas chromatography. SFA level ranged from 6.11 to 6.54 % of total fatty acids. MUFA was close to each other in the samples and composed the third largest fraction of 8.89 to 9.86 %. The *n*-6 PUFA constituted the second largest group of 35.4 to 39.0 % whereas the *n*-3 PUFA of 46.0 to 48.1 % formed the largest group. Most concentrated SFA was lignoceric acid, highest MUFA was erucic acid, highest *n*-6 was arachidonic acid whilst docosahexaenoic acid (DHA) was the highest *n*-3 PUFA. Cholesterol was the only sterol detected, 589 to 874 mg/100 g. The highest phospholipid was phosphatidylcholine having a range of 29.1 (60.4 %) to 20.2 (59.6 %) mg/100 g. 100 g bull brain would provide 8.56 g of DHA, 100 g hen brain would provide 9.47 g of DHA.

Introduction

Exactly when the individual animal species were domesticated is unknown. DNA sequencing technology suggests that the dog may have been domesticated from the wolf as long as 135, 000 years ago, but archaeological evidence suggests that dog was domesticated about 14,000 years ago (12000 B.C.). The earliest domestic food specie was the sheep (somewhere around 8000 B.C.), donkeys (4000 B.C.) followed closely by goats, hogs and cattle (6500 B.C.) Ilama (5500 B.C.), horses (3500 B.C.) and chickens (6000 B.C.)[1]. Indigenous chicken constitutes 80 % of the 120 million poultry type raised in the rural areas in Nigeria[2]. They are self-reliant and hardy birds with the capacity to withstand harsh weather conditions and adaptation to adverse environment. They are known to possess qualities such as the ability to hatch on their own, brood and scavenge for major parts of their food and possess appreciable immunity from endemic diseases. Their products are preferred by the majority of Nigerians because of the pigmentation, taste, leanness and suitability for special dishes. The Nigerian chicken is a light breed, often with single comb and that black and brown plumage, laced with various colours such as mottling are common. The different ecotypes are in two major categories based on body size and body weight as heavy ecotype and light ecotype. The heavy ecotype (Fulani ecotype) is found in the dry savannahs (Guinea and Sahel), Montane regions and cattle Kraals of the north and weigh about 0.9 -2.5 kg at maturity. The light ecotype are those chicken types from the swampy rainforest and derived savannah agro-ecological zones whose mature bodyweight ranges between 0.68 -1.5 kg[2]. Olawunmi et al.[3] found that the Fulani ecotype chicken was bigger in size than the Yoruba ecotype chicken: 1.76±0.4 and 0.79±021 kg for Fulani and Yoruba ecotypes respectively. Indigenous male chicken was also bigger in size than their female counterparts: 1.5±0.06 kg and 1.29±0.04 kg respectively[2].

There are many breeds of cattle found in Nigeria. They include White Fulani, Sokoto Gudale, Red Longhorn (Raheja), Shuwa cattle, Adamawa, Biu cattle, Chad, Nigerian Shorthorn (Muturu) and N'Dama. White Fulani is a strong big robust breed with a white coat with black ears and nuzzle, black tongue and eyebrows and often black hooves. The body colour is very variable from white, through red and roan to black. The head is held high, the hump pronounced and the tail fairly high. The horns are all shapes and lengths. These cattle are good milkers and good workers[4].

Meat animals yield, besides their carcasses, a considerable amount of parts which are biologically and hygienically fit for human consumption. These by-products are very different from the point of view of structure, proximate composition or functional or sensory properties, but they can all be used for food. They are generally consumed either as main ingredients in traditional dishes or as ingredients in meat products[5]. Among by-products which are edible is the brain which is consumed as direct meat products.

A considerable literature has been published on the health benefits of fish, oil-rich fish and fish oils and their constituent long chain (LC) *n*-3 PUFA. There is hardly any information on the contribution of the animal brain as a good source of LC *n*-3 and *n*-6 PUFA. The purpose of the present report was to explore evidence relating to the lipid composition of the brains of the bull and the hen as contributors to the availability of LC fatty acids when used as food.

Materials and methods

Sample collection

Five matured live hens (Yoruba ecotype) were purchased from Ado-Ekiti, Nigeria and brought to the laboratory. The bull brain (White Fulani) was collected from butchers who daily slaughted cattle for the meat from the slaughter house based in Ado – Ekiti.

Sample treatment

In the laboratory the hen's head was held on the stump and the head removed with an axe. At the end of bleeding, the hen was plucked, the head rinsed and dried in the oven for 5 h at 60 ^{o}C. After drying, the brain was extracted, ground, sieved and kept in freezer (-4 ^{o}C) in McCartney bottles pending analysis.

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Five birds (all free range) were used in the study. Their ages varied between 42-45 weeks with a weight range of 1.2 to 1.5 kg. The bull brain from the slaughter house was caught into bits for proper oven-drying until constant weight was reached. Drying was for 5 h at 70 ^{o}C. It was then ground, sieved and kept in freezer (-4 ^{o}C) pending analysis.

Extraction of lipid

0.25 g of each sample was weighed into the extraction thimble. 200 ml of petroleum ether (40-60 ^{o}C boiling range) was measured and then added to the dried 250 ml capacity flask. The covered porous thimble with the sample was placed in the condenser of the Soxhlet extractor arrangement that has been assembled[6]. The lipid was extracted for 5 h. The extraction flask with the oil was oven dried at 105 ^{o}C for I h. The flask containing the dried oil was cooled in the desiccator and the weight of the cooled flask with the dried oil was measured.

Preparation of methyl esters and analysis

50 mg of the extracted oil was saponified for 5 min at 95 ^{o}C with 3.4 ml of 0.5 M KOH in dry methanol. The mixture was neutralised by 0.7 MHCl. 3 ml of 14 % boron triflouride in methanol was added[6]. The mixture was heated for 5 min at 90 ^{o}C to achieve complete methylation process. The fatty acid methyl esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1 ml for analysis and 1 µl was injected into the injection pot of the GC. The fatty acid methyl esters were analysed using an HP 5890 powered with HP gas chromatograph (HP 5890 powered with HP ChemStation rev. A09.01[1206] software [GMI, Inc, Minnesota, USA]) fitted with a flame ionization detector. Nitrogen was the carrier gas with a flow rate of 20-60 ml/min. The oven programme was: initial temperature at 60 ^{o}C, first ramping at 10 ^{o}C/min for 20 min, maintained for 4 min, second ramping at 15 ^{o}C/min for 4 min and maintained for 10 min. The injection temperature was 250 ^{o}C whilst the detector temperature was 320 ^{o}C. A capillary column (30 m x 0.25 mm) packed with a polar compound (HP INNOWAX) with a diameter (0.25 µm) was used to separate the esters. Split injection type was used having a split ratio of 20:1. The peaks were identified by comparison with standard fatty acid methyl esters.

Sterol analysis

Sterol was analysed as described by AOAC[6]. The aliquots of the extracted fat were added to the screw-capped test tubes. The sample was saponified at 95 ^{o}C for 30 min, using 3 ml of 10 % KOH in ethanol, to which 0.20 ml of benzene had been added to ensure miscibility. Deionised water (3 ml) was added and 2 ml of hexane was added in extracting the non-saponifiable materials. Three extractions, each with 2 ml hexane, were carried out for 1 h, 30 min and 30 min respectively. The hexane was concentrated to 1 ml in the vial for gas chromatographic analysis and 1 µl was injected into injection pot of GC. The peaks were identified by comparison with standard sterols. The sterols were analysed using similar conditions as for fatty acid methyl ester analyses.

Phospholipids analysis

Modified method of Raheja, Kaur, Singh, & Bhatia el al.[7] was employed in the analysis of phospholipids. 0.01 g of the extracted fat was added to each test tube. To ensure complete dryness of the fat for phospholipids analysis, the solvent was completely removed by passing stream of nitrogen gas on the fat. 0.40 ml chloroform was added to the tube followed by the addition of 0.10 ml chromogenic solution. The tube was heated at 100 ^{o}C in water bath for 1 min 20 sec. The content was allowed to cool to the laboratory temperature and 5 ml hexane added and the tube shaken gently several times. The solvent and the aqueous layers were allowed to be separated. The hexane layer was recovered and concentrated to 1.0 ml for analysis. The phospholipids were analysed using an HP 5890 powered with HP gas chromatograph (HP 5890 powered with HP ChemStation rev. A09.01 [1206] software [GMI, Inc, Minnesota, USA]) fitted with a pulse flame photometric detector. Nitrogen was used as the carrier gas with a flow rate of 20-60 ml/min. The oven programme was: initial temperature at 50 ^{o}C, whilst the detector temperature was 320 ^{o}C. A capillary column (30 m x 0.25 mm) packed with a polar compound (HP) with a diameter (0.25 µm) was used to separate the phospholipids. Split injection type was used having a split ratio of 20:1 The peaks were identified by comparison with standard phospholipids.

Quality assurance

Standard chromatograms were prepared for sterols, phospholipids and fatty acid methyl esters which were then compared with respective analytical results; calibration curves were prepared for all the standard mixtures and correlation coefficient determined for each fatty acid parameter, same for sterols and phospholipids. Correlation coefficient should be > 0.95 for the result to be acceptable. It was performed with Hewlett Packard Chemistry (HPCHEM) software (GMI, Inc 6511 Bunker Lake Blvd Ramsey, Minnesota, 55303, USA).

Further on quality assurance, fatty acid values were subjected to calculation of uncertainty interval percentage. Certified reference materials (CRMs) play a critical role in validating the accuracy of nutrient data. A range of food CRMs with assigned values and uncertainty intervals (UIs) for many nutrients are currently supplied by several organizations[8]. The fatty acids whose UIs were available were evaluated in certified reference materials (CRMs): C18:2 members and C18:3[6, 8]. Certified reference material was available for cholesterol but none for phospholipids[8].

Calculation of fatty acid per 100 g in sample

At the data source and reference database levels, values for individual fatty acids are usually expressed as percentages of total fatty acids. At the user database levels, values per 100 g of food are required. A conversion factor derived from the proportion of the total lipid present as fatty acids is required for converting percentages of total fatty acids to fatty acids per 100 g of food. Total lipid level was multiplied by a conversion factor of 0.561 to convert it to total fatty acids [9]. For fatty acids, precision is best limited to 0.1 g/100 g of fatty acids[10].

Statistical analysis

Statistical analysis[11] was carried out to determine coefficient of variation in percent (CV %), linear correlation coefficient (CC), coefficient of determination (CD), linear regression coefficient (RC), coefficient of alienation (CA) in percent and index of forecasting efficiency (IFE) in percent. The CC was subjected to the table (critical) value at $r_{=0.05}$ to see if significant differences existed in the values of fatty acids, sterols and phospholipids between the bull and hen brains.

Results and discussion

Table I depicts total lipid and calculated total fatty acid levels on dry weight basis. The values of total lipids between the two samples were very close with the CV % of 4.44. The total fat of 42.5-45.2 g/100 g were close to the value in duck's meat and skin (43 %), higher than in chicken's meat and skin (18 %) but much lower than in beef fat (67 %), lamb fat (72 %) and pork fat (71 %)[12]. Fornias[5] reported the proximate composition

and energy value of cattle, pig and sheep by-products and lean beef, pork and lean meat of sheep. He reported the fat of cattle brain as 10.6 % wet weight with 77.6 % water content which on dry matter basis gave a fat content of 47.3 % in cattle brain; based on this, sheep brain has 38.1 % fat and pig brain has 42.6 % fat; all these values were close to the present report of 42.5 – 45.2 %.

Table II shows the saturated fats (SFA) and the monounsaturated fats (MUFA) of the samples. In the bull brain the following SFA recorded 0.0% value: C2:0, C3:0, C4:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0 and C20:0 with not detected (nd) for C5:0 and C6:0. In the hen brain, similar observations were made but the nd values were in C2:0, C3:0, C6:0, C8:0 and C12:0. For the MUFA, both samples had 0.0 % value for: C14:1 (*cis*-9), C16:1 (*cis*-9), C18:1 (*trans* - 6), C18:1 (*cis*-6), C18:1 (*trans* -9), C18:1 (*cis* -9) and C18:1 (*trans* -11). The two SFA members in the samples were behenic acid (C22:0) and lignoceric acid (C24:0) with respective values of 2.80 – 2.99 % and 3.31-3.54 % of total fatty acid composition. These gave a total SFA value as 6.11-6.54 % of the total fatty and a CV % of 4.77. The C22:0 and C24:0 have not been implicated in enhancing the level of low density lipoprotein (LDL) cholesterol unlike myristic (C14:0) and palmitic (C16:0) acids. The MUFA levels were contributed by gadoleic acid (C20:1), erucic acid (C22:1) and nervonic acid (C24:1). Their total values were: C20:1, 0.962-1.03 %; C22:1, 4.62-4.94 %; C24:1, 3.31-3.89 % and respective CV % values of 4.77, 4.77 and 11.3. Although the CV % values were low in both the SFA and MUFA showing homogenousity of result values, a close observation would show that the values in the hen brain were correspondingly higher than in the bull brain. Some literature SFA values were (% total fat): beef fat (43 %); lamb fat (50 %); pork fat (37 %); chicken's meat and skin (33 %); duck's meat and skin (27 %) and calf liver (30 %) with corresponding MUFA values of 48 %, 39 %, 41 %, 42 %, 54 % and 54 %[12]; all these SFA and MUFA values were much higher than the present SFA and MUFA levels in the brains of bull and hen. In Nigeria, herds are allowed to range over the grasslands and forests. Grass-finished beef tends to produce more favourable SFA.

Table II also contains polyunsaturated fatty acids (PUFA) composition of *n*-6 and *n*-3 in the samples. Among the *n*-6 family, C20:4 *n*-6, *cis* (arachidonic acid, AA) was the most concentrated in both samples with value range of 18.5-24.3 % with CV % of 19.1. Whilst total PUFA *n*-6 totalled 39.0 % in the bull brain, it totalled 35.4 % in the hen with CV % of 6.84. C18:2 *n*-6, *trans* (*trans*-linoleic acid or conjugated linoleic acid, CLA) has a value of 2.30 % (bull brain) but 0.113 % (hen brain) with CV % of 128, the highest CV % in Table II. One of the factors that affect the total lipid found in a serving of meat is highly dependent upon the feeding regimen[13]. CLA is a constituent of ruminant animals and exist as a general mixture of conjugated isomers of linoleic acid (LA). The *cis* – 9, *trans* -11 CLA isomer (rumenic acid or RA) accounts for up to 80-90 % of the total CLA in ruminant products[13]. Naturally occurring CLAs originate from two sources: bacterial isomerization and/or bio hydrogenation of PUFA in the rumen and the desaturation of *trans* –fatty acids in the adipose tissue and mammary gland[14, 15]. Microbial bio hydrogenation of LA and α-LA by anaerobic rumen bacterium *Butyrivibrio fibrisolvens* is highly dependent on rumen pH. Grain consumption decreases rumen pH, reducing *B. fibrisolvens* activity, conversely grass-based diets provide for a more favourable rumen environment for subsequent bacterial synthesis[13]. Rumen pH might help explain apparent differences in CLA content between grain (mostly for the hen) and grass (mostly for the bull) – finished meat products. CLA has been shown in actions to reduce carcinogenesis, atherosclerosis and onset of diabetes. In the *n*-3 PUFA group, C22:6 *n*-3, *cis* (docosahexaenoic acid, DHA) has the highest levels in both samples with a range of 35.9 – 37.3 % and CV % of 2.73 depicting the closeness of the results. Eicosatrienoic acid (C20: 3*n*-3, *cis*, ETA) was second in the *n*-3 group with values of 4.55-4.87 % and CV % of 4.77. C20:5*n*-3 (eicosapentaenoic acid, EPA) of value 3.31-3.54 was number 3 in the *n*-3 group. On the whole, total *n*-6 PUFA was 35.4-39.0 % (CV % = 6.84) and *n*-3 totalled 46.0 -48.1 % (CV % =3.16) whereas *n*-6 + *n*-3 totalled 83.5-85.0 %. Total PUFA from literature were: beef fat (4 %); lamb fat (5 %); pork fat (15 %); chicken's meat and skin (19 %); duck's meat and skin (12 %) and calf liver (26 %) [12]; all these values were much lower than the present report. In sheep brain, total PUFA was 20.5 %[12].

The human brain has a high requirement for DHA; low DHA levels have been linked to low brain serotonin levels, which are connected to an increased tendency for depression and suicide. Several studies have established a correlation between low levels of *n*-3 fatty acids and depression. High consumption of *n*-3 FAs is typically associated with a lower incidence of depression, a decreased prevalence of age-related memory loss and a lower risk of developing Alzheimer's disease[16]. Sinclair et al.[17] were the first to show that beef consumption increased serum concentrations of a number of *n*-3 fatty acids including, EPA, DPA and DHA in humans. Likewise, there are a number of studies that have been conducted with livestock which report similar findings, that is, animals that consume rations high in precursor lipids produce a meat product higher in the essential fatty acids[13]. For instance, cattle fed primarily grass significantly increased the *n*-3 FA of the meat and also produced a more favourable *n*-6 to *n*-3 ratio than grain-fed beef[13].

N-3 and *n*-6 fatty acids are essential, that is, humans must consume them in the diet. *N*-3 and *n*-6 compete for the same metabolic enzymes, thus the *n*-6: *n*-3 ratio will significantly influence the ratio of the ensuing eicosanoids (hormones), (like prostaglandins, leukotriene, thromboxane, etc.), and will alter the body's metabolic function[18]. Generally, grass-fed animals accumulate more *n*-3 than do grain-fed animals which accumulate relatively more *n*-6. Metabolites of *n*-6 are significantly more inflammatory (especially AA) than those of *n*-3. This necessitates that *n*-3 and *n*-6 are consumed in balanced proportion; healthy ratios of *n*-6: *n*-3 range from 1:1 to 4:1[19]. In the samples under discussion, 2*n*-6/3*n*-3 in the bull has a value of 1.02:1.00; and 0.125: 1.00 in the hen. However on the total *n*-6/*n*-3, bull was 0.848:1 and 0.736:1 in hen. Typical Western diets provide ratios of between 10:1 and 30:1, that is, dramatically skewed toward n-6[20]. Here were the ratios of *n*-6 to *n*-3 fatty acids in some common oils: canola 2:1, soybean 7:1, olive 3-13:1, sunflower (no *n*-3), flax 1:3, cottonseed (almost no *n*-3), peanut (no *n*-3), grape seed (almost no *n*-3) and corn oil 46 to1 ratio of *n*-6 to *n*-3.

Table II also contains the ratio of PUFA/SFA. Values here ranged between 12.8:1 and 13.9:1 with CV % of 5.82. The PUFA/SFA (P/S) is important in determining the detrimental effects of dietary fats. The higher the P/S ratio the more nutritionally useful is the dietary oil. This is because the severity of atherosclerosis is closely associated with the proportion of the total energy supplied by saturated fats and PUFA.

Researchers have debated which fatty acids should be called essential. A simple definition of an essential nutrient has already been given: it is a nutrient that the body cannot make at all or cannot make in sufficient quantities to meet physiological need. In the case of fatty acids, though, insisting on a clear distinction between essential and nonessential nutrients oversimplifies reality. The body can make some fatty acids only if others are supplied. Also, some may be essential only for growth or for disease prevention[21]. Until all have agreed on how to define essentiality, nutrition experts must be careful with their use of the term. The facts are these. The body's cells do not possess enzymes to make any of the *n*-6 or *n*-3 fatty acids from scratch; nor can they convert an *n*-6 fatty acid to an *n*-3 fatty acid or vice versa. They can start with the 18-carbon member of a series and make the larger fatty acids of that series, although this conversion is slowed by dietary imbalances and enzyme competition (as earlier in *n*-6/*n*-3 ratios). Therefore, if a cell needs a fatty acid of either omega series, it must have either that specific fatty acid or another in that series. The conversion process is slow, so the most effective way to sustain body stores of AA, EPA and DHA is to obtain them directly from foods [21]. In the present report for example: LA (C18:2*n*-6) < AA (C20:4*n*-6); also, α – LA (C18:3*n*-3) < EPA (C22: 5*n*-3), < DHA (C22:6*n*-3).

The fatty acids were further subjected to statistical analysis. Result showed a high positive and significant linear correlation coefficient (CC) (0.9998) at $r_{=0.05}$ and n-2 degrees of freedom. The coefficient of determination (CD) was also high (0.9996) showing that 99.96 % of variance in the bull brain was associated with the variance in the hen brain. The linear regression coefficient showed that for every unit increase in the bull brain fatty acid, there was a corresponding increase of 0.165 in the fatty acid of hen brain. The coefficient of alienation (CA) was low at 2.01 % with a corresponding high value of index of forecasting efficiency (IFE) with a value of 97.99 %. The IFE is actually a value of reduction of error of relationship between the two brains fatty acids; this meant that the error in the prediction of relationship was just 2.01 %. The implication of this was that the bull brain fatty acids could carry out adequately the functions of the hen brain fatty acids.

Table III shows the fatty acids distribution per 100 g bull brain and hen brain as food. The values produced from the SFA and MUFA were consistently higher in the hen brain than the corresponding bull brain. In the *n*-6, values were higher in LA, *trans*-LA, dihomo-γ-C20:3*n*-6 and AA in bull brain than the hen brain and vice versa for other *n*-6 fatty acids. For *n*-3 fatty acids, values in bull brain were all correspondingly lower than in the hen brain. The calculation accounted for all the total fatty acids as calculated by crude fat x the conversion factor for the samples; this was because no trace level of fatty acid was recorded.

The National Institute of Health has published recommended daily intakes of FAs; specific recommendations included 650 mg of EPA and DHA, 2.22 g/day of α – LA and 4.44 g/day of LA. However, the Institute of Medicine has recommended DRI (dietary reference intake) for LA (*n*-6) at 12 to 17 g and α – LA (*n*-3) at 1.1 to 1.6 g for adult women and men, respectively. Although seafood is the major dietary source of *n*-3 fatty acids, a recent fatty acid intake survey indicated that red meat also serves as a significant source of *n*-3 fatty acids for some populations[13]; this had been aptly demonstrated in the brains of both the red meat (from bull brain) and from white meat (from hen brain).

The energy in food is held in form of fat, carbohydrate, protein and alcohol. Each gram of fat contains approximately 9 kilocalories (38 kJ). This value was used to calculate the energy levels of the various fat samples[22]. The energy density in the samples due to fat were 964 kJ/100 g (about 230 kcal./100 g) in the bull brain (Table IV). The 1990 Canadian RNI (Recommended Nutrient Intakes) included specific amounts for 3*n*-3 fatty acids and 2*n*-6 fatty acids. For *n*-3 fatty acids, the RNI is 0.5 % of total energy or 0.55 g/1000 kcal; for *n*-6 fatty acids, the RNI is 3 % of total energy or 3.3 g/1000 kcal [21] For energy contribution in the samples, the following was observed in both samples: PUFA > MUFA >SFA. In the energy distribution, CC was 0.99998, CD showed a 99.995 % of variance in the association between bull and hen brains, RC was very high at 8.52 being more favourable for hen brain, CA of 0.70 %, IFE of 99.3 % and $r_{=0.05}$ being significant.

In the analysis of sterols, only cholesterol was detected in both samples with values of (mg/100 g): 874 in bull brain and 589 in hen brain; its grand mean was 732±201 and 27.5 CV %. Cholesterol is a fatty compound involved in the transport of fat in the blood stream and is also part of the structure of cell membranes of tissues of the body. It is not a dietary essential since adequate amounts are synthesised in the body from other dietary ingredients. Confusion has arisen between the terms blood cholesterol and dietary cholesterol. For most individuals dietary cholesterol has little or no effect on blood cholesterol levels because reduced synthesis in the body compensates for increased dietary intake[12]. However, there are individuals who are sensitive to dietary cholesterol[23] and most authorities advise a general reduction in cholesterol intake for everyone. These sterols recorded 0.0 mg/100 g in the samples: cholestanol, ergosterol, campesterol, stigmasterol, savenasterol and sitosterol.

Meat supplies about one third of the dietary cholesterol in many western diets with the remainder from eggs and dairy products. Since all these foods are valuable sources of nutrients there could be some nutritional risk in restricting their intake. Most authorities, but not all, recommend a reduction in dietary cholesterol to around 300 mg or less per day[12]; this is more than the level in 100 g in the samples under discussion. Some literature values of cholesterol were as shown (mg/100 g): fish (50-60), egg yolk (1260), meat and poultry (60-120), brain (2000 - 3000), liver (300 - 350)[12]. Sheep brain contains 2200 mg/100 g cholesterol level (Paul & Southgate, 1978). Garcia et al.[24] reported (cholesterol/100 g) 40.3 and 45.8 or 40300 and 45800 mg/100 g of tissue in pastured and grain-fed steers (castrated bulls), respectively ($p < 0.001$).

Table V shows the levels of the various phospholipids. Phosphatidylcholine (PC) was the most abundant phospholipid in the two samples forming levels of (mg/100 g): 29.1 (or 60.4 %) in bull brain and 20.2 (or 59.6 %) in hen brain. PC is the most abundant phospholipid in brain cell membranes comprising about 30 % of the total phospholipid content while phophatidylserine (PS) makes up less than 10 %. In the present report PS made up 4.99 mg/100 g (10.3 %) in bull brain but 0.665 mg/100 g (1.96 %) in the hen brain. The PC is the key building block of membrane bilayers, it is also the principal phospholipid circulating in plasma, where it is an integral component of the lipoproteins, especially the HDL [21]. PS has been shown to enhance mood in a cohort of young people during mental stress and to improve accuracy during tee-off by

increasing the stress resistance of golfers. The US Food and Drug Administration (USFDA) had stated that consumption of PS may reduce the risk of dementia in the elderly and may also reduce the risk of cognitive dysfunction in the elderly[25]. Phosphatidylethanolamine/cephalin (PE) was 4.58 mg/100 g (9.50 %) in bull brain but was 9.72 mg/100 g (28.6 %) in hen brain. PE is found in all living cells, although in human physiology it is found particularly in nervous tissue such as the white matter of brain, nerves, neural tissue and in spinal cord[25]. Phosphatidylinositol (PtdIns, PI) is a negatively charged phospholipid. PI can be phosphorylated to form phosphatidylinositol phosphate (PIP), phosphatidylinositol bisphosphate (PIP_2) and phosphatidylinositol trisphosphate (PIP_3). PIP, PIP_2 and PIP_3 are collectively called phosphoinositides. Phosphoinositides play important roles in lipid signalling, cell signalling and membrane trafficking[25]. PI was 14.7 mg/100 g (14.7 %) in bull brain but lesser in hen brain with a value of 2.27 mg/100 g (6.68 %). Lysophosphatidylcholine was low in both samples. Partial hydrolysis of PC with removal of only one fatty acid yields a lysophosphatidylcholine. The statistical result of Table V showed that the CC (0.8907) was significant at $r_{=0.05}$ and *n*-2 degrees of freedom; RC was 0.267; the CD was slightly high at 79.3 %; CA was 45.5 % with slightly higher IFE (54.5 %).

Table VI shows the uncertainty per cent (UIP) for both the fatty acids and cholesterol. In the fatty acids, the table (standard) UIP levels were correspondingly higher in C18: 2*n*-6 (*cis* and *trans*) for bull but not in hen, in C18:3*n*-3, table value was higher than in both samples; however table UIP was higher than in calculated cholesterol in both samples. Also the correlation determined for all the standards: fatty acids, phospholipids and sterols, all had values ranging as follows: 0.99833-0.99997 (fatty acids), 0.99909-0.99999 (phospholipids) and 0.99920-0.99994 (sterols); all the correlation values were greater than 0.95 which is the critical correlation for acceptance of these types of analytical results. Both the correlation values and the UIP values attested to the quality assurance of the determinations.

In conclusion, the findings of this study showed that both bull and hen brains possessed unequal distribution of all the parameters (fatty acids, phospholipids and sterols) determined. These long-chain fatty acids: AA, EPA and DHA were high in both samples but low in SFA and MUFA. These are good for brain development even in human beings, incidentally brains are preserved for children in Nigeria, and hence consumption of brain tissue will be an advantage for children. Cholesterol levels in the samples were moderate and PC was the principal phospholipid. Significant differences existed in the fatty acid, energy contribution and phospholipid levels between the bull and hen brains at $r_{=0.05}$.

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Table I. Crude fat and total fatty acid levels of bull and hen brains (g/100 g)

Parameter	Bull	Hen	CV %
Crude fat	42.5	45.2	4.44
Total fatty acid*	23.8	25.4	4.44

*Crude fat x 0.561
CV % = coefficient of variation

Table II. Fatty acid composition of the brain of hen and bull (% total fatty acid)

Fatty acid	Bull	Hen	CV %
C22:0 (Behenic acid)	2.80	2.99	4.77
C24:0 (Lignoceric acid)	3.31	3.54	4.77
Total SFA[a]	6.11	6.54	4.77
C20:1 (Cadoleic acid)	0.962	1.03	4.77
C22:1 (Erucic acid)	4.62	4.94	4.77
C24:1 (Nervonic acid)	3.31	3.89	11.3
Total MUFA[b]	8.89	9.86	7.28
C18:2*n*-6, *cis* (Linoleic acid)	2.27	0.297	109
C18:3*n*-6, *cis* (γ-Linolenic acid)	1.90	2.04	4.77
C20:2*n*-6, *cis* (Eicosadienoic acid)	0.962	1.03	4.81
C20:3*n*-6, *cis* (Dihomo-γ-linolenic acid)	3.91	3.45	8.75
C20:4*n*-6, *cis* (Arachidonic acid, AA)	24.3	18.5	19.1
C22:2*n*-6, *cis* (Docosadienoic acid)	3.31	10.0	71.1
n-6 PUFA[c], *cis*	36.7	35.3	2.75
C18:2*n*-6, *trans* (Rumenic acid)	2.30	0.113	128
n-6 PUFA, total	39.0	35.4	6.84
C18:3*n*-3 (α – Linolenic acid)	2.22	2.38	4.77
C20:3*n*-3 (Eicosatrienoic acid)	4.55	4.87	4.77
C20:5*n*-3 (Eicosapentaenoic acid, EPA)	3.31	3.54	4.77
C22:6*n*-3 (Docosahexaenoic acid, DHA)	35.9	37.3	2.73
n-3 PUFA, total	46.0	48.1	3.16
n-6 + *n*-3 PUFA	85.0	83.5	1.26
SFA + MUFA + PUFA, total	100	99.9	0.071
PUFA/SFA	13.9	12.8	5.82
n-6/*n*-3	1.02	0.125	111
Ratio (total fatty acid)	1:1	1:1	-

[a]SFA = saturated fatty acid; [b]MUFA = monounsaturated fatty acid; [c]PUFA = polyunsaturated fatty acid (essential fatty acid).

Table III. Fatty acid (g/100 g) bull and hen brains as food

Fatty acid	Bull	Hen	CV %
Behenı acid	0.667	0.759	9.19
Lignoceric acid	0.789	0.898	9.20
Total SFA	1.46	1.66	9.20
Gadoleic acid	0.229	0.261	9.20
Erucic acid	1.10	1.25	9.20
Nervonic acid	0.789	0.986	15.7
Total MUFA	2.12	2.50	11.7
Linoleic acid	0.541	0.075	107
γ- Linolenic acid	0.454	0.517	9.20
Eicosadienoic acid	0.229	0.261	9.20
Dihomo-γ- linolenic acid	0.931	0.875	4.32
Arachidonic acid	5.79	4.70	14.7
Docosadienoic acid	0.789	2.54	74.3
Rumenic acid	0.548	0.029	127
α- Linolenic acid	0.529	0.602	9.19
Eicosatrienoic acid	1.08	1.23	9.20
EPA	0.789	0.898	9.20
DHA	8.56	9.47	7.27
Total PUFA	20.2	21.2	3.27

Table IV. Energy distribution as contributed by the fatty acid groups in bull and hen brains (values in kJ/ 100 g)

Fatty acid group	Bull	Hen
SFA	55.3 (6.11 %)	63.0 (6.54 %)
MUFA	80.5 (8.89 %)	95.0 (9.86 %)
PUFA	769 (85.0 %)	806 (83.6 %)
Total (energy)	905	964

Table V. Phospholipid levels (mg/100 g) of bull and hen brains

Phospholipid	Bull	Hen	CV %
Phosphatidylethanolamine (PE)	4.58 (9.50 %)	9.72 (28.6 %)	50.8
Phosphatidylcholine (PC)	29.1 (60.4 %)	20.2 (59.6 %)	25.4
Phosphatidylserine (PS)	4.99 (10.3 %)	6.65 (1.96 %)	108
Lysophosphatidylcholine (LPC)	2.43 (5.05 %)	1.09 (3.20 %)	54.1
Phosphatidylinositol (PI)	7.10 (14.7 %)	2.27 (6.68 %)	73.0
Total	48.2	34.0	24.5

Table VI. Uncertainty interval as percentage of analytical results

Parameter		UIP (table)	UIP (bull)	UIP (hen)
Fatty acid:				
	C18:2*n*-6, *cis*	24.0	7.49	57.2
	C18:2*n*-6, *trans*	24.0	7.39	1.50
	C18:3*n*-3	16.3	6.30	5.88
Sterol:				
	Cholesterol	3.50	0.538	0.798