LIPID PROFILE OF THE BRAIN OF MALE FREE-ROAMING DOMESTIC DOG [*Canis lupus* VAR. *familiaris* (LINNEAUS, 1758)]

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Abstract: This study reported the determination of the lipid profile of the brain of the male free-roaming domestic dog (*Canis lupus* var. *familiaris*) edible portion on dry weight basis. Parameters determined were total crude fat, sterols and phospholipid levels. Calculated from the main results were total fatty acid, total energy (both in kJ and kcal); fatty acid in edible portion (EP); some fatty acid quality parameters such as MUFA/SFA, PUFA/SFA, EPA/DHA, n-6/n-3 (LA/α–LA), ∑n – 6/∑n – 3, AA/DGLA, EPA + DHA, EPSI (MUFA/PUFA), C16:0 : C18:1 cis – 9, C18:0 : C18:1 cis – 9, % C16:0 in total SFA, and total UFA (MUFA + PUFA). Sample contained 12.2 g/100g crude fat with corresponding value of 6.87 total fatty acid. Whilst total energy of crude fat was 453 kJ/100g, the value for total fatty acid was 254 kJ/100g. The following fatty acids were of high concentration (%total fatty acid): C16:0 (19.6), C18:0 (13.1), C16:1 cis – 9 (4.85), C18:1 cis – 6 (23.8), C18:1 cis – 9 (20.7), C18:2 cis -9, 12 (3.40) C20:4 cis -5, 8, 11, 14 (4.28) and C22:6 cis – 4,7,10,13,16,19 (2.89). The values for total SFA, MUFA and PUFA were 37.2, 50.3 and 12.5%, respectively. The corresponding energy values (kJ/100g) were 94.5 (37.2%), 128 (50.3%) and 31.7 (12.5%). The following parameters were nutritionally good in the sample: MUFA/SFA, PUFA/SFA, EPA/DHA, n-6/n-3 (LA/α – LA), ∑n – 6/∑n – 3, AA/DGLA, EPA + DHA, EPSI and TUFA. Among sterols, only cholesterol was significant at 1499 mg/100g (99.9987%). The total phospholipid value was high at 3452 mg/100g with members being high at 14.0 – 41.3% but very low in lysophosphatidylcholine (LPC) having a value of 3.57 mg/100g and 0.10%. The male *Canis lupus* var. *familiaris* brain under study could be said to be moderate in fat content (12.2 g/100g) with high composition levels of TUFA (62.8%), high cholesterol of 1499 mg/100g (99.9987%) as well as high phospholipid levels of total 3452 mg/100g that was dominated by phosphatidylcholine (PC) of 1426 mg/100g (41.3%).

Keywords: *Canis lupus* var. *familiaris*, male, lipid composition

Introduction

Canis is a genus of the canidae containing multiple extent species, such species, such as wolves, coyotes, jackals and dogs. Species of this genus are distinguished by their moderate to large size, their massive, well-developed skulls and dentition, long legs and comparatively short ears and tails (Heptner and Naumov, 1998). Scientific classification: Kingdom (Animalia), Phylum (Chordata), Class (Mammalia), Order (Carnivora), Family (Canidae), Tribe (Canini), Genus (*Canis*) (Linnaeus, 1758).

The origin of the domestic dog is not clear. It is known that the dog was the first domesticated species (Perri, 2016). The domestic dog is member of the genus *Canis* (canines), which forms part of the wolf-like canids (Lindblad-Toh *et al*., 2005), and is the most widely abundant terrestrial carnivore (Young *et al*., 2011). The closest living relative of the dog is the gray wolf and there is no evidence of any other canine contributing to its genetic lineage (Fan *et al*., 2016). The dog and the extant gray wolf form two sister clades (Vila, 1997), with modern wolves not closely related to the wolves that were first domesticated (Larson and Bradley, 2014). The archaeological record shows the first undisputed dog remains buried beside humans 14,700 years ago (Liane *et al*., 2015), with disputed remains occurring 36,000 years ago (Germonpre, 2009). These dates imply that the earliest dogs arose in the time of human hunter-gatherers and not agriculturists (Thalmann *et al*., 2013). Domestic dogs have been selectively bred for millennia for various purposes (Dewey and Bhagat, 2002).

Despite their descent from wolves and classification as Carnivora, dogs are variously described in scholarly and other writings as carnivores (Hazard, 1982) or omnivores (Dewey and Bhagat, 2002). Unlike obligate carnivores, dogs can adapt to a wide-ranging diet, and are not dependent on meat-specific protein nor a very high level of protein in order to fulfil their basic dietary requirements. Dogs will healthily digest a variety of foods, including vegetables and grains, and can consume a large proportion of these in their diet, however all-meat diets are not recommended for dogs due to their lack of calcium and iron (Dewey and Bhagat, 2002). Comparing dogs and wolves, dogs have adaptations in genes involved in starch digestion that contribute to an increased ability to thrive on a starch-rich diet (Axelsson *et al*., 2013).

Dog meat is consumed in some East Asian countries, including Korea, China (Wingfield-Hayes, 2002) and Vietnam (Vietnam's dog meat tradition, 2001) a practice that dates back to antiquity (Simoons, 1994). It is estimated that 13 – 16 million dogs are killed and consumed in Asia every year (How many dogs and cats are eaten in Asia?, 2012). Other cultures, such as Polynesia and pre-Columbian Mexico, also consumed dog meat in their history. However, Western, South Asian, African, and Middle Eastern cultures, in general, regard consumption of dog meat as taboo. In some places, however, such as in rural areas of Poland, dog fat is believed to have medicinal properties-being good for the lungs for instance (Day, 2009). Dog meat is also consumed in some parts of Switzerland (Schwabe, 1979). Proponents of eating dog meat have argued that placing a distinction between livestock and dogs is western hypocrisy, and that there is no difference with eating the meat of different animals (William, 2002; John, 2002).

In many parts of Nigeria, dog meat has become a delicacy and it is now in high competition with beef and chicken consumption. The present work reported the lipid (fatty acid, sterols and phospholipids) profile of the brain of domestic male dog (*Canis lupus* var. *familiaris*).

Materials and Methods

Collection of samples

Samples of the male dog head were collected from a butcher located at Iworoko Ekiti, Ekiti State, Nigeria in the month of

August, 2015. Iworoko Ekiti is a community located very close to Ekiti State University, Ado – Ekiti at about less than 2 kilometres to the University campus. The samples were authenticated at the Ekiti State University, Ado Ekiti in the Department of Zoology.

Sample treatment

Samples that were authenticated were dissected to remove the brain in the Chemistry laboratory, wrapped in aluminium foil and frozen at - 4ºC for 5 days before being prepared for analysis. The brain was later removed from the aluminium foil, cut into bits for proper oven-drying; the sample was later dried in the oven until constant weight was attained. Drying was for 5h at 70ºC. It was then grounded, sieved and kept in freezer (- 4ºC) pending analysis.

Extraction of lipid

0.25 g of the sample was weighed into the extraction thimble. 200 ml of petroleum ether (40-60°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 (AOAC, 2006). The lipid was extracted for 5 h. The extraction flask with the oil was oven-dried at 105ºC for 1 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°C with 3.4 ml of 0.5 M KOH in dry methanol. The mixture was neutralized by 0.7 M HCl. 3 ml of 14 % boron trifluoride in methanol was added (AOAC, 2006). The mixture was heated for 5 min at 90°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°C, first ramping at 10°C/min for 20 min, maintained for 4 min, second ramping at 15ºC/min for 4 min and maintained for 10 min. The injection temperature was 250°C whilst the detector temperature was 320°C. A capillary column (30 m, 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 standards of fatty acid methyl esters.

Sterol analysis

Sterol was analyzed as described by AOAC (2006). The aliquots of the extracted fat were added to the screw-capped test tubes. The sample was saponified at 90°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 used in extracting the non-saponifiable materials. Three extractions, each with 2 ml of 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 the injection pot of GC. The peaks were identified by comparison with standard sterols. The sterols were analyzed using similar conditions as for fatty acid methyl ester analysis.

Phospholipid analysis

Modified method of Raheja *et al*. (1973) was employed in the analysis of phospholipids. 0.01g of the extracted fat was added to each test tube. To ensure complete dryness of the fat for phospholipid 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°C in water bath for 1 min, 20 sec. The content was allowed to cool to the laboratory temperature and 5 ml hexane was 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 analyzed using an HP 5890 powered with HP gas chromatograph (HP 5890 powered with HP ChemStation rev. AO9.01 [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°C, whilst the detector temperature was 320ºC. A capillary column (30 m, 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 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).

Calculation of fatty acid as food 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 level, values per 100g of food are required. When the content of total fatty acids in food or fat is not given, it is necessary to calculate it by using fatty acid conversion factor (XFA). The conversion factor reflects the ratio between the sum of fatty acids and total lipids (TL) in the food (Weihrauch *et al*., 1977).

$$\text{FACID (g/100g EP)} = \text{TL (g/100g EP)} \times \text{XFA}$$

Total lipid (TL = crude fat) level was multiplied by a conversion factor of 0.561 to convert it to total fatty acids (Anderson, 1976). For fatty acids, precision is best limited to 0.1 g/100g of fatty acids (Greenfiel and Southgate, 2003). Further calculations were the conversion of the edible portion (EP) into two different units of energy: kJ/100g EP and kcal/100g EP.

Result and Discussion

In Table 1 we have the crude fat content and other lipid related calculations of the sample. The value of 12.2 g/100g of the crude fat showed the sample to be a moderate source of fat. However, the total fatty acid had a slightly larger share of 6.87 g/100g (56.3%) whereas other lipids had about 5.33 g/100g (43.7%). The total energy due to 12.2 g/100g crude fat was 453 kJ/100g (110 kcal/100g) in which the total fatty acid had a value of 254 kJ/100g (61.8 kcal/100g). However, other lipid content would just contribute 197 kJ/100g (48.0 kcal/100g) of energy. From literature, the crude fat in turkey-hen brain is 9.21 g/100g and for duck brain it is 8.84 g/100g (Adeyeye and Aye, 2015); in the brain of *Hippotragus equinus* (Roan antelope), crude fat is 8.65 g/100g (Adeyeye and Aye, 2013); all these earlier literature values were lower than the present report. On the other hand, the following brain crude fat results were higher than the present result (g/100g): 42.5 (bull) and 45.2 (chicken) (Adeyeye, 2012); on dry matter

basis, the following were recorded (g/100g) for: 47.3 (cattle brain), 38.1 (sheep brain) and 42.6 (pig brain) (Fornias, 1996).

Table 1: Crude fat and other lipid related calculated values in *Canis lupus* var. *familiaris* male brain

Parameter	Unit	Value
Crude fat	g/100g	12.2
Total fatty acid[a]	g/100g	6.87
Total energy[b]	kJ/100g	453
Total energy[c]	kcal/100g	110
Total fatty acid energy[d]	kJ/100g	254
Total fatty acid energy[e]	kcal/100g	61.8
Other lipid content	g/100g	5.33
Other lipid energy[f]	kJ/100g	197
Other lipid energy[g]	kcal/100g	48.0

[a]Crude fat x XFA = 12.24 x 0.561; [b]Total energy = crude fat x 37.0; [c]Total energy = crude fat x 9.00; [d]Total fatty acid energy = total fatty acid x 37.0; [e]Total fatty acid energy = total fatty acid x 9.00; [f]Other lipid energy = other lipid value x 37.0; [g]Other lipid energy = other lipid value x 9.00

Table 2: Saturated and monounsaturated fatty acid profile and corresponding edible portion (EP) of the male brain of *Canis lupus* var. *familiaris*

Fatty acid	% total fatty acids	EPg/100g[a]
C2:0	–	–
C3:0	–	–
C4:0	–	–
C5:0	–	–
C6:0	0.00	0.00
C8:0	0.00	0.00
C10:0	0.00	0.00
C12:0	0.00	0.00
C14:0	3.51	0.2411
C16:0	19.6	1.35
C18:0	13.1	0.9000
C20:0	0.4664	0.0320
C22:0	0.4304	0.0296
C24:0	0.0531	0.0036
SFA	37.2	2.56
C14:1 (cis-9)	0.1530	0.0105
C16:1 (cis-9)	4.85	0.3332
C18:1 (cis-6)	23.8	1.64
C18:1 (cis-9)	20.7	1.42
C20:1 (cis-11)	0.4777	0.0328
C22:1 (cis-13)	0.1480	0.0102
C24:1 (cis-15)	0.0531	0.0063
MUFA (cis)	50.1	3.44
C18:1 (trans-6)	0.1678	0.0115
C18:1 (trans-9)	0.0152	0.0010
C18:1 (trans-11)	0.00	–
MUFA (trans)	0.1829	0.0126
MUFA (total)	50.3	3.45

[a]Edible portion of the fatty acid

Table 2 shows the results for saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) of the edible portion (EP) of the domestic male dog brain sample. The SFA was 37.2% of total fatty acids. This 37.2% SFA was contributed mainly from saturated fatty acids: C14:0 (3.51%), C16:0 (19.6%) and C18:0 (13.1%). Minor SFA contributors were C20:0, C22:0 and C24:0. C2:0, C3:0, C4:0 and C5:0 were not detected whereas 0.00% of total fatty acid was observed in C6:0, C8:0, C10:0 and C12:0. SFA with C12:0, C14:0 and C16:0 are known to be primary contributors to elevated blood cholesterol, and so contribute to cardiovascular diseases; C14:0 being the major culprit. SFA with 12, 14 or 16 carbons generally constitute about 25% of the total fat in animal foods. All the 12, 14 and 16 carbons SFA constituted a value of 23.11% being lower than 25% (the usual minimum value in animals). C18:0 (13.1% of total fatty acid) may not be as hypercholesterolemic as the other SFA (apparently because it is converted to oleic acid) (Bonanome and Grundy, 1988).

The C16:0 is most prevalent (usually) SFA in our diet and is present to some extent in essentially all fats. The value of C16:0 in the total SFA was 52.7% showing its pre-eminence in the total SFA. Considering the influence of C16:0 on the lipoprotein profile, 16:0 is intermediate, that is, it can be neutral when placed on a triglyceride molecule with PUFA, MUFA or 18:0, or cholesterol-raising when attached along with 12:0 + 14:0. In high amounts, 16:0 can even raise TC and LDL when substituted for 18:0, MUFA or PUFA in people who already have elevated TC or who eat large amounts of cholesterol. Hence, general advice has been to remove as much SFA from diet as possible (Hayes, 2002); although Enig and Fallon (2000) had enumerated many important roles in the body chemistry. In bull and chicken, the SFA values were low with respective values of 6.11% and 6.54% being contributed by only two SFA: C22:0 (behenic acid) (2.80 – 2.99%) and C24:0 (lignoceric acid) (3.31 – 3.54%) (Adeyeye, 2012). The brain of roan antelope contains 46.3% SFA (Adeyeye and Aye, 2013); also SFA in turkey-hen brain is 41.4 and it is 43.3% in duck brain (Adeyeye and Aye 2015).

The edible portion of the fatty acid was generally a reflection of the various SFA components. The total edible fatty acid contributed by the sample was 2.56 EPg/100g which came mainly from C16:0 (1.35 EPg/100g), C18:0 (0.9000 EPg/100g) and C14:0 (0.2411 EPg/100g).

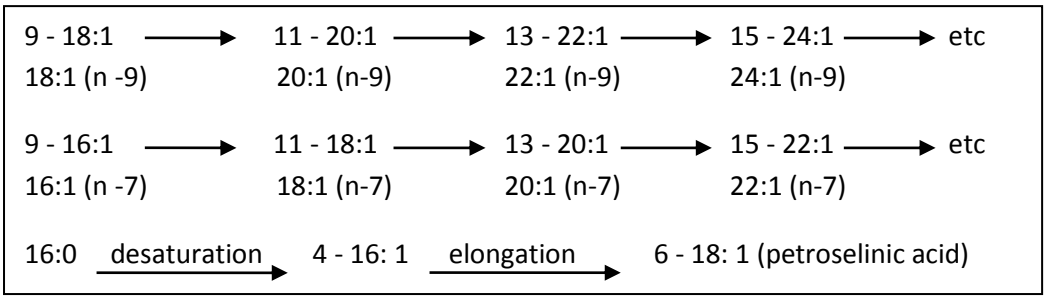
In the same Table 2, we have the values for MUFA contribution. Total MUFA was 50.3% of the total fatty acid (FA). Major contributors for MUFA were C16:1 cis – 9 (4.85%), C18:1 cis – 6 (23.8%), C18:1 cis – 9 (20.7%) and C20:1 cis – 11 (0.4777%); whereas minor contributors were C14:1 cis – 9 (0.1530 %), C22:1 cis – 13 (0.1480%) and C24:1 cis – 15 (0.0531%). Trans-MUFA values were generally low at C18:1 trans – 6 (0.1678%), C18:1 trans – 9 (0.0152%), C18:1 trans – 11 (0.00) and total trans-MUFA was 0.1829% of total FA. The cis-MUFA added to trans-MUFA gave a total value of 50.3% of total FA. Total MUFA values in bull and chicken were very much lower than the present results; also all the MUFA in both brains were contributed by C20:1 (cadoleic acid) (0.962 – 1.03%), C22:1 (erucic acid) (4.62 – 4.94%) and C24:1 (nervonic acid) (3.31 – 3.89%) resulting in the total value for bull as 8.89% and chicken as 9.86% (Adeyeye, 2012). In turkey-hen brain and duck brain, the total MUFA values were: 40.4% (turkey-hen) and 45.4% (duck) (Adeyeye and Aye, 2015) which were both lower than the present report. The total MUFA in the roan antelope brain was 44.3% (Adeyeye and Aye, 2013).

A number of epidemological studies comparing disease rates in different countries have suggested an inverse association between MUFA intake and mortality rates to CVD (Hu *et al*., 1997; Kris-Etherton, 1999). Oleic acid (9c – 18:1 or 18:1 (n – 9) is by far the most abundant monoenoic fatty acid in plant and animal tissue, both in structural lipids and in depot fats. This usual observation was in the reverse in this report as

C18:1 cis – 6 (23.8%) was the highest concentrated monoenoic FA whereas C18:1 cis – 9 (20.7%) was in the second position. 30% of the FA content in conventionally produced beef is composed of oleic acid (Whetsell *et al*., 2003), a MUFA that elicits a cholesterol-lowering effect among other healthful attributes including a reduced risk of stroke and a significant decrease in both systolic and diastolic blood pressure in susceptible populations (Kris-Etherton, 1999). Oleic acid also formed the second highest concentration in turkey and duck brains with values of 13.9 – 15.1% with turkey value being higher than the duck value (Adeyeye and Aye, 2015). Both bull and chicken brains lacked the presence of both C18:1 cis – 6 and C18:1 cis – 9 (Adeyeye, 2012). However, in roan antelope brain C18:1 cis – 6 (18.5%) was lower than C18:1 cis – 9 (22.3%) (Adeyeye and Aye, 2013). Olive oil contains up to 78% of oleic acid, and it is believed to have especially valuable nutritional properties as part of the Mediterranean diet. It has a number of important biological properties, both in the free and esterified form. Oleic acid is the biosynthetic precursor of a family of fatty acids with the (n – 9) terminal structure and with chain-lengths of 20 – 24 or more.

Petroselinic acid (6c – 18:1) formed the largest single group in the MUFA with a value of 23.8% of total FA. Petroselinic acid occurs up to a level of 50% or more in seed oils of the Umbelliferae family, including carrot, parsley and coriander. *In vitro* studies by Weber *et al*. (1995) revealed that triacylglycerols containing petroselinoyl [18:1 (n – 12)] moieties are hydrolysed by pancreatic lipase at much lower rates than other triacylglycerols. Weber *et al*. (1995) data showed that petroselinic acid (6c – 18:1) from dietary triglycrides is absorbed by rats as readily as oleic acid, but the former reduces the concentration of arachidonic acid (AA) in tissue lipids which suggests [in view of earlier studies (Mohrhauer *et al*., 1967)] petroselinic acid mediated inhibition of AA synthesis. Another important MUFA is the 16-carbon palmitoleic acid which has strong antimicrobial properties. It is found almost exclusively in animal fat. It occupied concentration level of 4.85% in the sample forming the third highest concentrated cis-MUFA. C16:1 is beneficial in reducing bad cholesterol (LDL) but it behaves like a saturated fatty acid and not as unsaturated FA in its effect on HDL-cholesterol (Nestle *et al*., 1994). It also reduces the fat deposition in blood vessels and blood clot formation (Grundy, 1994). Gadoleic acid – trivial name for cis-cos-9-enoic acid (20:1 n – 11) is a common but minor constituent of animal tissues and fish oils (as here with a value of 0.4777%) often accompanied by the 13-isomer. It is also found in rapeseed oil and seed oils of related species. Erucic acid is a fatty acid that is apparently responsible for a favourable response of persons with nervous system disorders (Christensen *et al*., 1988). The administration of erucic acid in the diet reduces the serum levels and brain accumulation of very-long-chain SFAs (such as C26:0) responsible for demyelination (Rasmussen *et al*., 1994; Sargent *et al*., 1994). In the Table 2, the edible portions of the fatty acids were individually low due to the corresponding levels of the FAs; SFA value was 2.56 EPg/100g and MUFA value was 3.45 EPg/100g.

The production of longer chain fatty acids of the n – 9 family and n – 7 family as well as the production of petroselinic acid are shown in scheme 1.



Scheme 1: Production of longer chain n-9 and n-7 families and petroselinic acid

Table 3 contains the PUFA n – 6 and n – 3 FA profile and corresponding edible portion (EP) of the sample. In the n – 6 PUFA group, the following FAs predominated (%): C18:2 cis – 9, 12 (3.40), C18:3 cis – 6, 9, 12 (0.3388), C20:3 cis – 8, 11, 14 (0.5443) and C20:4 cis – 5, 8, 11, 14 (4.28) with minor values from C20:2 cis – 11, 14 (0.0661) and C22:2 cis – 13, 16 (0.0531) giving us a total n – 6 PUFA cis as 8.68%. C18:2 cis – 9, trans – 11 was 0.1967% giving grand total value of n – 6 PUFA as 8.88%. The major PUFA concentration in the sample came from n – 6 PUFA (8.88%) because total value of n – 3 PUFA was just 3.61% being contributed majorly by C22:6 cis – 4, 7, 10, 13, 16, 19 with a value of 2.89%. Total n – 6 + n – 3 PUFA gave a value of 12.5%. In the sheep brain, the following values were reported: C18:2 (0.4%), C18:3 (-), C20:3 (1.5%), C20:4 (4.2%) C20:5 (0.7%), C22:5 (3.4%), C22:1 (0.6%), C22:4 (0.8%) and C22:6 (9.5) (Paul and Southgate, 1978). Some of the sheep brain FAs are comparable with the domestic dog brain FAs. The essential FAs affect the fluidity, flexibility and permeability of the membranes; they are the precursor of the eicosanoids, are necessary for maintaining the impermeabilty barrier of the skin and are involved in cholesterol transport and metabolism. Knowledge of the significance of the long-chain PUFA of the n – 3 type, particularly EPA and DHA, for human health has increased considerably since the 1970s (Stansby, 1990a,b). The n – 6 series are derived from LA and the n – 3 series from α-LA. Physiologically more important than these parent FAs are their elongated and denaturatd derivatives of metabolites. The eicosanoids are hormone –like compounds and include prostaglandins, thromboxanes and leukotrienes. Several eicosanoids originate from arachidonic acid (AA) which can be synthesized from LA. By virtue of their competitive inhibition in the enzyme systems, FAs of n – 3 type especially EPA and DHA, can slow down the eicosanoid overproduction and thus prevent or cure health disorders (Lands, 1986; FAO/WHO, 1994; Adeyeye and Aremu, 2017). The PUFA n – 3 FAs have antiatherosclerotic efficacy (Singer, 1994). There is evidence suggesting the long-chain n – 3 PUFAs also have beneficial effects on disease other than those of the heart and of the blood vessels. They include: inflammatory diseases; nephritis, strokes; arthritis, lupus erythematosus;

multiple sclerosis; cancer; skin diseases and asthma (Brenner, 1990).

Table 3: PUFA[a] n-6 and n-3 fatty acid profile and corresponding edible portion (EP) of the male brain of *Canis lupus* var. *familiaris*

Fatty acid	% total fatty acids	EP g/100g
C18:2 (cis-9,12)	3.40	0.2336
C18:3 (cis-6, 9, 12)	0.3388	0.0233
C20:2 (cis-11, 14)	0.0661	0.0045
C20:3 (cis-8, 11, 14)	0.5443	0.0374
C20:4 (cis-5, 8, 11, 14)	4.28	0.2939
C22:2 (cis-13, 16)	0.0531	0.0036
n-6 PUFA (cis)	8.68	0.5960
C18:2 (cis-9, trans-11)	0.1967	0.0135
n-6 PUFA (total)	8.88	0.6098
C18:3 (cis-9, 12, 15)	0.3861	0.0265
C20:3 (cis-11, 14, 17)	0.2853	0.0196
C20:5 (cis-5, 8, 11, 14, 17)	0.0531	0.0036
C22:6 (cis-4, 7, 10, 13, 16, 19)	2.89	0.1985
n-3 PUFA (total)	3.61	0.2482
n-6 + n-3 PUFA	12.5	0.8576

[a]PUFA = polyunsaturated fatty acid (Essential fatty acid)

In Table 3, FAs with significant concentration with corresponding good EPg/100g concentrations came from C18:2 cis – 9, 12; C18:3 cis – 6, 19, 12; C20:4 cis – 5, 8, 11, 14 and C22:6 cis – 4, 7, 10, 13, 17, 19. The EPg/100g was 0.5960 in n – 6 PUFA cis, 0.6098 in n – 6 PUFA (total), 0.2482 in n – 3 PUFA (total) and 0.8576 in n – 6 + n – 3 PUFA. The PUFA values predominated in the bull and chicken brains where n – 6 PUFA cis had values of 36.7% (bull) and 35.3% (chicken); C18:2n – 6 , trans (Rumenic acid) had respective values of 2.30% and 0.113% giving total n – 6 PUFA as 39.0 (bull) and 35.4 (chicken). Also the total n – 3 PUFAs were 46.0% (bull) and 48.1% (chicken) leading to n – 6 + n – 3 PUFA being 85.0% (bull) and 83.5% (chicken) (Adeyeye, 2012). The total PUFA in roan antelope was 9.26% (Adeyeye and Aye, 2013); this is close to our value of 12.5%. N – 6 + n – 3 PUFA in turkey brain is 18.2% and it is 11.3% in duck brain (Adeyeye and Aye, 2015). High consumption of omega – 3 FAs is typically associated with a lower incidenc of depression, a decreased prevalence of age-related memory loss and a lower risk of developing Alzheimer's diseases (Kalmijin, 1997; Yehuda *et al*., 1996; Laugharne, 1996).

Table 4: Energy values (and percentage values) of the fatty acid profile of *Canis lupus* var. *familiaris* male brain (kJ/100g and kcal/100g, EP SFA and MUFA)

Fatty acid	Energy in kJ/100g (% value)	Energy in kcal/100g (% value)
C14:0	8.92 (3.51)	2.17 (3.50)
C16:0	49.8 (19.6)	12.1 (19.5)
C18:0	33.3 (13.1)	8.10 (13.1)
C20:0	1.19 (0.4685)	0.2884 (0.4652)
C22:0	1.09 (0.4291)	0.2661 (0.4292)
C24:0	0.1350 (0.0531)	0.0328 (0.0529)
SFA	94.5 (37.2)	23.0 (37.2)
C14:1 (cis-9)	0.3889 (0.1531)	0.0946 (0.1526)
C16:1 (cis-9)	12.3 (4.84)	3.00 (4.84)
C18:1 (cis-6)	60.5 (23.8)	14.7 (23.7)
C18:1 (cis-9)	52.6 (20.7)	12.8 (20.6)
C20:1 (cis-11)	1.21 (0.4764)	0.2954 (0.4765)
C22:1 (cis-13)	0.3762 (0.1481)	0.0915 (0.1476)
C24:1 (cis-15)	0.1350 (0.0531)	0.0328 (0.0529)
MUFA (cis)	127 (50.1)	31.0 (50.1)
C18:1 (trans-6)	0.4265 (0.1679)	0.1038 (0.1674)
C18:1 (trans-9)	0.0386 (0.0152)	0.0094 (0.0152)
C18:1 (trans-11)	–	–
MUFA (trans)	0.4648 (0.1829)	0.1131 (0.1829)
MUFA (total)	128.0 (50.3)	31.1 (50.3)

In Table 4 we have energy values and their percentage levels of the FAs in kJ/100g and kcal/100g EP for the SFA and MUFA. It is interesting to note that the percentages of the energy values were correspondingly equivalent to the FA values. Values that were significant in the fatty acid levels were also significant in the energy levels. Most energy values were low. All the observations made in Table 4 applied to the observations in Table 5 with exception that energy values in Table 5 were generally lower than in Table 4.

Table 5: Energy values (and percentage values) of the fatty acid profile of *Canis lupus* var. *familiaris* male brain in kJ/100g and kcal/100g (EP n-6 PUFA and n-3 PUFA)

Fatty acid	Energy in kJ/100g (% value)	Energy in kcal/100g (% value)
C18:2 (cis-9, 12)	8.64 (3.40)	2.10 (3.39)
C18:3 (cis-6, 9, 12)	0.8612 (0.3391)	0.2095 (0.3379)
C20:2 (cis-11, 14)	0.1680 (0.0661)	0.0409 (0.0660)
C20:3 (cis-8, 11, 14)	1.38 (0.5433)	0.3365 (0.5427)
C20:4 (cis-5, 8, 11, 14)	10.9 (4.29)	2.65 (4.27)
C22:2 (cis-13, 16)	0.1350 (0.0531)	0.0329 (0.0529)
n-6 PUFA (cis)	22.1 (8.62)	5.37 (8.68)
C18:2 (cis-9, trans-11)	0.5000 (0.1969)	0.1216 (0.1961)
n-6 PUFA (total)	22.6 (8.88)	5.49 (8.88)
C18:3 (cis-9, 12, 15)	0.9814 (0.3864)	0.2387 (0.3850)
C20:3 (cis-11, 14, 17)	0.7252 (0.2855)	0.1764 (0.2845)
C20:5 (cis-5, 8, 11, 14, 17)	0.1350 (0.0531)	0.0328 (0.0529)
C22:6 (cis-4, 7, 10, 13, 16, 19)	7.35 (2.89)	1.79 (2.89)
n-3 PUFA (total)	9.18 (3.61)	2.23 (3.61)
n-6 + n-3 PUFA	31.7 (12.5)	7.72 (12.5)

Table 6: Some quality parameters of the fatty acids of *Canis lupus* var. *familiaris* male brain from Tables 2 and 3

Parameter	Value
MUFA/SFA	1.35
PUFA/SFA	0.3360
EPA/DHA	0.0184
n-6/n-3 (LA/α-LA)	8.81
Σn-6/Σn-3	2.46
AA/DGLA	7.86
EPA +DHA	2.89
ESPI[a] (MUFA/PUFA)	4.02
C16:0:C18:1cis-9	0.9469
C18:0:C18:1 cis-9	0.6329
% C16:0 in ΣSFA	52.7
% C18:0 in ΣSFA	35.2
TUFA[b]=(MUFA+PUFA)	62.8
TFA[c] (SFA + MUFA + PUFA)	100
Total EPg/100g	6.87
Total energy (kJ/100g FA)	254
Total energy (kcal/100g FA)	6.18

[a]Essential PUFA status index; [b]Total unsaturated fatty acid; [c]Total fatty acid

Some quality parameters of the FAs of domestic male dog brain extracted from Tables 2 and 3 can be observed in Table 6. The MUFA/SFA ratio is an important aspect of phospholipid compositions and changes to the ratio also have effects on such disease state as cardiovascular disease, obesity, diabetes, neuropathological conditions and cancer. It's value in this report was 1.35 which could be said to be very positive. 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 oil. This is

because the severity of atherosclerosis is closely associated with the proportion of the total energy supplied by SFA and PUFA fats (Honatra, 1974). The value of P/S in this report is slightly low at 0.3360. The n – 6 and n – 3 compete for the same enzymes and have different biological roles; the balance between n – 6 and n – 3 FAs in the diet is of considerable importance (FAO/WHO, 1994). The ratio of n – 6 to n – 3 or specifically LA to α-LA in the diet should be between 5:1 and 10:1 (FAO/WHO, 1994) or 4 – 10 g of n – 6 FAs to 1.0 g of n – 3 FAs (Canadian Government Publishing Centre, 1990). As LA is almost always present in foods, it tends to be relatively more abundant in animal tissues. Our LA/α-LA in this result was close to the ratio of 10:1 with a value of 8.81 under FAO/WHO (1994) rating and also in the CGPC (1990) rating of 4 – 10g (n – 6) FAs to 1.0g of n – 3 FAs we have a value of 2.46. The desaturation and elongation leading to the production of EPA and DHA as well as AA (Fig. 1, Simopoulos, 1991) might not be impaired by the LA/α-LA of 8.81:1. The AA/DGLA had a value of 7.86 which could be said to be good. A high ratio between AA and DGLA as an indicator of Δ - 5 desaturase activity in the skeletal muscle phospholipids has been related to good insulin activity (Benatti *et al*., 2004). The following are used as status markers to reliably assess the functional PUFA status (Benatti *et al*., 2004). The best known marker is mead acid [trivial name for all-cis-icosa-5, 8, 11-trienoic acid (20:3n – 9)].

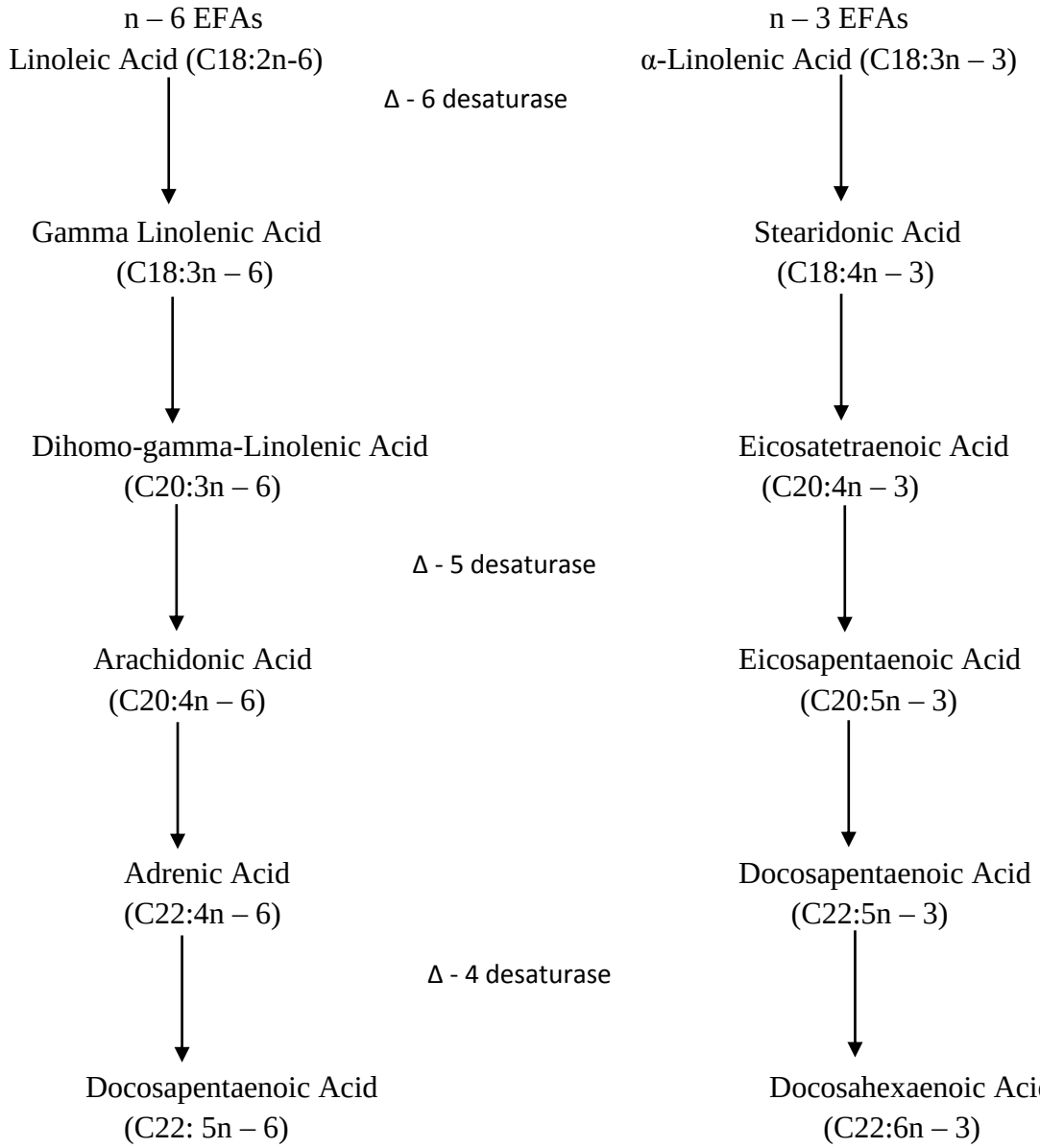


Fig. 1: Linoleic (C18:2n – 6) and α-linolenic (C18:3n – 3) acid metabolism and elongation (Simopoulos, 1991)

The synthesis of this FA is promoted if there are insufficient concentrations of LA and α-LA to meet the need for the synthesis of long-chain PUFA. EPA and DHA inhibit mead acid synthesis; the presence of mead acid indicates a general shortage of all essential PUFA. Mead acid was not detected in the sample, EPA/DHA ratio being 0.0184. Another suitable indicator of essential PUFA status is the PUFA status index (EPSI) which is this ratio: MUFA/PUFA; it had a value of 4.02 which was above average. The higher the EPSI status index the better the essential PUFA status. Finally, if there is a functional shortage of DHA, the body starts to synthesize the most comparable long-chain PUFA of the n – 6 family,

osbond acid (C22:5 n – 6). Therefore, under steady state conditions, the ratio between DHA and osbond acid is a reliable indicator of the functional DHA status (Neuringer *et al*., 1986). Therefore, the PUFA in *Canis lupus* var. *familiaris* brain could not cause functional distress: it contained no osbond acid.

The sterol levels in the sample can be seen in Table 7. Cholesterol was the only significant sterol with a value of 1499 mg/100g and percentage value of 99.9987. Cholesterol level in this sample compared well with the values in egg yolk (1260 mg/100g) and brains generally (2000 – 3000 mg/100g) (Bender, 1992); sheep brain (2200 mg/100g) (Paul and Southgate, 1978); bull brain (874 mg/100g) (Adeyeye, 2012); turkey-hen brain (1234 mg/100g) and duck brain (1267 mg/100g) (Adeyeye and Aye, 2015); brain of roan antelope (1234 mg/100g) (Adeyeye and Aye, 2013). Cholesterol is a high molecular weight alcohol that is manufactured in liver and most human cells. In conjunction with SFA, cholesterol in the membrane gives the cells necessary stiffness and stability. When the diet contains excess of PUFA, this replaces SFA in the cell membrane, so that the cell wall actually becomes flabby. The bile salts are made from cholesterol. Bile is vital for digestion and assimilation of fats in the diet. Cholesterol does act as antioxidant thereby protects us against free radical damage that leads to heart disease and cancer; this is the likely reason for the fact that cholesterol levels go up with age. Babies and children need cholesterol-rich foods throughout their growing years to ensure proper development of their brain and other parts of the nervous system. Dietary cholesterol plays an important role in maintaining the health of the intestinal wall (Alfin-Slater and Aftergood, 1980). This is why low-cholesterol vegetarian diets can lead to leaky gut syndrome and other intestinal disorders. Most authorities, but not all, recommend a reduction in dietary cholesterol to around 300 mg or less per day (Bender, 1992).

Table 7: Sterol levels (mg/100g) of edible portion of *Canis lupus* var. *familiaris* male brain

Sterol	Value	Percentage level
Cholesterol	1499	99.9987
Cholestanol	4.75e-4	3.17e-5
Ergosterol	1.98e-3	1.32e-4
Campesterol	5.50e-4	3.67e-5
Stig-masterol	1.67e-3	1.12e-4
5-Avenasterol	8.86e-3	5.91e-4
Sitosterol	6.43e-3	4.29e-4
Total	**1499**	**100**

Table 8: Phospholipid levels (mg/100g) of edible portion of *Canis lupus* Var. *familiaris* male brain

Phospholipid	Value	Percentage level
Phosphatidylethanolamine (PE)[a]	483	14.0
Phosphatidylcholine (PC)[a]	1426	41.3
Phosphatidylserine (PS,Ptd-L-Ser)	840	24.3
Lysophosphatidylcholine (LPC)	3.57	0.10
Phosphatidylinositol (PI, PtdIns)	699	20.3
Total	**3452**	**100**

[a]PE is also called cephalin and PC is also called lecithin

Phospholipid levels of the EP of *Canis lupus* var. *familiaris* are shown in Table 8. The highest concentrated phospholipid was phosphatidylcholine (PC) with a value of 1426 mg/100g (41.3%) out of 3452 mg/100g. the PC is the 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 (Whitney *et al*., 1994). The closest level to this is 840 mg/100g contributed by phosphatidylserine (PS) forming a percentage level of 24.3. PS (Ptd-L-Ser) 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 (Adeyeye, 2011). Phosphatidylinositol (PI or PtdIns) occupied the third position with a value of 699 mg/100g (20.3%). PI can be phosphorylated to form phosphatidylinositol phosphate (PIP), phosphatidylinositol biphosphate (PIP2) and phosphatidylinositol triphosphate (PIP3). PIP, PIP2 and PIP3 are collectively called phosphoinositides. Phosphoinositides play important roles in lipid signaling, cell signaling and membrane tracking. The fourth position is occupied by phosphatidylethanolamine (PE) with a value of 483 mg/100g (14.0%). 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 (Adeyeye, 2011). Lysophosphatidylcholine (LPC) was the least concentrated in the sample (3.57 mg/100g; 0.10%). Partial hydrolysis of PC with removal of only one FA yields a lysophosphatidylcholine molecule.

Conclusion

Canis lupus var. *familiaris* had average level of crude fat, moderate SFA and high MUFA but low in PUFA. Very low trans-FAs were observed. Good ratios were observed for MUFA/SFA, PUFA/SFA, LA/α-LA, AA/DGLA, EPSI and TUFA. All the quality status were met by the PUFA. Cholesterol and phospholipids were both high. Dog meat has become a delicacy in Nigeria, the results of the brain lipid analysis will add to available nutritional information of the dog meat.

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