Available online at www.elixirpublishers.com (Elixir International Journal)

Food Science

Elixir Food Science 54 (2013) 12555-12568

ISSN: 2229-712X

Health implications of the consumption of the dietary lipids in the testes of bulls, bucks and African giant pouch rats

Emmanuel Ilesanmi Adeyeye

Department of Chemistry Ekiti State University, P.M.B. 5363, Ado-Ekiti, Nigeria.

ARTICLE INFO

Article history:

Received: 1 November 2012;

Received in revised form:

3 January 2013;

Accepted: 15 January 2013;

Keywords

Lipids composition,
Bulls,
bucks,
pouch rats,
Testes,
Health implications

ABSTRACT

The levels of fatty acids, phospholipids and sterols were determined in the testes of bulls, bucks (goats) and African giant pouch rats found in Nigeria. Results showed testes weights variations were (gram wet weight): 48.6-48.5 (bulls); 23.4-23.5 (bucks); 5.72-6.04 (African giant pouch rats). Crude fat range was 2.65-3.00 g/100 g dry weight; SFA varied from 27.0-48.0 % of total fatty acids; total unsaturated fatty acids varied from 52.0-73.0 %; PUFA range was 13.2-15.3 %; PUFA/SFA range was 0.274-0.565; MUFA/SFA range was 0.810-2.13; AA/DGLA range was 18.8-74.5; LA/ALA range was 2.81-19.1 whereas n-6/n-3 range was 4.38-10.4 and EPSI (PUFA/MUFA) range was 0.265-0.370. In the phospholipids, lecithin (phosphatidylcholine) was highest in all the samples with values of 236-307 mg/100 g or 44.7-45.7 %. Among the sterols only cholesterol was of any significant level with values of 260-378 mg/100 g or 99.992-99.994 %. Chi-square (X^2) analysis showed that SFA, AA/DGLA and LA/ALA were significantly different among the samples. In phospholipids, the following parameters were signicantly different among the samples: Cephalin, Lecithin, Ptd-L-Ser (PS) whereas all phospholipid parameters were significantly different among themselves in the bulls, bucks and the African giant pouch rats. Cholesterol was also significantly different among the samples.

© 2013 Elixir All rights reserved.

Introduction

The slaughter of meat animals yields many edible by-products, which are valuable sources of protein and other nutrients required in the diet. These by-products are also very important from an economic point of view, since they can make a significant contribution to cost reduction in the meat industry, particularly if they are used as ingredients in meat products.

There is a tendency at present, to utilize these by-products for food, to the persistent food crisis affecting most underdeveloped countries, particularly as regards their low consumption of animal protein, and in relation to the growing problem of environmental pollution.

In many developing countries meat animals are frequently slaughtered only for the carcass, whereas a number of by-products which can be obtained quite easily could help to improve the supply of low-cost, high-protein foods for people.

As regards the utilization of edible slaughter by-products, most studies have concentrated on protein extraction, involving costly processing techniques of little or no practical interest to developing countries. Good profits can be achieved, however, by utilizing by-products as ingredients in meat products [1].

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 main ingredients in meat products.

The adjective edible can have a number of interpretations. By-products which are regarded as edible in a given region could be considered inedible in another. The latter is generally so in affluent countries, where most by-products are regarded as low-quality foods, the consumption of which is restricted to either low-income or specific ethnic groups. The acceptance of by-products is also influenced by the consumer habits, aesthetic preferences, religions precepts and food standards in force in a given region or country [1].

Testes are obtainable in cattle, not in pigs, but also obtainable in sheep and consumption is direct as meat products [1]. By-product yields vary widely depending on species, sex, live weight and method of recovery.

The testes (testicles) are the primary organs of reproduction in males. Testes are considered primary because they produce male gametes (spermatozoa) and male sex hormones (androgens). Testes do not remain in the body cavity but descend from their site of origin, near the kidneys, down through the inguinal into the scrotum [2]. The testis of the bull is 10-13 cm long, 5-6.5 cm wide and weighs 300-400 g. The testis is of similar size in boars, but is smaller in rams, bucks (goats) and stallions.

The testicles of calves, lambs, cocks, and other animals are eaten in many parts of the world, under a wide variety of euphemistic culinary names. Testicles are a by-product of the castration of young male animals raised for meat, so were probably a late spring seasonal specialty [3], though nowadays they are generally frozen and available year-round.

Testicles are cooked in a variety of ways: sautéed and sauced, fricasseed, deep-fried with breading or batter, in pies, poached, roasted, etc. Before cooking, they are generally scalded, skinned and soaked in cold water [3].

Testicles are known by a wide variety of euphemisms, including ‘stone’, ‘mountain oysters’, ‘prairie oysters’, etc. [3].

Tele:
E-mail addresses: eiadeyeye@yahoo.com

© 2013 Elixir All rights reserved

Lamb testicles in particular are often called 'lamb fries' or simply 'fries', the French term animelles is occasionally encountered. In UK, sweetmeat is commonly used (compare sweetbread). In the Greece and Cyprus, testicles (Greek α μελετητα) are often served grilled. In the USA, bull testicles are usually served breaded and deep-fried as an appetizer, under the name "Rocky Mountain oysters". In Okinawa, Japan, goat testicles are usually boiled and sliced, into sashimi, it is called 'Tamaashi' [3].

The literature is very lean on information concerning works on testicles, some available are mostly biological reports. They included: Costa and Silva who studied the wild boars seminiferous tubules morphometric and functional characteristics [4]; Millar and Fairall [5], they determined the effects of different planes of nutrition on spermatogenesis and androgenesis in males during the mating season, and to establish whether there were associated changes in anterior pituitary and hypothalamic hormone secretion,; Cook et al. [6] determined the effects of a recombinant fussion protein anti-GnRH vaccine on testicular development, feedlot performance and carcass quality of beef bulls; Lin et al. [7] examined the composition of the phospholipid molecular species of ethanolamine and choline glycerophospholipids, including their diacyl, alkanylacyl and alkylacyl subclasses, in the testis of prepubertal (juvenile, 2 years old) and young adult (7-8 years old) monkeys; Wallace and Lascelles [8] analysed the jugular blood plasma and testicular lymph collected from conscious rams.

The present work reports on the dietary lipids composition of the testes of bulls, bucks (goats) and African giant pouch rats. It is hoped that this work will contribute information to consumers of testes on their dietary lipids profiles and may likely contribute to information on food composition tables.

Materials and methods

The bulls and bucks testes samples were obtained fresh from the butchers at the butchers' market in Ado Ekiti, Nigeria whereas testes of African giant pouch rats came from the whole organism as obtained from a local animal hunter commissioned for the purpose.

The samples were properly washed to remove blood, dirt and other impurities which could affect the desired use of the samples. The testes were weighed fresh. The samples were then cut into bits and dried until constant weight. The dried samples were grinded into flour and kept in the freezer pending analyses.

About 0.25 g of each sample part was weighed into the extraction thimble and the fat extracted with petroleum ether (40-60 ^{o}C boiling range) using a Soxhlet apparatus [9]. The extraction lasted 5-6 h.

The crude fat extracted was converted to the methyl ester using the boron trifluoride method [10]. The gas chromatographic conditions for the analyses of FAME (fatty acid methyl esters) were as follows: The GC was the HP 5890 powered with HP ChemStation rev AO9.01 [1206] software [GMI, Inc, Minnesota, USA] fitted with a flame ionisation detector (FID). A split injection with split ratio of 20:1 was used. GC inlet temperature was 250 ^{o}C with an oven programme of 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 (maintained for 10 min) and detector temperature at 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. The peaks were identified by comparison with standard fatty acid methyl esters. Carrier gas used was nitrogen.

The sterol analysis was as described by AOAC [11]. The aliquots of the processed fat were added to the screw-capped test tubes. The samples were 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 and 2 ml of hexane was added in extracting the non-saponifiable materials. The 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 chromatography analysis and 1µl was injected into the injection pot of GC. The GC conditions of analyses were similar to the GC conditions for methyl esters analyses. The peaks were identified by comparison with standard sterols.

The method of Raheja et al. [12] was employed in the analyses of the phospholipids content determination. The GC conditions for analyses of phospholipids were similar to FAME analyses except in the following: Column type was HP5, oven programme initial temperature at 50 ^{o}C, second ramping at 15 ^{o}C/min for 4 min, maintained for 5 min and the detector was pulse photometric detector (PFPD).

For the purpose of ensuring the accuracy of the results obtained, the followings 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 fatty acids, sterols and phospholipids. Correlation is a statistical index that shows the quality assurance of the calibration curve performed. It was prepared with the Hewlett Packard Chemistry (HPCHEM) software (GMI, Inc 6511 Bunker Lake Blud Ramsey, Minnesota, 55303, USA).

Statistical analyses [13] were carried out to determine mean, standard deviation, coefficient of variation in per cent. Further statistical analysis was carried out using the Chi-square (X^2) method as appropriate, the α value for the X^2 was $\alpha_{=0.05}$.

Results and discussion

The weight levels (g wet weight) of the testes are shown in Table I. Wide variation existed among the samples with coefficient of variation per cent (CV %) of 82.6. The testes weights were reflections of the live weight of the samples from where they were obtained, bull testes > buck testes > African giant pouch rat testes. There is literature on data showing the yields of testicles obtained in Cuba: cattle (0.46 kg, average live weight: 380 kg); pigs (-); sheep (0.22 kg, 27 kg average live weight) [14, 15]. Costa and Silva [4] got a testis weight of 20.2±3.7 g from wild boars. In the investigation of Millar and Fairall [5] they got testes weights (g) as follows: sexually active hyrax: high plane diet (86.2 ±8.2); low plane diet (48.2±5.2) and sexually quiescent hyrax (5.6±1.8). The present work had bull testes (48.5±0.049) close to the value of 48.2±5.2 g reported for sexually active hyrax on. low plane diet [5] and also the African giant pouch rat testis of 23.4±0.06 g was close to 20.2±3.7 g from wild boars [4].

In Table II the testes crude fat content (dry weight) levels are shown. The values were close with CV % of 6.20. Again the trend in testes sizes also showed in the crude fat content with trend: bull (3.00 g/100 g) > buck (2.82 g/100 g) > pouch rat (2.65g/100 g). We also have in literature the proximate composition and energy value of testicles from cattle: water

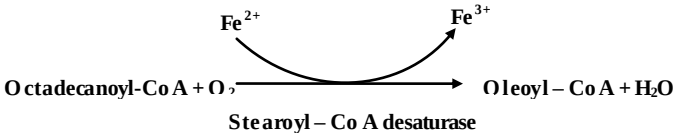
(86.8 %), protein (10.3 %), fat (1.7 %), energy value (cal) (56) [16, 17].

Results in Table III depict the levels of saturated fatty acid (SFA) in the three testes samples. The following fatty acids (FAs) were not detected: C2:0, C3:0, C4:0 in all the samples but C5:0 was not detected in bull and pouch rat but recorded 0.00% in the buck testes. Also, the following FAs recorded 0.00 % level in the samples: C6:0, C8:0, C10:0 and C12:0. Palmitic acid (C16:0), was the overall highest saturated FAs in all the samples ranging between 17.9 – 25.4 % with a trend of buck > bull > African giant pouch rat. This acid accounts for 27% of the FAs in beef. There is a very strong evidence that palmitic acid raises serum cholesterol levels [18] and that this occurs predominantly by increasing bad cholesterol (LDL) levels. This fatty acid accounts for most cholesterol-raising activity from fat sources, thereby increasing the risk of atherosclerosis, cardiovascular diseases, and stroke [19]. Stearic acid (18:0) is the second highest SFA with range value of 5.40-20.0 % and a trend of bull > buck > African giant pouch rat. The acid accounts for about 18 % of the FA in beef. Several studies have shown that the stearic acid effect on total cholesterol is minimal and not detrimental to human health [20, 21, 22, 23]. For practical purposes, stearic acid is essentially neutral in its effects on serum total cholesterol, similar to oleic acid [18]. It is not clear why stearic acid does not raise cholesterol level as do other SFAs. A possible reason could be that it is rapidly absorbed into tissue compared with other SFAs [18]. However, it has been observed in dogs, rats, and hamsters that stearic acid or stearic acid-rich glycerides are absorbed less efficiently than SFAs of shorter chain length or their glycerides [24]. Some investigators have speculated that stearic acid may be thrombogenic (causes blood clotting). This effect has not been proven [18]. Also, the effects of stearic acid on hypertension, cancer, obesity and other illnesses are unknown [25]. Beef is also a source of two SFAs, lauric (C12:0) and myristic (C14:0), that are related to human health issues. Lauric and myristic FAs are responsible for raising bad cholesterol levels in blood serum [18] and have been shown to be strongly correlated with early heart attack [26]. However, the percentages of lauric (0.00-0.00 %) and myristic (0.00 -1.48 %) acids in testes were small. These results suggest that testes have no more cholesterol-raising effect than chicken or fish and therefore, testes need not to be eliminated from cholesterol-lowering diets [27]. Other minor SFAs were arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0) were all low in values with respective levels of 0.461-1.83 %, 0.426-1.69 % and 0.053-0.208 %. The lowest CV % was recorded in C16:0 (18.8 %) and highest in C14:0 (88.3 %) and others in between recorded 54.6-71.5 % (for SFAs having percentage values greater 0.00 %).

The results for the monounsaturated fatty acids (MUFAs) are shown in Table IV. Petroselinic acid (6c-18:1) occurs up to a level of 50 % or more in seed oils of the Umbelliferae family, including carrot, parsley and coriander. Kleiman and Spencer [28] gave the levels of petroselinic acid (PA) as 24-37 % in the various species of Umbelliferae and Araliaceae. The unusual position of the double bond in this FA provides an opportunity for production, through cleavage of this bond, of valuable raw materials- lauric and adipic acids. The US has no domestic source of lauric acid and, in 1979, imported about half billion Ib [29] of lauric-rich oils, about half of which goes into surfactants and the other half into edible products. Petroselinic acid range in the present samples was 16.3-24.2 %. The data of Weber et al. [30] on fat absorption and fecal excretion indicate that petroselinic acid from dietary triacylglycerols is absorbed as readily as oleic acid. Reduction in the concentration of arachidonic acid in the lipids of heart, liver and blood with concomitant increase in the concentration of linoleic acid suggests petroselinic acid-mediated inhibition of arachidonic acid synthesis.

Trans-18:1 levels in the samples were trans petroselinic acid (C18:1 trans-6) (0.166-0.657 %), elaidic acid (C18:1 trans-9) (0.015-1.49 %), vaccenic acid (C18:1 trans-11) (0.00-0.00 %). Tissues of ruminant animals, such as cows, sheep and goats, can contain a number of different 18:1 isomers like: C18:1 trans-9 (5.0 %) and C18:1 cis-9 (85 %), C18:1 trans-11 (47 %) and C18:1 cis-11 (47 %) [31] with the cis-isomers, 9-and 11-18:1 slightly predominating as might be expected. 11t-18:1 makes up 50 % of trans monoenes in ruminant tissues (which can comprise 10-15 % of the total monoenes or 3-4 % of the total fatty acids. In the present report C18:1 trans-11 had a range of 0.00-0.00 % of the total fatty acids. cis-Vaccenic acid [11c-18:1 or 18:1 (n-7)] is a common monoenoic fatty acid of bacterial lipids, and it is usually present but as a minor component of plant and animal tissue. It is occasionally a more abundant constituent of plants, for example those containing appreciable amounts of its biosynthetic precursor, 9-16:1 (e.g. the fruit of sea buckthorn). Although cis-vaccenic acid was not in these results, however its biosynthetic precursor (9-16:1) had values range of 2.13-6.90 %. Note that vaccenic acid per se is the trans isomer. 11-cis-eicosenoic acid [11-20:1 or 20:1 (n-9), gondoic] is a common if minor constituent of animal tissues and fish oils, often accompanied by the 13-isomer. It is also found in rapeseed oil and seed oils of related species. In the present results, while gondoic acid [C20:1 (cis-11)] had a range of 0.472-1.87 % the values for erucic acid [C22:1(cis-13)] range from 0.053-0.208 %.

In nearly all higher organisms, including many bacteria, yeasts, algae, plants and animals, double bonds are introduced into fatty acids by an aerobic mechanism that utilizes preformed fatty acids as the substrate. Molecular oxygen and a reduced pyridine nucleotide (NADH or NADPH) are required cofactors. Thus in animals and yeasts, the coenzyme A ester of octadecanoic (stearic) acid is converted directly to oleoyl-CoA by a concerted removal of hydrogen atoms from carbons 9 and 10 (D-stereochemistry in each instance). The stearoyl-CoA desaturase system is in the endosplasmic reticulum membrane with the active centre exposed to the cytosol, and consists of three proteins, cytochrome b5 reductase, cytochrome b5 and the desaturase, which contains two atoms of iron at the active site.



Membrane- bound enzymes are notoriously difficult to purify, but the evidence suggests that the yeast Δ9 desaturase consists of two membrane spanning regions with the bulk of the protein protruding into the cytosol. The enzyme has much in common with hydroxylases and contains eight essential histidine residues that coordinate with the di-iron centre at the active site. The cytochrome b5 component is fused to the desaturate and is believed to facilitate electron transfer from NADH reductase to the catalytic di-iron core. Palmitoleate is synthesized from palmitate by a similar mechanism. Subsequently, oleate can be

chain elongated by two carbon atoms to give longer-chain fatty acids of the (n-9) family, while palmitoleate is the precursor of the (n-7) family of fatty acids. In mammalian systems the elongases are known to be distinct enzymes that differ from those involved in the production of longer-chain polyunsaturated fatty acids. Alpha- and beta-oxidation can also occur to give shorter chain components of the two families.

9-18:1 ⟶ 11-20:1 ⟶ 13-22:1 ⟶ 15-24:1 ⟶ etc.

18:1(*n*-9) 20:1(*n*-9) 22:1(*n*-9) 24:1(*n*-9)

9-16:1 ⟶ 11-18:1 ⟶ 13-20:1 ⟶ 15-22:1 ⟶ etc.

16:1(*n*-7) 18:1(*n*-7) 20:1(*n*-7) 22:1(*n*-7)

Petroselinic acid (6-18:1) in seed oils of the Umbelliferae is synthesised by an enzyme that removes hydrogens from position 4 of palmitate, before the resulting 4-16:1 is elongated by two carbon atoms.

16:0 desaturation 4-16:1 elongated 6-18:1 petroselinic acid

Elaidic acid can raise bad cholesterol (LDL) in serum [32, 33, 23]. Trans-fatty acids do not have beneficial properties compared to cis-fatty acids. Trans-fatty acids may behave similar to SFAs. Studies have shown that foods enriched in C18:1 trans resulted in higher bad cholesterol (LDL) levels compared with C18:1 cis [34, 23]. Whereas C18:1-trans raised bad cholesterol (LDL) equivalent to SFAs, it had no effects on good cholesterol (HDL) [23]. Trans-vaccenic acid is important in the human bodies' production of conjugated linolenic acid (CLA). Palmitoleic acid (2.13-6.90 %) is also found in rich amounts in macadamia nuts, olive, canola and peanut oils. This monounsaturated fatty acid is beneficial in reducing bad cholesterol (LDL) and it behaves like a saturated and not as unsaturated fatty acids in its effect on HDL cholesterol [35]. It also reduces the fat deposition in blood vessels and reduces blood clot formation [18]. As shown earlier, all these C18: fatty acids may be elongated and desaturated in adipose tissue to produce long chain fatty acids (C22 and C20), which are beneficial to human health [36]. Oleic acid (C18:1) is the primary monounsaturated fatty acid in beef and accounts for about 33 % of the FA in beef. In the present report oleic fatty acid had a range of 15.9-21.2 % with the following trend: African giant pouch rat > buck > bull testes values. The FA is also found in rich amounts in olive, canola and peanut oils. Available evidence indicates that while most SFAs raise serum cholesterol concentrations the oleic acid does not [37]. For practical purposes, it is convenient to use the neutrality of oleic acid as a baseline with which to judge the responses of other FAs. The fact that the body synthesizes a large quantity of oleic acid suggests that it has a variety of biological uses, and to this extent the concept of neutrality of oleic can be extended to imply safety [18]. In several studies on the relative carcinogenicity of fatty acids or their ability to suppress the immune system, oleic acid was the FA with the least negative effect [18]. One reason why oleic acid may not raise serum cholesterol concentrations is because it is a favoured substrate for the liver enzyme that converts cholesterol to an inactive form (the Acyl CoA transferase: cholesterol acyltransfrerase) [18].

Table V depicts the polyunsaturated fatty acid (PUFA) n-6 and n-3 composition of the testes samples. The major important polyunsaturated FAs found in beef are linoleic acid (LA) (C18:2) (about 3.5 %), alpha – linolenic acid (ALA) (C18:3) (1.5 %), arachidonic acid (AA) (C20:4) (about 1 %), eicosapentaenoic acid (EPA) (C20:5) (< 1 %), docosapentaenoic acid (DPA-3) (C22:5) (< 1 %) and docosahexaenoic acid (DHA) (C22:6) (< 1 %) [38]. ALA (C18:3) is classified as a short-chain omega-3 FA and is also found in nuts and seeds. EPA, DPA-3 and DHA are found predominantly in foods of marine origin and are classified as long-chain omega-3 FAs. The meat and milk of grazing animals has been reported to contain significantly more omega-3 fatty acids than does meat and milk of animals fed conserved forages and grains. This higher content of omega-3 FAs may be beneficial to human health [39, 40]. Linoleic acid (C18:2) (about 3.5 %) is also found in corn, sunflower oil, safflower oil and soybeans. AA (C20:4) (about 1 %) is found in brain, liver, glandular and egg lipids. Both of these fatty acids belong to the omega-6 family of fatty acids. The present levels of LA were higher than the literature value cited above, the range was 4.25-8.79 %, this was also the case in AA with values of 2.07-5.34 %. For the n-3 FAs, EPA range was 0.053-0.208 % whereas DHA was ND to 0.966 % and ALA range was 0.382-1.51 %.

The omega-3 FAs, like ALA (C18:3), appear to have little direct value for human health. However, the human body can add 2 or 4 carbons to these 18-carbon chains fats to produce 20- or 22-carbon chain omega-3 FA. Thus, ALA (C18:3) is a precursor for EPA (C20:5) and DHA (C22:6) FAs, which are important for human health. It has been suggested that ALA has a beneficial effect on cardiovascular heart disease [41, 25]. However, other studies reported no evidence of ALA having a positive effect on cardiovascular heart disease [42, 43]. Although ALA supplementation causes an increase in the blood and plasma levels of ALA, EPA and DPA, no benefit has shown on either risk factors for cardiovascular diseases or on the secondary prevention of cardiovascular heart disease [43]. ALA (C18:3) may help balance LA (C18:2) and be beneficial.

For many years linoleic acid (LA) (C18:2; omega-6) was thought to be the preferable FA for the diet because it was considered to be the most effective cholesterol-lowering FA. However, despite an increase in LA intake (from about 4 % to 7 %), there has been a growing reservation about recommending its consumption, due to no proven long-term safety [18]. In humans, high supplemental intakes of LAs can lower good cholesterol concentration and may increase the risk for cholesterol gallstones. In addition, the presence of LA in bad cholesterol lipids makes them more prone to oxidation, which could promote atherosclerosis. Because of these detrimental effects, current recommendations have been moderated and now caution that intakes of this fatty acid should not exceed current concentrations (about 7 % of total energy intake) [18]. However, surprisingly, recent information from the American Heart Association indicates that LA has a noticeable effect on lowering cholesterol further than oleic and palmitic acids when plasma cholesterol levels are high (> 200 mg/dl). They suggest that at a 10 % calorie intake in the form of PUFAs, LA achieves a maximal effect on cholesterol lowering [44]. It also has been suggested [45] that a higher intake of LA appears to protect against stroke, possibly through potential mechanism of decreased blood pressure, reduced platelet aggregation and enhance deformability of erythrocyte cells.

LA and ALA are FAs that can be transformed to CLA (conjugate linolenic acid) by bacteria in the rumen [46] hence, rumenic acid. CLA is a collective term describing a mixture of positional and conjugated isomers of LA involving a double bond at positions 8 and 10, 9 and 11, 10 and 12, or 11 and 13 [47]. Each of these positional C18 isomers can occur in cis-trans, trans-cis, cis-cis and trans-trans forms [47]. In beef and milk

samples, the cis -9, trans- 11 and the cis-10, trans-12 CLA are the predominant forms. Interest in CLA research has increased in the past few years as a result of reports of CLA consumption providing several health benefits [48]. Because plants do not synthesize CLA, ruminant fats in milk or meat are the primary dietary CLA sources for humans [49]. The predominant CLA in ruminant fats is the cis-9, trans-11 isomer that accounts for more than 80 % of total CLAs [50]. It has been found that CLA is an antioxidant, which also reduces circulating cholesterol in mice [51]. Other literature reports that CLA has a positive effect by reducing cardiovascular risk, protects against atherosclerosis, is anti-carcinogenic, reduces intake, reduces body content of adipose tissue and lipid, and enhances the immune system [52, 53, 54, 51, 55]. The level of CLA in the testes samples ranged from 0.195-0.771 % with the trend as pouch rat > bull > buck.

It has been suggested that AA (C20:4) is detrimental to human health [56]. However, it promotes inflammation that is an important protective response when one is injured. It also forms the basis of anti-inflammatory prostaglandins that the body uses, to reduce inflammation [57]. The amount of AA in beef is very low (< 0.5 % of total fat); thus, great amounts of beef have to be consumed to detect any contradictory effect. AA in the testes was much higher than 0.5 % in the beef, values ranged from 2.07-5.34 % with the trend of pouch rat > buck >bull.

Two other omega-3 FAs in the samples, DHA (C22:6) and EPA (C20:5), have been reported to have health benefits [58]. These omega-3 FAs have been shown to prevent cancer [59], and cardiovascular disease [60], as well as being therapeutic for arthritis [61], autoimmue disease [62], inflammatory effects [63] and depression [64]. DHA is also important during pregnancy for infant and brain development [65, 66] and reduces the incidence of premature birth [67]. EPA lowers blood cholesterol [69] and reduces blood clotting, allowing better blood circulation [69]. Thus, there is a benefit from the production of additional DHA and EPA by the body's elongation and desaturation of shorter chain FAs (C18:3 omega-3; 18:2 omega-6; 18:1) in humans as mentioned above. In the testes EPA was detected in all the three samples (0.053-0.208 %) whereas DHA was only detected in the buck (0.966 %).

Erucic acid (C22:1; about 1 % in beef fat) is a fatty acid that is apparently responsible for a favourable response of persons with nervous system disorders [70]. The level of erucic acid in this report ranged from 0.146-0.580 % with a trend of pouch rat > bull > buck. The administration of erucic acid in the diet will reduce the serum levels and brain accumulation of very long chain SFAs (such as 26:0) responsible for demyelination [71, 72]. Accumulation of certain long-chain FAs is associated with degenerative diseases of the central nervous system, such as behenic acid (C22:0; about 1 % in beef fat) and lignoceric acid (C24:0; about 1 %) as well as that of the unsaturated members of the C22 and C24 group [44]. Behenic acid levels ranged from 0.426-1.69 % in the testes sample; lignoceric acid levels ranged from 0.053-0.208 % in the testes. Accumulation occurs because enzymes needed to maintain turnover of these FAs are lacking [73]. Behenic acid has been detected to be a cholesterol-raising SFA factor in humans [74].

Table VI contains the summary of the results in Tables III, IV and V as well as some ratio values. The relative amounts of PUFA and SFA in dietary oils is important in nutrition and health. The ratio of PUFA/SFA (P/S ratio) is therefore 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 SFAs and PUFAs [75]. The present PUFA/SFA levels ranged from 0.274-0.565 which were low for bull and buck but averagely normal for the pouch rat. From several in vivo and in vitro studies with different animal species it is well known that ALA, LA and oleic acid (18:1n-9) compete for the same Δ^6- desaturase in the metabolic cascade. Dietary studies on rats and other animals have shown that ALA is a strong suppressor of n-6 FA metabolism, whereas 10 times as much LA is required to give an equal suppression of n-3 metabolism [76]. The n-6 and n-3 FAs have critical roles in the membrane structure [77] and as precursors of eicosanoids, which are potent and highly reactive compounds. Since they compete for the same enzymes and have different biological roles, the balance between the n-6 and n-3 FAs in diet can be of considerable importance [78]. The ratio of n-6 to n-3 or specifically LA to ALA in the diet should be between 5:1 and 10:1 [78] or 4-10 g of n-6 FAs to 1.0 g of n-3 FAs [79]. As LA is almost always present in foods, it tends to be relatively abundant in animal tissues. This is supported in the present report as follows: C18:2 (n-6) ranged as follows: 12.0-13.1 % whereas C18:3 (n-3) ranged as 1.15-2.84 %. In turn, these FAs are the biosynthetic precursors in animal systems of C20 and C22 PUFAs, with 3-6 double bonds, via sequential desaturation and chain-elongation steps (desaturases in animal tissues can only insert a double bond on the carboxyl side of an existing double bond [80]). On the overall n-6/n-3 ratios, the values of 4.38-10.4 would serve well than the LA/ALA with values of 2.81-19.1 which fell within the level of either being low or high. Whilst it would be easy for the body to synthesise AA [20:4 (n-6)] from [18:2 (n-6)], it may be difficult to synthesise the n-3 PUFA series especially eicosapentaenoic acid [20:5 (n-3) or EPA] because of the low level of C18:3 (n-3) and so the diet must be enhanced in this PUFA. However, the 2n-6/3n-3 fell within the above ratio as 10.4: 1 (bull), 7.81:1 (buck) and 4.38 (pouch rat). The relative proportion of MUFA/SFA is an important aspect of phospholipids compositions and changes to this ratio have been claimed to have effects on such disease states as cardiovascular disease, obesity, diabetes, neuropathological conditions and cancer. The MUFA/SFA levels in the samples ranged from 0.810-2.13 which were better than in the PUFA/SFA levels. For example, MUFA/SFA have been shown to have cytoprotective actions in pancreatic β-cells. cis-Monoenoic acids have desirable physical properties for membrane lipids in that they are liquid at body temperature, yet are relatively resistant to oxidation. They are now recognised by nutritionists as being beneficial in the human diet.

Several clinical studies show that insulin resistance is related to muscle phospholipids fatty acid composition [81, 82, 83]. Insulin resistance is characterized by specific changes of the composition of FAs in the serum lipids and in the skeletal muscle membranes. Impaired insulin sensitivity is associated with high proportions of palmitic acid (16:0) and low levels of LA in serum. In addition, there are apparent changes of the FA desaturase activities, suggesting an increased activity of the Δ-9 and Δ-6 desaturases and a decreased activity of the Δ-5 desaturase. Experimental studies have indicated that insulin activates the Δ-9 and Δ-6 desaturases. In experimental diabetes and in spontaneously diabetic rats, there are reduced activities of Δ-9, Δ-6 and Δ-5 liver microsomal desaturases, which are restored after insulin treatment [84, 85]. Insulin-deficient patents with type-1diabetes have high levels of LA and low levels of the

metabolites including AA in their serum lipids, with an increase of AA and a normalization of the PUFA after insulin treatment [86]. A high ratio between AA and DGLA [dihomo-gamma-linolenic acid, C20:3n-6 (cis-8, 11, 14)], as an indicator of Δ-5 desaturase activity in the skeletal muscle phospholipids has been related to good insulin sensitivity. Moreover, in-vitro studies, evidenced that an increased unsaturation and a decreased ratio of n-6 to n-3 FAs in the muscle membrane are compatible with an increased membrane fluidity, findings that have been linked to the presence of an increased insulin receptors and an increased insulin binding [87, 88]. Table VI gave the AA/DGLA values range as 18.8-74.5 which were all favourable to high AA/DGLA ratio leading to good insulin sensitivity.

For the assessment of essential PUFA status of an individual, the total amount of the various EFA and PUFA in plasma or erythrocyte phospholipids is a useful indicator [89]. In general, if insufficient essential PUFA are available to meet PUFA requirements, the body starts to synthesise certain FAs that are hardly present if the EFA and PUFA status is adequate. Therefore, these FAs can be essential PUFA status markers. The best known marker is Mead acid (C20:3n-9). The synthesis of this FA is promoted if there are insufficient concentrations of LA and ALA 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. No Mead acid (5z, 8z, 11z)-Eicosa-5, 8, 11- trienoic acid was detected in the samples. Another suitable indicator of essential PUFA status is the essential PUFA status index (EPSI), which is the ratio between all essential PUFA (the sum of all n-3 and n-6 FAs) and all nonessential unsaturated FAs (the sum of all n-7 and n-9 FAs). The higher the EPSI the better the essential PUFA status. The EPSI values in the testes ranged from 0.265-0.370. Finally, if there is a functional shortage of DHA, the body starts to synthesise the most comparable long-chain PUFA of the n-6 family, osbond acid (22:5n-6). Therefore, under steady state conditions, the ratio between DHA and osbond acid (all-cis-4, 7, 10, 13, 16) is a reliable indicator of the functional DHA status [90]. Osbond acid was not detected in any of the samples.

The statistical analysis of the results in Table VI as summarised in Table VII showed that the results in the row columns were either significantly different or not at $\alpha = 0.05$. Significant results were indicated in SFA, AA/DGLA and LA/ALA. It should be noted particularly for cases where there are more than two categories or groups that X^2 cannot indicate or specify where the significant difference lies, a situation similar to that found in ANOVA. However, post hoc tests that provide solution to the problem when encountered in ANOVA cannot be applied to chi-square test. In case of X^2-test, the category that contributes the highest proportion is declared as one that differs significantly from others [13]. Hence, significant difference was due to bull in SFA, buck in AA/DGLA and buck in LA/ALA.

Table VIII shows the level of various phospholipids in the samples. Phospholipids are not essential nutrients: they are just another lipid and, as such, contribute 9 kcalories per gram of energy. Minor contributor to the phospholipids level was lysophosphatidylcholine that contributed less than 1.0 mg/100 g in each of the teste samples. Actual values for the lysophosphatidylcholine ranged from 0.116-0.134 mg/100 g (0.019-0.022 %). The total phospholipids level ranged from 516-687 mg/100 g showing the testes to be high in phospholipids content. The highest concentrated phospholipid was lecithin (phosphatidylchlorine) with values ranging from 236-307 mg/100 g or 44.7- 45.7 %; this was slightly followed by Ptd-L-Ser (PS) (phosphatidylserine) with values ranging from 132 – 180 mg/100 g or 25.6 – 26.2 % and for the third position: bull (116 mg/100 g, 16.9 %) in cephalin (PE) (phosphatidylethanolamine) > bull (84.1 mg/100 g, 12.2 %) in Ptd Ins (PI) (phosphatidylinositol); in buck (84.4 mg/100 g, 14.3 %) in Ptd Ins (PI) > buck (84.7 mg/100 g, 14.2 %); in pouch rat (85.4 mg/100 g, 16.5 %) > pouch rat (63.1 mg/100 g, 12.2 %). The CV % were close with values ranging from 0.854 – 30.2.

Lecithin is usually the most abundant phospholipid in animals and plants, often amounting to almost 50 % of the total, and as such it is the key building block of membrane bilayers. This observation is true for lecithin values in these results with percentage values ranging from 44.7 – 45.7 %. Phosphatidylcholines (PC) are a class of phospholipids that incorporate choline as a head group. They are a major component of biological membranes and can be easily obtained from a variety of readily available sources such as egg yolk or soy beans from which they are mechanically extracted or chemically extracted using hexane. They are also a member of the lecithin group of yellow-brownish fatty substances occurring in animal and plant tissues. Phosphatidylcholines are such a major component of lecithin that in some contexts the terms are sometimes used as synonyms. However, lecithin extract consists of a mixture of phosphatidylcholine and other compounds. It is also used along with sodium taurocholate for stimulating fed- and fasted-state biorelevant media in dissolution studies of highly-lipophilic drugs. Phosphatidylcholine is more commonly found in the exoplasmic or outer leaflet of a cell membrane. It is thought to be transported between membranes within the cell by Phosphatidylcholine Transfer Protein (PCTP) [91]. Phosphatidylcholine also plays a role in membrane-mediated cell signalling and PCTP activation of other enzymes [92]. At birth and throughout infancy, phosphatidylcholine concentrations are high (as high as 90 % of the cell membrane), but it is slowly depleted to as low as 10 % of the cellular membrane in the elderly. As is such, some researchers in the fields of health and nutrition have begun to recommend daily supplementation of phosphatidylcholine as a way of slowing down senescence [93] and improving brain functioning and memory capacity [94]. In addition to the increased caloric burden of a diet rich in fats like phosphatidylcholine, a recent report has linked the microbial catabolites of phosphatidylcholine with increased atherosclerosis through the production of choline, trimethylamine oxide and betaine [95]. Cephalin 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. The US Food and Drug Administration (USFDA) has stated that consumption of PS may reduce the risk of cognitive dysfunction in the elderly [96]. Phosphatidylserine (Ptd-L-Ser or PS) is a phospholipid component, usually kept on the inner-leaflet, the cytosolic side, of cell membranes by an enzyme called flippase. When a cell undergoes apoptotic cell death PS is no longer restricted to the cytosolic part of the membrane, but becomes exposed on the surface of the cell. Early studies of PS distilled the chemical from bovine brain. Because of concerns about Bovine Spongiform Encephalopathy, however, modern studies and commercially available products are made from soybeans. The FAs attached to the serine in the soy product are not identical to those in the bovine product, which is also impure. However, preliminary studies indicate that the soy

product is at least as effective as that of bovine origin [97, 98]. PS has been demonstrated to speed up recovery, prevent muscle soreness, improve well-being, and might possess ergogenic properties in athletes involved in cycling, weight training and endurance running. Soy-PS, in a dose dependent manner (400 mg), has been reported to be an effective supplement for combating exercise-induced stress by blunting the exercise-induced increase in cortisol levels [99]. PS supplementation promotes a desirable hormonal balance for athletes and might attenuate the physiological deterioration that accompanies overtraining and/or overstretching [100]. In recent studies, 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 [101]. First pilot studies indicate that PS supplementation might be beneficial for children with attention-deficit hyperactivity disorder [102, 103]. Traditionally, PS supplements were derived from bovine cortex (BC-PS); however, due to the potential transfer of infectious diseases, soy-derived PS (S-PS) has been established as a save alternative. Soy-derived PS is generally Recognised As safe (GRAS) and is a safer nutritional supplement for older persons if taken up to a dosage of 200 mg three times daily [104]. Phosphatidylserine has been shown to reduce specific immune response in mice [105, 106]. The average daily PS intake from the diet in Western countries is estimated to be 130 mg. Annexin-A5 is a naturally-occurring protein with avid binding affinity for PS. Labeled-annexin- A5 enables visualization of cells in the early- to mid-apoptotic state in-vitro or in vivo. Another PS binding protein is Mfge8. Technetium-labeled annexin-A5 enables distinction between malignant and benign tumours whose pathology includes a high rate of cell division and apoptosis in malignant compared with a low rate of apoptosis in benign tumors. In Table IX, the X^2 values at the row column showed the following phospholipids as being significantly different among the samples: cephalin, lecithin, Ptd-L-Ser (PS), and vertical columns for the samples all at $\alpha = 0.05$.

The sterol results in Table X showed the values to be high for cholesterol in all the samples with values ranging from 260-378 mg/100 g representing percentage levels of 99.992-99.994 % making other types of sterols very irrelevant since they constituted 0.006-0.008 % of the total sterols in the samples. Cholesterol is a high-molecular weight alcohol that is manufactured in the liver and in most human cells. Like SFAs, the cholesterol we make and consume plays many vital roles. Along with SFA, cholesterol in the cell membrane gives our cells necessary stiffness and stability. This is why serum cholesterol levels may go down temporarily when we replace SFA with polyunsaturated oils in the diets [107]. Cholesterol acts as a precursor to vital corticosterols, hormones that help us deal with stress and protect the body against heart disease and cancer; and to the sex hormones like androgen, testosterone, estrogen and progesterone. Cholesterol is a precursor to vitamin D, a very important fat-soluble vitamin needed for healthy bones and nervous system, proper growth, mineral metabolism, muscle tone, insulin production, reproduction and immune system function. The bile salts are made from cholesterol. Bile is vital for digestion and assimilation of fats in the diet. Recent research shows that cholesterol acts as an antioxidant [108]. This is the likely explanation for the fact that cholesterol levels go up with age. As an antioxidant, cholesterol protects us against free radical damage that leads to heart disease and cancer. Cholesterol is needed for proper function of serotonin receptors in the brain [109]. Serotonin is in the body's natural "feel-good" chemical, low cholesterol levels have been linked to aggressive and violent behaviour, depression and suicidal tendencies. Mother's milk is especially rich in cholesterol and contains a special enzyme that helps the baby utilise the nutrient. Babies and children need cholesterol-rich foods throughout their growing years to ensure proper development of the brain and nervous system. Dietary cholesterol plays an important role in maintaining the health of the intestinal wall [110]. This is why low-cholesterol vegetarian diets can lead to leaky gut syndrome and other intestinal disorders.

Cholesterol levels in literature from many animal protein sources were either lower or higher than the testes cholesterol levels. Values in mg/100 g were: fish (50-60), egg yolk (1260), meat and poultry (60-120), brain (2000-3000), liver (300-350) [111]; others were rabbit, lean (71), brain, sheep (2200), liver: ox (270), sheep (430), pig (260) and calf (370) [112]. Most authorities, but not all, recommend a reduction in dietary cholesterol to around 300 mg or less per day [111]; only the cholesterol in pouch rat (260 mg/100 g) fell within this value. The X^2-test showed that significant differences ($\alpha = 0.05$) existed among the cholesterol levels in the testes samples.

Quality assurance

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, thus attesting to the quality assurance of the determinations.

Conclusion

The findings of this study showed that the samples testes sizes were functions of the liveweight of the corresponding animals. The crude fat levels were of slight unequal distribution but generally low at 2.65-2.82 g/100 g. The SFA was less than the total unsaturated fatty acids in all the testes samples; the trans C18:1 was also generally low at 0.181-2.15 % making the fats good for human health. The phospholipids were generally high and will promote the good health of its consumers. The cholesterol was the only sterol of significance and was generally slightly higher than the recommended daily intake of 300 mg per day (except in African giant pouch rat). On the whole testes would serve as good animal by-products in dietary fat sources.

References

1. Fornias OV. Edible by-products of slaughter animals. FAO animal production and Health Paper 123. Rome, Italy: FAO of the United Nations, Rome, 1996.

2. Sorenson AM. Animal reproduction: principles and practices. New York, USA: McGraw-Hill; 1979.

3.http://en.wikipedia.org/w/index.php.?title=Testicle(food)&ldid =48310326

4. Costa DS, Silva JFS. Wild boars (Sus scrofa scrofa) seminiferous tubules morphometry. Brazilian Archives of Biology and Technology (An International Journal) 2006; 49(5): 739-745.

5. Millar R, Fairall N Hypothalamic, pituitary and gonadal hormone production in relation to nutrition and the male hyrax. J. Reprod. Fetil. 47: 339-341 (1976).

6. Cook RB. Popp JD, Kastelic JP, Robbins S, Harland R. The effects of active immunization against gnRH on testicular development, feed lot performance and carcass characteristics of beef bulls. Journal of Animal Science. 2000; 78: 2778-2783.

Table I-Weight levels (g wet weight) of the testes of bull, buck and African giant pouch rat

Parameter	Animal			Mean	SD	CV %
	Bull	Buck	Pouch rat			
Testes	48.5±0.04	23.4±0.06	5.88±0.23	25.9	21.4	82.6

SD = standard deviation, CV % = coefficient of variation

Table II-Crude fat levels of the testes of bull, buck and African giant pouch rat (g/100 g dry weight)

Parameter	Animal			Mean	SD	CV %
	Bull	Buck	Pouch rat			
Crude fat	3.00	2.82	2.65	2.82	0.18	6.20

Table III- Saturated fatty acid (SFA) composition of the testes of the bull, buck and African giant pouch rat (% total fatty acids)

Fatty acid	Bull	Buck	Pouch rat	Mean	SD	CV %
Acetic C2:0	-	-	-	-	-	-
Propionic C3:0	-	-	-	-	-	-
Butyric C4:0	-	-	-	-	-	-
Valeric C5:0	-	0.00	-	-	-	-
Caproic C6:0	0.00	0.00	0.00	0.00	0.00	0.00
Caprylic C8:0	0.00	0.00	0.00	0.00	0.00	0.00
Capric C10:0	0.00	0.00	0.00	0.00	0.00	0.00
Lauric C12:0	0.00	0.00	0.00	0.00	0.00	0.00
Myristic C14:0	1.15	1.48	0.00	0.876	0.776	88.3
Palmitic C16:0	25.3	25.4	17.9	22.9	4.29	18.8
Stearic C18:0	20.0	17.3	5.40	14.2	7.78	54.6
Arachidic C20:0	0.740	0.461	1.83	1.01	0.722	71.5
Behenic C22:0	0.682	0.426	1.69	0.142	0.102	71.5
Lignoceric C24:0	0.084	0.053	0.208	0.115	0.082	71.5

- = not detected (or not determined as the case may be).

Table IV – Monounsaturated fatty acid (MUFA) composition of the testes of the bull, buck and African giant pouch rat (% total fatty acids)

Fatty acid	Bull	Buck	Rat	Mean	SD	CV%
Myristoleic C14:1 (*cis*-9)	0.989	0.617	0.600	0.735	0.220	29.9
Palmitoleic C16:1 (*cis*-9)	3.22	2.13	6.90	4.08	2.50	61.1
trans-Petroselinic C18:1 (*trans*-6)	0.266	0.166	0.657	0.363	0.260	71.5
Petroselinic C18:1 (*cis*-6)	17.4	16.3	24.2	19.3	4.30	22.3
Elaidic C18:1 (*trans*-9)	0.024	0.015	1.49	0.511	0.851	167
Oleic C18:1 (*cis*-9)	15.9	20.2	21.2	19.1	2.79	14.6
Vaccenic C18:1(*trans*-11)	0.00	0.00	0.00	0.00	0.00	0.00
Gondoic C20:1 (*cis*-11)	0.757	0.472	1.87	1.03	0.740	71.5
Erucic C22:1 (*cis*-13)	0.235	0.146	0.580	0.320	0.229	71.5
Nervonic C24:1 (*cis*-15)	0.084	0.053	0.208	0.115	0.082	71.5

Table V-Polyunsaturated fatty acid (PUFA) *n-6* and *n-3* composition of the testes of the bull, buck and African giant pouch rat (% total fatty acids)

Fatty acid	Bull	Buck	Rat	CV %
Linoleic C18:2*n-6* (*cis* 9, 12)	8.79	7.30	4.25	34.2
Rumenic C18 : 2*n-6* (*cis-9, trans*-11)	0.312	0.195	0.771	71.5
Gamma-linolenic (GLA) C18:3*n-6*(*cis*-6, 9, 12)	0.536	0.334	1.33	71.5
Eicosadienoic C20 :2*n-6*(*cis*-11, 14)	0.105	0.065	0.259	71.5
Dihomo-gamma-linolenic C20:3*n-6*(*cis*-8, 11, 14)	0.110	0.069	0.273	71.5
Arachidonic C20 :4*n-6* (*cis*-5, 8, 11, 14)	2.07	5.13	5.34	43.7
Docosadienoic C22 :2*n-6* (*cis*-13, 16)	0.084	0.0526	0.208	71.5
Alpha linolenic C18 :3*n-3* (*cis*-9, 12, 15)	0.612	0.382	1.51	71.5
Eicosatrienoic (ETE) C20:3*n-3* (*cis*-11, 14, 17)	0.452	0.282	1.118	71.5
Eicosapentaenoic (EPA) C20:5*n-3 n-3* (*cis*-5,8,11,14,17)	0.084	0.053	0.208	71.5
Docosahexanoic (DHA) (22:6*n-3*(*cis*-4, 7, 10,13,16,19)	-	0.966	-	-

Table VI-Summary of Tables III, IV and V into their appropriate groupings

Parameter	Bull	Buck	Pouch rat	Mean	SD	CV %
SFA	48.0	45.1	27.0	40.0	11.4	28.4
MUFA (*cis*)	38.6	39.9	55.6	44.7	9.45	21.1
MUFA (*trans*)	0.290	0.181	2.15	0.874	1.11	127
MUFA (total)	38.9	40.1	57.7	45.5	10.5	23.2
PUFA (*n-6*)	12.0	13.1	12.4	12.5	0.573	4.57
PUFA (*n-3*)	1.15	1.68	2.84	1.89	0.864	45.7
PUFA (total)	13.2	14.8	15.3	14.4	1.11	7.71
EPSI*	0.338	0.370	0.265	0.324	0.054	16.7
AA/DGLA**	18.8	74.5	19.6	37.6	31.9	84.9
LA/ALA^{+}	14.3	19.1	2.81	12.1	8.38	69.4
n-6/n-3	10.4	7.81	4.38	7.54	3.05	40.4
EPA/DHA^{++}	-	0.054	-	-	-	-
PUFA/SFA	0.274	0.329	0.565	0.389	0.154	39.7
MUFA+PUFA	52.0	54.9	73.0	60.0	11.4	18.9
MUFA/SFA	0.810	0.888	2.13	1.28	0.743	58.2

*= Essential PUFA Status Index; ** Arachidonic acid/Dihomo-gamma-linolenic acid; +=Linoleic acid/Alpha linolenic acid; ++ = Eicosapentaenoic acid/Docosahexaenoic acid.

Table VII-Chi-square (X^2) analysis of the results in Table VI

Parameter	X^2calculated	X^2table at $\alpha = 0.05$	Remark
SFA	6.44	5.99	Result significant
MUFA	4.88	5.99	Result insignificant
PUFA	0.172	5.99	Result insignificant
AA/DGLA	54.2	5.99	Result insignificant
EPSI	0.018	5.99	Result insignificant
LA/ALA	11.6	5.99	Result significant
n-6/n-3	2.46	5.99	Result insignificant
PUFA/SFA	0.428	5.99	Result insignificant
MUFA/SFA	0.865	5.99	Result insignificant

Table $\alpha = 0.05$ is 5.99 at 2 degrees of freedom.

Table VIII-Phospholipid levels (mg/100 g) of the testes of bull, buck and African giant pouch rat

Phospholipid	Bull	Buck	Pouch rat	Mean	SD	CV%
Cephalin (PE)	116 (16.9)	84.7(14.2)	63.1 (12.2)	87.9	26.6	30.2
Lecithin	307 (44.7)	271 (45.3)	236 (45.7)	271	35.7	13.2
Ptd-L-Ser (PS)	180 (26.2)	156 (26.2)	132 (25.6)	156	23.9	15.3
Lysophosphatidyl-choline	0.134 (0.019)	0.122 (0.020)	0.116(0.022)	0.124	0.009	7.32
PtdIns (PI)	84.1 (12.2)	85.4 (14.3)	85.4(16.5)	85.0	0.726	0.854
Total	687 (100)	597 (100)	516 (100)	600	85.3	14.2

PE = phosphatidylethanolamine; Lecithin = phosphatidylcholine; PS = phosphatidylserine,; PI = phosphatidylinositol; Values in brackets are percentage values.

Table IX-Chi-square (X^2) analysis of the results in Table VIII

Parameter	X^2 calculated	X^2 table at $\alpha = 0.05$	Remark
Cephalin (PE)	16.0	5.99^{+}	Result significant
Lecithin	9.38	5.99^{+}	Result significant
Ptd-L-Ser (PS)	7.29	5.99^{+}	Result significant
Lysophosphatidyl-choline	0.001	5.99^{+}	Result insignificant
PtdIns (PI)	0.012	5.99^{+}	Result insignificant
Total	23.7	5.99^{++}	Result significant
Bull	384	9.49^{++}	Result significant
Buck	342	9.49^{++}	Result significant
African giant pouch rat	300	9.49^{++}	Result significant

$^{+}$ = α = 05 table value at 2 degrees of freedom;
$^{++}$ = α = 05 table value at 4 degrees of freedom.

Table X-Sterol levels (mg/100 g) of the testes of bull, buck and African giant pouch rat

Sterol	Bull	Buck	Pouch rat	Mean	SD	CV %
Cholesterol	378(99.994)	350(99.994)	260(99.992)	329	61.6	18.7
Cholestanol	3.97817e-4	0.00	0.00	-	-	-
Ergosterol	2.83683e-4	0.00	0.00	-	-	-
Campesterol	5.04494e-3	5.00840e-3	4.99736e-3	5.0169e-3	2.4903e-5	4.9638e-1
Stigmasterol	1.664034e-3	1.61389e-3	1.59547e-3	1.624463e-3	3.5482e-5	2.18
5Avenasterol	8.8544e-3	8.84749e-3	8.84500e-3	8.848877e-3	4.725e-6	5.339845e-2
Sitosterol	6.39776e-3	6.40409e-3	6.39129e-3	6.397713e-3	6.40e-6	1.00037782e-1
Total	378	350	260	329	61.6	18.7

7. Lin Don S, Neuringer M, Connor WE. Selective changes of docosahexanoic acid-containing phospholipid molecular species in monkey testis during puberty. Journal of Lipid Research 2004; 45: 529-535.
8. Wallace JC, Lascelles AK. Composition of testicular and epididymal lymph in the ram. J. Reprod. Fertil. 1964; 8: 235-242.
9. AOAC. Official methods of analysis 18th ed. Washington DC, USA: Association of Official Analytical Chemists, 2005; 920.39 (A).
10. AOAC. Official methods of analysis 18th ed. Washington DC, USA: Association of Official Analytical Chemists, 2005; 969.33 and 996.06.
11. AOAC. Official methods of analysis 18th ed. Washington DC, USA: Association of Official Analytical Chemists, 2005; 970.51.
12. Raheja RK, Kaur C, Singh A, Bhatia I.S. New colorimetric method for the quantitative estimation of phospholipids without acid digestion. Journal of Lipid Research. 1973; 14, 695-697.
13. Oloyo RA. Fundamentals of research methodology for social and applied sciences. Ilaro, Nigeria: ROA Educational Press; 2001.
14. Venegas O, Pérez D, de Hombre R, Villavicencio Núñez, Rendimientos y calidad de la carne de carnero. Memorias III Conferencia Internacional de Ciencia y Tecnologia de Alimentos. 1992; T-1, 4. 97.
15. González AM, Tablas de rendimiento. Reporte annual. Institute de Investigaciones para la Industria Alimenticia, Cuba, 1989; 1.4.
16. US Department of Agriculture. Composition of Foods: beef products; raw, processed, prepared. Washington, DC: USDA Agriculture Handbook No. 8-10; 1970.
17. Koniecko ES, Handbook of meat analysis 2nd ed. Avery, New Jersey, 1985.
18. Grundy S.M. Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. American Journal of Clinical Nutrition. 1994; 60 (Suppl): 986S-990S.
19. Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca JA, Huth PJ. Dietary conjugate linoleic acid reduces plasma lipoprotein and early aortic atherosclerosis in hypercholestoremic hamster. Artery. 1997; 22, 266-277.
20. Bonanome A, Grundy S. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. New England Journal of Medicine. 318, 244-248 (1988).
21. Zock PL, Katan MB. Hydrogenation alternatives: effects of trans-fatty acids and stearic acid versus linoleic acid on serum lipids and lipoprotein in humans. Journal of Lipids Research. 1992; 33: 399-410.
22. Kris-Etherton PM, Deer J, Mitchell DC, Mustad VA, Russell ME, McDennell ET, Slabsky D, Pearson TA. The role of fatty acids saturation on plasma lipids, lipoproteins: 1. Effects of whole food diets high in cocoa butter, olive oil, soybean oil, dairy butter and milk chocolate on the plasma lipids of young men. Metabolism 1993; 42: 121-129.
23. Judd JT, Baer DJ, Clevidence BA, Kris-Etherton P, Muesing RA, Iwane M. Dietary cis and trans monounsaturated and saturated FA and plasma lipids and lipoproteins in men. Lipids 2002; 37(2): 123-131.
24. Kitchevsky D. Stearic acid metabolism and atherogenesis: history. American Journal Clinical Nutrition. 1994; 60 (Suppl): 997S-1001S.
25. Hu FB, Stampfer MJ, Manson JE, Rimm E.B, Wolk A, Colditz GA, Hennekens CH, Willett WC. Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. American Journal of Clinical Nutrition. 1999; 69: 890-897.
26. Kromhout D, Menotti A, Bloemberg B, Aravanis C, Blackburn H, Buzina R, Dontas AS, Fidanza F, Giamoaoli S, Jansen A. et al., Dietary saturated and trans-fatty acids and cholesterol and 25-year mortality from coronary heart disease: the Seven Countries Study. Preventive Medicine. 1995; 24, 308-315.
27. Denke MA. Role of beef and beef tallow, an enriched source of stearic acid, in a cholesterol-lowering diet. American Journal Clinical Nutrition.1994; 60 (Suppl): 1044S-1009S.
28. Kleiman R, Spencer GF. Search for new industrial oils: XVI. Umbelliflorae- seed oils rich in petroselinic acid. American Oil Chemists' Society. 1982; 59(1): 29-38.
29. US Department of Agriculture. Agricultural Statistics. Washington, DC: USDA; 1979.
30. Weber N, Richter Klaus-Dieter, Schulte E, Mukherjee KD. Petroselinic acid from dietary triacyglycerols reduces the concentration of arachidonic acid in tissue lipids of rats. Nutrient Metabolism,1995; 1563-1568.
31. Hay JD, Morrison WR. Positional isomers of cis and trans-monoenoic fatty acids from ox (steer) perinephric fat. Lipids. 1973; 8, 94-95.
32. Abbey M, Nestel PJ. Plasma cholesterol ester transfer protein activity is increased when trans-elaidic acid is substituted for cis-oleic acid in the diet. Atherosclerosis. 1994; 106: 99-107.
33. Muller H, Jordal O, Seljeflot I, Kieruff P, Kirkhus B, Ledsaak O, Pedersen JI. Effect on plasma lipids and lipoproteins of replacing partially hydrogenated fish oil with vegetable fat in margarine. British Journal Nutrition 1998; 80: 243-251.
34. Nicolosi RJ, Wilson TA, Rogers EJ, Kritchevsky D. Effects of specific fatty acid (8:0, 14:0, cis-18:1) on plasma lipoproteins, early atherogenic potential and LDL oxidative properties in the hamster. Journal Lipids Research. 1998; 39: 1972-1980.
35. Nestel P, Clifton P, Noakes M. Effects of increasing dietary palmitoleic acid compared with palmitic and oleic acids on plasma lipids of hypercholesterolemic men. Journal Lipid Research. 1994; 35: 656-662.
36. Burdge GC, Wootton SA. Conversion of alpha-linolenic and eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Britain Journal of Nutrition. 2002; 88, 411-420.
37. Grundy SM. Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. American Journal of Clinical Nutrition. 1994; 60 (Suppl): 986S-990S.
38. Enser M, Hallett KG, Hewett B, Fursey GA, Wood JD, Harrington G. Fatty acids content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. Meat Science. 1998; 49(3): 329-341.
39. Kelly ML, Berry JR, Dwyer DA, Griinari JM, Chouinard PY, Van Amburgh ME, Bauman DE. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. Journal Nutrition. 1998; 128(5): 881-885.
40. Dhiman TR. Factors enriching CLA concentration in ruminant food product. IFT Annual Meeting Technical Program Abstracts. 1999; 82-84.

41. Ascherio A. Epidemiologic studies on dietary fats and coronary heart disease. American Journal of Medicine. 2002; 113 (Suppl): 9B, 9S-12S.
42. Renaud S, Lanzmann-Petithory D. Dietary fats and coronary heart disease pathogenesis. Curr Atheroscloe Rep. 2002; 4: 419-424.
43. Sanderson P, Finnegan YE, Williams CM, Calder PC, Burdge GC, Wootton SA, Griffin BA, Millward DJ, Pegge NC, Bemelmans WJE. UK food standards agency α-linolenic acid workshop report. British Journal of Nutrition. 2002; 88: 573-579.
44. Whetsell MS. Human health effects of fatty acids in beef. Pasture-Based Beef Systems for Appalachia. Virginia: West Virginia University; 2003.
45. Iso H, Sato S, Umemura U, Kudo M, Koike K, Kitamura A, Imano H, Okamura T, Naito T, Shimamoto T. Stroke. 2002; 33: 2086-2093.
46. Kepler CR, Hirons KP, McNeill JJ, Tove SB. Intermediates and products of the biohydrogenation of linoleic acid by Butyrivibrio fibrisolvens. Journal of Biological Chemistry. 1966; 241: 1350-1354.
47. Eulitz K, Yurawecz MP, Sehat N, Fritsche J, Roach JAG, Mossoba MM, Kramer JKG, Adlof R.O, Ku Y. Preparation, separation and confirmation of the eight geometrical cis/trans conjugated linoleic acid isomers 8, 10-through 11, 13-18:2. Lipids. 1999; 34: 873 -877.
48. Kramer KG, Sehat N, Dugan MER, Mossoba MM, Yurawecz MP, Roach JG, Eulitz K, Aalhus JL, Schaefer AL, Ku Y, Distribution of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed a commercial CLA mixture by gas chromatography and silver ion-high-performance liquid chromatography. Lipids. 1998; 33: 549-558.
49. Herbein JH, Loor JJ, Wark WA. An opportunity for pasture-based dairy farms? Abingdon, VA, Mid-Atlantic dairy grazing field day and workshop; July 11th , 2000.
50. Chin SF, Liu W, Strorkson JM, Pariza M. Dietary sources of conjugate dienoic isomers of linoleic acid, a recognized class of anticarcinogens. Journal of Food Composition and Analysis. 1992; 5: 185-197.
51. West DB, Delany JP, Camet PM, Blohm F, Truett AA, Scineca J. Effect of conjugated linoleic acid on body fat and energy metabolism in the mouse. American Journal of Physiology.1998; 275: R667-R672.
52. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S. et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature. 1994; 372(6508), 739-746.
53. Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca VA, Huth PJ, Dietary conjugate linoleic acid reduces plasma lipoprotein and early aortic atherosclerosis in hypercholestoremic hamster. Artery. 1997; 22: 266- 277.
54. Ip C, Chin SF, Scimeca JA, Pariza M. Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Research. 1991 51: 6118-6124.
55. Cook ME, Miller CC, Park Y, Pariza M. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. Poultty Scieice. 1993; 72: 1301-1305.
56. Barham JB, Edens MB, Fonteh AN, Johnson MM, Easter L, Chilton FH. Addition of eicosapentaenoic acid to γ-linolenic acid-supplemented diets prevents serum arachidonic acid accumulation in humans. Journal of Nutrition. 2000; 130; 1925-1931.
57. Fallon S, Enig MG. Tripping lightly down the prostaglandin pathways. Price-Pottenger Nutrition. Foundation Health Journal. 1996; 20(3): 5-8.
58. Mantzioris E, Clenad LG, Gibson RA, Neuman MA, Demasi M, James MJ. Biochemical effects of a diet containing foods enriched with n-3 fatty acids. American Journal of Clinical Nutrition. 2002; 72: 42-48.
59. Hardman WE. Omega-3 fatty acids to augment cancer therapy. Journal of Nutrition. 2002; 132(11 Suppl): 3508S-3512S.
60. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 2000; 56(8): 365-379.
61. Kremer JM. N-3 fatty acid supplements in rheumatoid arthritis. American Journal of Clinical Nutrition. 2000; 71(1Suppl): 349S-351S.
62. Harbige LS, Fisher BA. Dietary fatty acid modulation of mucosally-induced tolerogenic immune responses. Proc Nutr Soc. 2001; 60(4): 449-456.
63. Grimm H, Mayer K, Mayser P, Eigenbrodt E. Regulatory potential of n-3 fatty acids in immunological and inflammatory processes. British Journal of Nutrition. 2002; 87(Suppl 1): S59:67.
64. Puri BK, Counsell SJ, Hamilton G, Richardson AJ, Horrobin DF. Eicosapentaenoic acid in treatment-resistant depression associated with symptom remission, structural brain changes and reduced neuronal phospholipids turnover. Int J Clin Pract. 2001; 55(8): 560-563.
65. Horrocks LA, Yeo YK. Health benefits of docosahexaenoic acid (DHA). Pharmacol Res. 1999; 40(3): 211-225.
66. Ewinwright R. 2002. In: M.S. Whetsell, Human health effects of fatty acids in beef. Pasture-Based Beef Systems for Appalachia, West Virginia University, 2003; 1-8.
67. Allen KG, Harris MA. The role of n-3 fatty acids in gestation and parturition. Exp Biol Med. (Maywood) 2011; 226(6): 498-506.
68. Pal S, Thomson AM, Bottema CD, Roach PD, Polyunsaturated fatty acids down regulate the low density lipoprotein receptor of human HepG2 cells. Journal of Nutrition and Biochemistry. 2002; 13(1): 55-63.
69. Heller AR, Fisher S, Rossel T, Geiger S, Siegert G, Ragaller M, Zimmermann T, Koch T. Impact of n-3 fatty acid supplemented parenteral nutrition on haemostasis patterns after major abdominal surgery. British Journal of Nutrition. 2002; 87(Suppl): S95-101.
70. Christensen E, Hagve TA, Christophersen BO. The Zellweger syndrome: deficient chain-shortening of erucic acid [22:1(n-9)]. Biochem Biophys Acta. 1988; 959(2): 134-142.
71. Sargent JR, Coupland K, Wilson R. Nervonic acid and demyelinating diseases. Med Hypotheses. 1994; 42: 237-242.
72. Ramussen M, Moser AB, Borel J, Khangoora S, Moser HW. Brain, liver and adipose tissue erucic and very long chain fatty acids levels in adrenoleukodystrophy patients treated with glyceryl trierucate and trioleate oils (Lorenzo's oil). Neurochem Res. 1994; 19: 1073-1082.
73. Lord RS, Bralley JA. Copyright 2001 Metametrix Inc. Metametrix is a service mark registered with the United States Patent and Trademark Office (www.metametrix.com).

74. Cater NB, Denke MA. Behenic acid is a cholesterol-raising saturated fatty acid in humans. American Journal of Clinical Nutrition. 2001; 73(1): 41-44.
75. Honatra D. Dietary fats and arterial thrombosis. Haemostasis. 1974; 2: 21-52.
76. Holman RT. The slow discovery of the importance of omega 3 essential fatty acids in human health. Journal of Nutrition. 1998; 128: 427S-433S.
77. Kinsella JE. Possible mechanisms underlying the effects of n-3 polyunsaturated fatty acids. Omega-3 News. 1990; 5: 1-5.
78. WHO/FAO. Fats and oil in human nutrition. Report of a joint expert consultation. FAO Food and Nutrition Paper 57. Rome: WHO/FAO; 1994.
79. Canadian Government Publishing Center. Nutrition recommendations: the report of the Scientific Review Committee. Ottawa: Canadian Government Publishing Center; 1990.
80. Berg JM, John L, Typoczko L, Lubert S. Biochemistry (6^{th} ed). New York: W.A. Freeman and Company; 2007.
81. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. New Engl J Med. 1993; 328: 238-244.
82. Storen LH, Baur LA, Kriketos Ad, Pan DA, Cooney GJ, Jenkins AB, Calvert GD, Campbell LV. Dietary fats and insulin action. Diabetologia. 1996; 39: 621-631.
83. Vessby B, Tengblad S, Lithell H. Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. Diabetologia. 1994; 37: 1044-1050.
84. Eck MG, Wynn JO, Carter WJ, Faas FH. Fatty acid desaturation in experimental diabetes. Diabetes 1979; 28: 479-485.
85. Mimouni V, Poisson JP. Altered desaturase activities and fatty acid composition in liver microsomes of spontaneously diabetic Wistar BB rat. Biochem Biophys Acta. 1992; 1123: 296-302.
86. Bassi A, Avogadro A, Crepaldi C, Zambon S, Marin R, Macdonald I., Manzato E. Short-term diabetic ketosis alters n-6 polyunsaturated fatty acid content in plasma phospholipids. J Clin Endocrinol Metab. 1992; 81: 1650-1653.
87. Ginsberg BH, Jabour J, Spector AA. Effects of alterations in membrane lipid unsaturation on the properties of the insulin receptor of Ehrlich ascites cells. Biochem Biophys Acta. 1982; 690: 157-164.
88. Grunfeld C, Baird KL, Khan CR., Maintenance of 3T3-L1 cells in culture media containing saturated fatty acids decreases insulin binding and insulin action. Biochem Biophys Res Commun. 1981; 103: 219-226.
89. Hornstra G, Essential fatty acids, pregnancy and pregnancy complications: a roundatable discussion. In Sinclair A., Gibson R. (eds): Essential fatty acids and eicosanoids. Champaign: American Oil Chemists' Society; 1992, pp177-182.
90. Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effects of prenatal and postnatal omega-6 fatty acid deficiency on retina and brain in rhesus monkeys. Proc Natl Acad Sci USA. 1986; 83: 4021-4025.
91. K.W. Wirtz, Phospholipid transfer proteins. Ann. Rev. Biochem. 60(13), 73-99 (1991).
92. Kanno K, Wu MK, Agate DA, Fanelli BK, Wagle N, Scapa EF, Ukomadu C, Cohen DE. Interacting proteins dictate function of the minimal START domain phosphatidylcholine transfer protein/StarD2. J Biol Chem. 2007; 282(42), 30728-30736.
93. Mei-Chu H, Koji S, Riki Y, Masao S, Katsumi I. Learning behaviour and cerebral protein kinase C, antioxidant status, lipid composition in senescene-accelarated mouse: influence of a phosphatidylcholine-vitamin B_{12} diet. British Journal of Nutrition. 2001; 86, 163-171.
94. Chung Shu-Ying, Tomoe M, Eiko U, Kayoko U, Rieko H, Noriko Y, Yasnunobu M, Toyohiko K, Shigeru Y. Administration of phosphatidylcholine increases brain acetycholine concentration and improves memory in mice with dementia. The Journal of Nutrition. 1995; 125: 1484-1489.
95. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Duqar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Allayee JD et al., Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011; 472(7341): 57-63.
96. Adeyeye EI, Oyarekua MA. Lipid profile of the skin and muscle of freshwater sardine (Pellenula afzeliusi) nutritional/dietary implications. Bangladesh J Sci Ind Res. 2011; 523- 532.
97. Blockland A, Honig W, Brouns F, Jolles J. Cognition-enhancing properties of subchronic phosphatidylserine (PS) treatment in middle-aged rats: comparison of bovine cortex PS with egg PS and soybean PS. Nutrition. 1999; 15(10): 778-783.
98. Crook TH, Klatz RM (eds). Treatment of age-related cognitive decline: effects of phosphatidylserine in anti-aging medical therapeutics. 2. Chicago: Health Quest Publications; 1998.
99. Jäger R, Purpura M, Kingsley M. Phospholipids and sports performance. Journal of the International Society of Sports Nutrition. 2007; 4, 5. doi.10.1186/1550-2783-4-5.
100. Starks MA, Starks SL, Kingsley M, Purpura M, Jäger R. The effects of phosphatidylserine on endocrine response to moderate intensity exercise. Journal of the International Society of Sports Nutrition. 2008; 5, 11.doi:10.1186/1550-2783-5-11.
101. Jäger R, Purpura M, Geiss KR, Weiβ M, BaumeisterJ, Amatulli F, Schröder L, Herwegen H. The effect of phosphatidylserine on golf performance. Journal of the International Society of Sports Nutrition. 2007; 4: 23.doi:10.1186/1550-2783-4-23.
102. Hirayama S, Masuda Y, Rabeler R, Effect of phosphatidylserine administration on symptoms of attention-deficit/hyperactivity disorder in children. Agro Food. 2006; 17(5): 32-36.
103. Vaisman N, Kaysar N, Zaruk-Adasha Y, Pelled D, Brichon G, Zwingelstein G, Bodennee J. Correlation between changes in blood fatty acid composition and visual sustained attention performance in children with inattention effect of dietary n-3 fatty acids containing phospholipids. The American Journal of Clinical Nutrition. 2008; 87(5): 1170-1180.
104. Jorissen BL, Brouns F, Van Boxtel MP, Riedel WJ. Safety of soy-derived phosphatidylserine in elderly people. Nutr Neurosci. 2002; 5(5): 337-343.
105. Hoffmann PR, Kench JA, Vondracek A. et al., Interaction between phosphatidylserine and the phosphatidylserine receptor inhibits immune responses in vivo. J. Immunol. 2005; 174(3): 1393-1404.
106. Carr DJ, Guarcello D, Blalock JE. Phosphatidylserine suppresses antigen-specific IgM production by mice orally administered sheep red blood cells. Proc Soc Exp Biol Med. 1992; 200(4): 548-554.

107. Jones PJ. Regulation of cholesterol biosynthesis by diet in humans. The Am J Clin Nutr. 1997; 66(2): 438-446.
108. Cranton EM, Frackelton JP. Free radical pathology in age-associated diseases: treatment with EDTA chelation, nutrition and antioxidants. Journal of Holistic Medicine. 1984; Spring/Summer 6(1): 6-37.
109. Engelberg H. Low serum Cholesterol and suicide. Lancet. 1992 March 21; 339: 727-728.
110. Alfin-Slater RB, Aftergood L. Lipids, modern nutrition in health and disease 16th ed. Lea and Febiger; 1980.
111. Bender A. Meat and meat products in human nutrition in developing countries. FAO Nutrition Paper 53. Rome: FAO; 1992.
112. Paul AA, Southgate DAT. McCance and Widdowson's the Composition of Foods 4th ed. London: HMSO; 1978.