

EVALUATION OF POLYUNSATURATED TO SATURATED FATTY ACID RATIO IN THE FAT OF DIFFERENT CHICKEN TYPES

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ABSTRACT

Samples were taken from the liver and the abdominal fat of the carcasses of male and female broilers (meat type), layers (egg type) and locals (dual purpose type). Lipids were extracted and subjected to volumetric analysis, iodine number determination, thin layer chromatography (TLC) with iodine staining and high performance liquid chromatography – mass spectrometry (HPLC-MS) to determine the polyunsaturated to saturated fatty acid (P/S) ratio. The study concluded that female chickens, meat type chickens and abdominal fat samples had higher ($P < 0.05$) lipid quantities than male chickens, layer and dual purpose chickens and liver samples, respectively. The P/S ratio of lipids extracted from the abdominal fat and from the liver did not differ ($P < 0.05$) from each other.

Keywords: P/S ratio, liver, abdominal fat, broiler, layer, dual purpose, chicken.

INTRODUCTION

Excessive intake of food having high saturated fatty acid (SFA) as a fraction of the total fatty acid composition predisposes humans to excess cholesterol accumulation by increasing low density lipoprotein cholesterol (National Cholesterol Education Program, 1991; Sacks and Byers, 2001). This has led to the reduction in the consumption of red meat due to its high content of SFA which predisposes to arteriosclerosis. On the contrary, consumption of chicken meat is increasing. Different parts of the chicken have been reported to have varied amounts of fats (USDA, 2007) therefore, it is important to investigate the fatty acid composition of different chicken types. The objective of this study was to evaluate the effect of type, sex and carcass sampling sites on P/S ratio of chicken carcass fat.

MATERIALS AND METHODS

A total of thirty six chickens were used. Twelve meat chicken type were obtained from the Poultry Unit of the Obafemi Awolowo University Teaching and Research farm, Ile-Ife, Osun State. Twelve egg and twelve dual purpose chickens were obtained from Sabo and Oja Ife markets, Ile-Ife respectively. The body weights range of the chickens were: 2.5 kg-2.7 kg (female meat type), 2.3 kg-2.5 kg (male meat type), 3.3 kg-3.5 kg (male egg type), 3.2 kg-3.4 kg (female egg type), 1.8 kg-2.0 kg (female dual

purpose type), and 3.0 kg-3.2 kg (male dual purpose type). There were six experimental units (female meat type, male meat type, female layer type, male layer type, female dual purpose, male dual purpose).

The birds were slaughtered, liver and abdominal fat samples were taken from each experimental unit with 3 replicates per sample making a total of 108 samples. Samples were wrapped in black cellophane bags to prevent moisture and oil loss, and light penetration, which could cause oxidation. The wrapped samples were sealed in a tightly covered bowl and kept in a freezer at -20°C .

Lipid was extracted and stored in the freezer (20°C) following the procedure of Folch *et al.* (1957) at Poultry Meat Research Laboratory, Departments Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. Volumetric analysis of extracted lipid using titration method was carried out following the procedure of Egan *et al.* (1991) to determine the saturated, monounsaturated and polyunsaturated fatty acids fraction.

At Lipid and Natural Products Laboratory, Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria, Iodine number determination of lipid extracted from liver samples was done following Hanus' procedure and Thin Layer Chromatography analysis was carried out on the lipid extract. It involves the separation of lipid fractions on silica gel

prepared glass plate. The separated spots on silica gel plates were identified by staining with vanillin-sulphuric acid solution to identify all spots and iodine crystals to identify unsaturated spots.

Fresh lipid extract was collected and dissolved in 5ml of chloroform poured into a 5-10 ml glass vial and sent to the Faculty of Health and Wellness Science, Biotechnology Laboratory, Cape Peninsula University of Technology, South Africa. Extracted lipid was dissolved in chloroform (1 ml) was mixed with 6 ml of 2 M sodium methoxide (or sodium hydroxide) in anhydrous methanol and shaken vigorously. The solution was heated in a steam bath at 500C for about 60 minutes. Afterwards, 10 ml of 2 M methanolic solution of hydrochloric acid was added and the sample reheated. Distilled water (5 ml) was added and the required esters were extracted into n-hexane. The hexane layer was washed with 4 ml dilute potassium bicarbonate (5%) to remove excess acid and dried over anhydrous sodium sulphate. The gradient elution was prepared using 0.1% formic acid in water as solvent A and acetonitrile as solvent B. The gradient was set up as follows: 2% B to 100% B (30 min); held at 100% B (30 – 36 min); 2% B (37 – 45 min). Thermo Fischer Scientific C18 column (5 µm; 4.6 × 150 mm, Bellefonte, USA) was used through which the samples were eluted. The injection rate was 0.6 µl per min at oven temperature of 300C. Samples eluted from the HPLC column were passed into a mass spectrometer for fragmentation and structure determination. A mass spectrometer with specifications ESI QTOF (Bruker Daltonik GmbH, Germany) was used. Mass spectrometer settings include negative mode with Electrospray voltage +3500 V at dry gas temperature of 300 °C, dry gas flow of 9 lmin⁻¹; nebulizer gas pressure 35 psi.

The experiment was designed as a randomized complete block design. Data collected were subjected to analysis of variance using General Linear Model (GLM) and T-test procedures in the SAS statistical package. Duncan's multiple range test was used to determine the significant differences among the means.

RESULTS AND DISCUSSION

From the result shown in table 1, abdominal fat for meat type chicken gave the highest quantity

of lipid extract while abdominal fat of dual purpose chicken had the lowest quantity of lipid extract. Lipid extracted from the liver of the three chicken types considered did not differ significantly from each other. To consider the overall effect of abdominal fat and liver on the quantity of lipid extracted regardless of chicken type, abdominal fat had significantly high quantity of lipid extracted (%) and liver lipid quantity was very low (table 1). From this result, abdominal fat having the highest fat composition (Pikul *et al.*, 1985) could be a good representation of the lipid quantity in chickens.

From this study, there is no significant difference in the polyunsaturated to saturated fatty acid (P/S) ratio between the sample sites considered (liver and abdominal fat) within the three chicken types considered and also regardless of chicken type. This implies that sample sites for lipid extraction and the interaction between sample site and chicken type did not have an effect on the P/S ratio. This agreed with the result of Kralik *et al.* (2005) that observed no significant difference in P/S ratio of the sample sites considered (breast and thigh muscle). It however disagreed with Zlender *et al.* (2000) who reported that there was a significant difference in P/S ratio between sample sites considered (breast muscle with skin and leg muscle with skin) at $P < 0.05$.

Sex considered within chicken type had significant effect on the degree of unsaturation of lipid using iodine number determination (Table 2). Female and male meat type was found to have the highest and the lowest degrees of unsaturation, respectively. But considering the overall effect of sex on the degree of unsaturation, sex had no significant effect on the degree of unsaturation of lipid. This is in agreement with the report of Rondelli *et al.* (2004) that sex has no effect on the fatty acid composition of broiler chicken, although they observed significant differences when they subjected their data to multivariate analysis.

Considering the number of carbon atoms in the fatty acids with two or more double bonds (PUFA) in the chicken liver fat (table 2), female meat type had a similar PUFA with female dual purpose except for linoleic acid (C18:2) present in female meat type but absent in female dual purpose, and α -linolenic acid (C18:3) present in female dual purpose but absent in female meat

type. The carbon atoms which are PUFA in male egg type and male dual purpose were also similar but differ slightly with the presence of C28:6 in male egg type which is absent in male dual purpose type.

Christie (2011) reported that TLC analysis can also be quantified using standards with similar degree of unsaturation. Cod liver oil with iodine number of 157 had R_f of 0.92 while olive oil with iodine number of 86 had R_f of 0.84. Female meat type had R_f of 0.94 close to R_f of cod liver oil (0.92). This can possibly mean that they have a similar fatty acid composition. Cod liver oil has high degree of unsaturation (iodine number 157) relative to olive oil (iodine number 86). Male egg type and male dual purpose with R_f of 0.68 and 0.80, respectively had close R_f to olive oil (0.84). These lipid extracts can also possibly have similar fatty acids composition.

CONCLUSION

From this study, it can be concluded that: female chickens had the highest lipid content than male chickens; fat from the abdominal site had higher lipid quantity than fat from the liver; meat type chickens had the highest lipid content as compared with egg type chickens which had higher lipid content than dual purpose chickens; there was no difference in the polyunsaturated to saturated fatty acid ratio of lipids extracted from abdominal fat and from the liver. Across the three chicken types and sex considered, there was no difference in the degree of unsaturation of the lipids extracted from different chicken types and sexes. Quantitatively, lipids extracted from egg type chickens had the highest carbon number polyunsaturated fatty acids as C28:6, while lipids extracted from meat type and dual purpose chickens had higher carbon number polyunsaturated fatty acids as C22:6.

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Table 1: Percent lipid content and Polyunsaturated to saturated fatty acid ratio of lipid extracts of two sampling sites from three chicken types

Chicken type	Sampling Site	SFA (%) (±SEM)	PUFA(%) (±SEM)	P/S ratio (±SEM)	Lipid content (%)
Meat type	Abdominal fat	2.3±0.10	0.9±0.02 ^{ab}	0.39±0.05 ^a	34.67±2.09 ^a
	Liver	2.2±0.00	0.9±0.05 ^{ab}	0.41±0.05 ^a	8.17±1.66 ^c
Egg type	Abdominal fat	2.1±0.15	0.7±0.00 ^{ab}	0.33±0.45 ^a	30.50±3.89 ^{ab}
	Liver	2.0±0.25	0.6±0.05 ^b	0.30±0.01 ^a	5.33±0.61 ^c
Dual purpose type	Abdominal fat	2.2±0.35	0.9±0.25 ^{ab}	0.41±0.06 ^a	25.67±4.27 ^b
	Liver	2.4±0.35	1.1±0.00 ^a	0.46±0.04 ^a	6.17±0.79 ^c
Mean across site	Abdominal fat	0.38±0.03			30.28 ^a
	Liver	0.38±0.03			6.56 ^b

^{a, b, ab} Means in the same column having the same superscript do not differ significantly at P<0.05. Meat type = broiler chicken, egg type = layer chicken, dual purpose = local chicken

Table 2: Saturated fatty acid and polyunsaturated fatty acid composition of lipid extract from three chicken types

Chicken type	Sex	Iodine no ±SEM	SFA	PUFA
Meat type	Female	239 ^a ±85.81	C12:0, C22:0	C18:2,C20:4,C20:5,C22:5,C22:6
	Male	41 ^b ±5.13	C12:0, C22:0	C18:2,C20:4,C20:5,C22:6
	±SEM	139.8±67.11 ^a	-	-
Egg type	Female	122 ^{ab} ±20.05	C20:0, C22:0	C18:2,C18:3,C20:4,C22:5,C22:6
	Male	166 ^{ab} ±11.74	C14:0, C20:0, C22:0	C18:2,C18:3,C20:4,C20:5,C22:5,C22:6, C28:6
	±SEM	144.0±15.97 ^a	-	-
Dual purpose	Female	203	C10:0, C20:0, C22:0	C18:3,C20:4,C20:5,C22:5,C22:6
	Male	122 ^{ab} ±6.36	C20:0, C22:0	C18:2,C18:3,C20:4,C20:5,C22:5,C22:6
	±SEM	111.6±49.91 ^a	-	-

Meat type = broiler chicken, egg type = layer chicken, dual purpose = local chicken

^{a, b, ab} Means in the same column having the same superscript do not differ significantly at P<0.05. Meat type = broiler chicken, egg type = layer chicken, dual purpose = local chicken.