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Comparative Studies on Fatty Acid Composition of Domestic and Hybrid Chickens and Their Effect on Blood Parameters in Experimental Rats

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University of Rajshahi

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THESIS SUBMITTED FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY
IN THE
INSTITUTE OF BIOLOGICAL SCIENCES
RAJSHAHI UNIVERSITY, BANGLADESH

BY
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Bangladesh

June, 2015

MAHBUBA KHATUN

COMPARATIVE STUDIES ON FATTY ACID COMPOSITION OF DOMESTIC AND HYBRID CHICKENS AND THEIR EFFECT ON BLOOD PARAMETERS IN EXPERIMENTAL RATS

Ph.D Thesis

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2015

DEDICATED TO
MY
RESPECTED PARENT

SULTANA MONOWARA

&

LATE. ABU BAKAR SIDDIQUE

DEDICATED TO
MY
RESPECTED PARENTS

SULTANA MONOWARA

&

LATE. ABU BAKAR SIDDIQUE

DECLARATION

I hereby declare that the whole works now submitted as a thesis entitled **Comparative studies on fatty acid composition of domestic and hybrid chickens and their effect on blood parameters in experimental rats** in the institute of Biological Sciences, Rajshahi University for the degree of Doctor of Philosophy is the result of my own investigation. The thesis has not been concurrently submitted for other degree.

June, 2015
Rajshahi

(Mahbuba Khatun)
Candidate

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18 June, 2015

CERTIFICATE

This is to certify that the thesis entitled **Comparative studies on fatty acid composition of domestic and hybrid chickens and their effect on blood parameters in experimental rats** is the record of bonafide research carried out at Institute of Biological Sciences, Rajshahi University under my supervision. All the data presented in this thesis are based on her own observations and no portion thereof has been previously published or submitted for any other degree.

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M. A. Islam

Professor Dr. Mohammad Amirul Islam
Supervisor

CONTENTS

LIST OF FIGURES

LIST OF TABLES

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Mahbuba Khatun
(The Author)

ABSTRACT

Chicken meat is generally thought to be health friendly due to its favorable fatty acid composition leading to an antiatherogenic lipidemic status. Beneficial effect of the chicken oil on hyperglycemia has also been claimed through its effect on oxidative stress and insulin resistance. To further explore the potential benefits, the effects of the oil, extracted from domestic and hybrid chicken for their fatty acid composition as well as for their effects on glycemic and lipidemic status in diabetic model rats were studied.

The macro and micronutrient contents of these two chicken species (10 chickens of each species) were also compared. All macronutrients such as ash, moisture, total protein, total carbohydrate and total lipid were found to be high for the hybrid chicken. Lipid content was significantly higher (around 40%) in hybrid chicken than that of domestic chicken. Except for potassium and manganese which were found to be high in hybrid chicken (127.8 µg/kg and 9.74 µg/kg respectively) than domestic chicken (86.83 µg/kg and 6.83 µ/kg respectively), all other micronutrients such as iron, calcium, zinc and lead were higher for domestic chicken. Zinc content of hybrid chicken was significantly lower (around 50%) than that of domestic chicken.

The average amount of oil content of two species were extracted with soxhlet apparatus using n-hexane as an extracting solvent and it was found to be 3.54% for domestic chicken and 4.84% for hybrid chicken. Chemical characteristics of the extracted oil were investigated by analyzing various parameters such as iodine value, saponification value, acid value, peroxide value and percentage free fatty acid. Iodine value and unsaponifiable matter were higher for hybrid chicken (77.92% and 11.14% respectively) than those of domestic chicken (60.56% and 3.5% respectively). Again saponification value, acid value, peroxide value and percentage free fatty acid were higher for domestic chicken than hybrid chicken.

Lipid and protein content of different meat portion (Breast, Thigh, Drumstick and Wings) of chicken were analyzed and compared. Lipid content was higher in thigh meat (3.65±0.57 and 5.43±0.9 for domestic and hybrid chicken) and protein content was higher in breast meat (16.54±1.5 and 19.27±1.3) for both of the chickens.

An interventional design was used to evaluate the hypoglycemic and hypolipidemic effects. Seven groups of rats (n=6 in each group) were studied: Group: NCD - normal (non-diabetic rats), DC- Diabetic Control (Alloxan induced diabetic rat without treatment), DG- Diabetic + Glibenclamide, DDO- Diabetic + Domestic chicken oil (1% of total diet), DHO- Diabetic + Hybrid chicken oil (1% of total diet), DDF- Diabetic + Domestic chicken flesh(1% of total diet), DHF- Diabetic + Hybrid chicken flesh (1% of total diet).

Diabetes was induced in Wister adult rats with alloxan (55 mg/kg bw) and fasting blood glucose above 11.5 mmol/L after three days were considered as diabetic. The rats were fed for 21 days with a dose for glibenclamide as 0.6 mg/ kg body weight, for chicken oil as 1% of total diet and an amount of dried flesh of chicken equivalent to 1% of chicken oil (calculated from oil content, Table 4.4). End point (21^{th} day) was compared with baseline (7^{th} day) of the study.

Blood glucose level was significantly decreased for DDO and DHO group. There were significantly lower blood glucose value in DG and DDO groups at endpoint compared to baseline (mmol/l, mean ± SD, Baseline vs Endpoint, 16.0 ± 1.12 vs 10.9 ± 0.08; P< 0.001 and 15.06 ± 1.09 vs 11.9 ± 0.7 ; P<0.001, for the DG and DHO groups at Endpoint compared to Baseline (mmol/l, mean ± SD, Baseline vs Endpoint, 16.0 ± 1.12 vs 10.9 ± 0.08; P< 0.001 and 16.11 ± 0.71 vs 12.8 ± 0.56; P< 0.001).

There were also significant effect on body weight was observed for DDO and DHF group. For DG and DDO groups at Endpoint compared to Baseline (gm, mean ± SD, Baseline vs Endpoint, 94.54 ± 0.54 vs 117.9 ± 0.63; P< 0.001 and 94.49 ± 2.98 vs 113.52 ± 2.64; P< 0.05, for DG and DHF groups at

Endpoint compared to Baseline (gm, mean ± SD, Baseline vs Endpoint, 94.54 ± 0.54 vs 117.9 ± 0.63; P< 0.001 and 94.16 ± 1.63 vs 114.56 ± 2.54; P< 0.05).

Chicken oil also showed hypolipidemic effects. A significant decrease in cholesterol level had seen for DDO and DHO treatment group. 69% and 82% reduction of cholesterol level were observed for domestic and hybrid chickens oil treatment respectively in diabetic rats whereas 44% and 62% reduction were seen for domestic and hybrid chicken flesh. In case of glibenclamide, it was 89%. LDL level was reduced significantly for DDO and DHO treatment group by 65% and 71%, for DG group, it was 77%. HDL level was increased significantly for DHO and DHF treatment group by 70% and 62%. Triglyceride level was reduced significantly for DHO group by 30%, Whereas Triglyceride level was reduced by 86% for glibenclamide treatment.

A significant reduction of Uric acid level was observed for DHO group by 56% compared with control group. Serum urea levels were reduced significantly for DDO and DHO treatment group by 45% and 54%. Creatinine level was also reduced significantly for DDO and DHO by 41% and 62%.

Administration of chicken oil and flesh also maintained SGPT and SGOT level. A significant reduction was found in SGPT level for DHO and DHF treatment group by 60% and 51% respectively. Similar effect had seen for SGOT level. The level was decreased significantly by 43% and 42% for DHO and DHF. So, both types of chicken oils have a beneficial impact on liver function test.

The antiatherogenic fatty acid content in the domestic chicken oil was as follows: arachidonic acid (1.7%), oleic acid (75%) and stearic acid (1.84%). In contrast the proatherogenic fatty acids were as follows: myristic acid (0.24%), palmitic acid (19%) and behenic acid (0.85%). On the other hand, the antiatherogenic fatty acid content in the hybrid chicken oil was as follows: arachidonic acid (0.5%), oleic acid (48%), palmitoleic acid (28%) and stearic acid (4.7%). In contrast, the proatherogenic fatty acids were as follows: myristic acid (0.34%), palmitic acid (17%) and behenic acid (0.2%).

Both, the domestic and hybrid chicken oils contain predominant amount of MUFAs and PUFAs. Chicken meat and flesh seem to have hypoglycemic and hypolipidemic effects on experimental diabetic rats. So, consumption of chicken might be more beneficial to human health.

INTRODUCTION

1.1 General Introduction

Lipids are important constituents of food and living cells. Dietary lipids play important roles in the energy production process of animal tissues as a source of essential fatty acids (EFA). Essential fatty acids are polyunsaturated fatty acids, among which n-3 fatty acids have potential health benefits (Nettleton 1995, Harris 1989). Other polyunsaturated fatty acids and monounsaturated fatty acids are also associated with many health benefits like protection against coronary heart diseases. According to American Heart Association (AHA), regular dietary intake of n-3 fatty acids have some protective health effects such as decrease in platelet aggregation, reduction in triglyceride levels, retardation of atherosclerosis and anti-inflammatory effects (Etherton *et al* 2002).

A study (McKenney *et al* 2007) shows that patients with high triglycerides and poor coronary artery status were given 4 grams a day of a combination of EPA and DHA along with some monounsaturated fatty acids. Those patients with very unhealthy triglyceride levels (above 500 mg/dl) reduced their triglycerides on average by 45% and their VLDL cholesterol by more than 50%.

It has been reported that an adequate intake of DHA and EPA is particularly important for mother during pregnancy and lactation. During this time the mother must supply the baby's required DHA and EPA because they are

unable to synthesise these essential fatty acids themselves. DHA makes upto15% to 20% of the cerebral cortex and 30% to 60% of retina so it is an absolute nutrient for pregnant mother necessary for normal development of the fetus and baby. The constant drain on a mother's DHA reserves can easily lead to a deficiency and some researchers revealed that preeclampsia (pregnancy-related high blood pressure) and postpartum depression could be linked to a DHA deficiency. There is some evidence that an insufficient intake of ω-3 fatty acids may increase the risk of premature birth and an abnormal low birth weight. There is also emerging evidence that low levels of ω-3 fatty acids are associated with hyperactivity in children. Experts recommend that women should get at least 500-600 mg of DHA every day during pregnancy and lactation (John *et al* 2011).

It has also been revealed that daily supplementation with 2.7 gm of EPA and 1.8 gm of DHA can markedly reduce the number of tender joints and extend the time before fatigue sets in (Navarro *et al* 2000).

Patients with ulcerative colitis have abnormally low blood levels of EPA. Clinical trials have shown that the supplementation with fish oil (2.7gm of EPA and 1.8 gm of DHA daily) can reduce the severity of the condition by more than 50% and enable many patients to discontinue anti-inflammatory medication and steroids (Aslan and Triadafilopouos 1992).

EFA also combat for weight loss. It has shown that supplementing the diet with 2.2 gm of EPA and 1.4 gm of DHA daily will stabilize weight in patients with inoperable pancreatic cancer (Albino and Anthony 2000).

Essential fatty acids are closely associated with the risk of diabetes. It was reported that Type 2 Diabetes is strongly associated with proinflammatory products in obese tissue and it has been established that insulin resistance results from inflammation of the adipose tissue in which cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and (IL)-6 as well as products such as PAI-1 (plasminogen activator inhibitor-1) are part of the development and progression of T2D. In this regard, omega-3 fatty acids should be beneficial and prevent T2D because they have the potential of suppressing the production of these proinflammatory products in macrophages and adipocytes of adipose tissue (Bjarne 2011).

It has been also reported that people with diabetes are 6 times more likely to suffer a first heart attack and 3 to 8 times more likely to die from heart disease than those without the disease. For this reason, diabetes treatment focuses on managing glucose and insulin levels and preventing heart disease. Omega-3 fatty acids are highly effective when used to reduce blood sugar, improve insulin action, lower blood pressure and lipids, and improve circulation (Joyce & Nettleton 1995).

Generally essential fatty acids have lots of beneficial health effects like on cellular developments, formation of healthy cell membranes, and blockage of

tumor formation in animals, as well as block the growth of human breast cancer cells. It also assists in the development and function of the brain and nervous system and they help to regulate proper thyroid and adrenal activities. Essential fatty acid regulates blood pressure, immune response and liver function, as well as help with blood clotting and breaking down cholesterol. So, one should take these essential fatty acids in sufficient amount through diet because mammals cannot synthesize essential fatty acids in the body and the only source of EFA is diet.

There are main two sources of essential fatty acids. These are vegetable source and animal source. Among vegetable source various type of vegetable oils are included such as soya bean oil, mustard oil, rapeseed oil etc. In animal source fish, meat and eggs are the commonest sources of EFAs. Among them fish is a very rich source of essential fatty acids. Fish meat is a good source of essential amino acids and EFAs. It is also a good source of B vitamins and also that of the A and D vitamins in the case of fatty species. Some fresh water species such as carp have high thiaminase activity so the thiamine content in these species is usually low. As for minerals, fish meat is considered as a valuable source of calcium and phosphorus in particular but also of iron, copper and selenium. Saltwater fish have a high content of iodine. But excess intake of fish can cause side effects including belching, bad breath, heartburn, nausea, loose motion, rash and sea food allergy. Fish also has relatively high price than other animal sources. So in addition to fish other animal sources of fatty acids should be considered.

Actually animal meat quality depends on its fatty acid composition. Beef, Lamb and Mutton contain very high amount of saturated fatty acids, but chicken meat contents relatively lower amount of saturated fat. Thus chicken meat is commonly regarded both by health professionals and the general public as a healthy type of meat, well-liked, and the consumption is increasing (Kosthold 2004). Chicken meat may be a good source of healthier meat with EFA content because chicken meat is not only an important source of macronutrients but also a very good source of some micronutrient like vitamins (A, B3, B5, B6) and minerals (selenium, iron, sodium). Moreover, Chicken meat does not contain trans fats that contribute to coronary heart disease which can be found in high amounts in beef and lamb.

In recent years, consumption of chicken has increased due to its nutritional characteristics, such as its high protein (around 20 g/100 g raw meat without skin), low fat (around 5 g/100 g raw meat without skin), high content in essential fatty acids and fat soluble vitamins, coupled with a favorable saturated and unsaturated fatty acid ratio (Buzby and Farah 2006). Very few studies have been carried out in abroad on fatty acid composition in chicken meat. Actually study of fatty acid composition of chicken meat is important to find a way to produce healthier meat.

It has been reported that chicken is good for diabetic patients because of its low fat content. Diabetes is a major threat to global public health that is rapidly getting worse and the biggest impact is on adults of working age in developing

countries. Diabetes has become one of the major causes of premature illness and death in most countries, mainly through the increased risk of CVDs. In 2000, there were 171 million people with diabetes worldwide and by 2030 this figure is expected to be more than double, to reach a total of 366 million (Rheeder 2006). Statistical projections for Bangladesh suggest that the number of diabetics will rise from 3.2 million in 2000 to 11.1 million in the year 2030 (Wild *et al* 2004). The high fat content in red meats causes elevated levels of cholesterol which can lead to blockages in the arteries resulting in coronary heart disease. Diabetics are at a higher risk of developing coronary heart disease and should replace red meat in their diets with lean white meats like chicken. This has a beneficial effect on both the heart and the kidneys, two organs that are at greater risk in diabetics (anonymous 2014).

Hybrid chicken contains low iron which may be beneficial for health. Chicken meat is white lean meat and generally its iron content is lower than red meat. Excess iron can cause oxidative stress mediate apoptosis of pancreatic islets with a resultant decrease in insulin secretion capacity. Pancreatic islets have an extreme susceptibility to oxidative damage, perhaps because of the nearly exclusive reliance on mitochondrial metabolism of glucose for glucose-induced insulin secretion and low expression of the antioxidant defense system as shown in fig 1.1. Iron can also accumulate in and contributes to atherosclerosis plaques formation in Apo E deficiency. So, low iron content may be a beneficial side of chicken meat as iron overload can cause diabetes and CVDs.

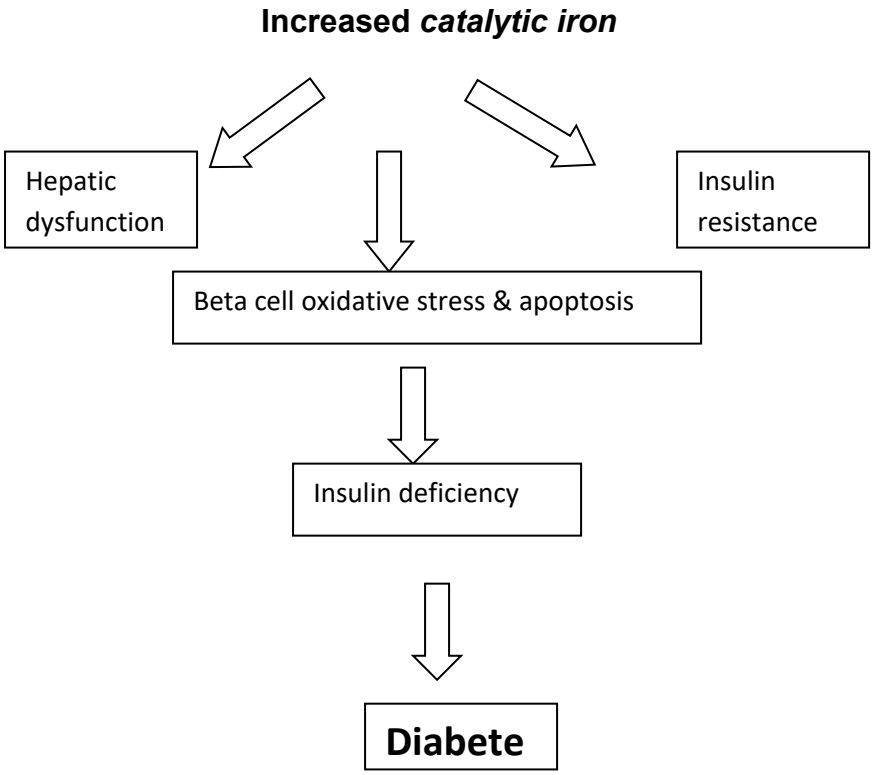


Figure: 1.1. Oxidative stress mediated apoptosis of pancreatic islets

Several studies have been done on variation in feeds of hybrid chickens to get the desirable fatty acid composition, as we know biosynthesis of fatty acid in our body also depends on dietary lipids. A study (Anna *et al* 2011) shows that broiler meat can be considered as a good source of EFA through maintaining the dietary balance between *omega*-6 and *omega*-3 fatty acids and increasing the food content of Se and very long chain *omega*-3 fatty acids in their feed. In this study sixty newly hatched chickens were fed wheat-based diets containing 40 g rapeseed oil/kg, 10 g linseed oil/kg and varying amounts of

selenium enriched yeast for three weeks. It resulted in meat with a favorable ratio between *omega*-6 and *omega*-3 fatty acids and with selenium concentration ranging from 0.2 to 0.6 mg/kg which is beneficial both in prophylactic and therapeutic uses and might be useful for some groups of patients e.g. those with ischemic pain.

Another study (Gallardo *et al* 2012) shows that feed supplement with seeds rich in α-linolenic acid, a ω3 fatty acid with 18 carbons, a precursor of long-chain fatty acids such as EPA, DPA, and DHA results a desirable fatty acid composition in the meat.

Another study *(*Zlender *et al* 2008) shows that animal feed enriched by nutritionally important fatty acids can improve the nutritive value of animal fats. Adding linseed oil rich in the α-linolenic acid results in a significant increase in the amount of this fatty acid in chicken lipids. Adding herring oil to chicken feed increases the shares of eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic (DHA) acids. Fatty acid composition also depends on the nature of food with its environment. Grass fed chickens, reared outdoors have a more favorable fatty acid composition than those of the extensive indoor rearing chickens. The share of essential fatty acids as well as polyunsaturated omega-3 and omega-6 fatty acids is greater among outdoor rearing chickens than that of indoor rearing ones and they have favorable fatty acid composition, which probably should also be reflected in the more favorable lipid composition of such chickens. This poultry

meat is an important provider of the essential polyunsaturated fatty acids (PUFAs). Domestic chickens are particularly good source of EFA because of their diet.

1.2 Rationale

Bangladesh is an agro-based developing country in the Southeast Asian region and livestock especially poultry is a promising sector for employment generation and poverty reduction in this country (GOB 1999). About 30 years back the contribution of poultry to the total animal protein was about 22 to 27 percent in the country (Ahmed & Haque 1990). About 89% of the rural households that rear livestock were also found to rear poultry (BBS 1996). Poultry meat and eggs are used chiefly as human food and poultry meat alone contributes 29% of the total meat production in Bangladesh (BBS 2001). It was in 1995 that the poultry industry started in an organized manner. In Bangladesh, just in twenty years, the poultry industry has seen exponential growth. Now about 18.6% GDP comes from agriculture sector, one-third is from the poultry industry. In our daily life we need to take meat for getting animal protein. According to the Food and Agriculture Organization, each person should take 56 kilogram of meat and 365 eggs every year. But in Bangladesh, per capita intake of meat is only 11.27kg and that for egg is only 30 per year. As a result, people suffer from malnutrition. It is evident that a substantial majority of the population suffers from varying degrees of malnutrition, including protein-energy malnutrition, micro-nutrient deficiencies, iodine deficiency disorder, iron deficiency, anemia, and vitamin deficiencies. A

major proportion of these nutritional disorders might be prevented by increase intake of meat produced by the poultry industries. Fish and cattle production require a longer time. But poultry production is relatively faster and easier. Accordingly, chicken meat fatty acid may be a good source of fatty acids. The fatty acid composition of a chicken, however varies depending on its genotype, feed quality, environment and also cooking procedure. Though hybrid chickens, which we get from poultry production, are the most popular and commonly consumed chickens, there is a strong belief among the common people that domestic chickens are much more beneficial for health than the hybrid chickens. So, specific and comparative studies are needed for different varieties of chicken species to understand their meat composition, specially the fatty acid composition. In the above context, it is important to conduct a study on the lipid and fatty acid composition of domestic and hybrid chickens and, at the same time, to investigate the biological effects of the oil of these chickens.

1.3 OBJECTIVES

1.3.1 General Objectives

The general objective of the study is the comparative studies on fatty acid composition of domestic and hybrid chickens and their effect on blood parameters in experimental diabetic rats.

1.3.2 Specific Objectives

1. To determine the nutritional composition, such as ash, moisture, total protein, lipid, total sugar, reducing sugar, glycogen and mineral contents of both the domestic and hybrid chicken species.
2. To extract and purify the fat from both chicken species (domestic and hybrid).
3. To analyze the saponification value, iodine value, unsaponifiable matter, acid value and peroxide value of the isolated fat of the two chicken species.
4. To isolate and identify the fatty acid composition of domestic and hybrid chicken oil.
5. To investigate the comparative hypoglycemic effect of the selected chicken species (Flesh and Oil diet) on blood parameters such as blood sugar, SGPT, SGOT, urea, uric acid and creatinine in alloxen induced Wister rats.
6. To observe the comparative hypolipidemic effect of the selected chicken species (Flesh and Oil diet) on blood parameters such as Lipid profile (Total Cholesterol, TG, HDL and LDL) in alloxen induced Wister rats.

REVIEW OF LITERATURE

Bangladesh is one of the high density countries of the world has a population of 150 million people within the area of 143,000 km2. About eighty percent people of this country still live in villages and are extremely poor. Both the government and a variety of non-governmental organizations are actively promoting poultry development at all levels. Small-scale poultry production has developed in a large number of developing countries around the world as an important source of earning for the rural poor peoples. In the last few years, the recognition of small-scale commercial poultry production helps to accelerate the pace of poverty reduction riding in new height in Bangladesh. The poultry industry has been successfully becoming a leading industry of the country. The sector is also growing rapidly for last two decades though it started farming during mid sixties in this country. It has already capable to rise at an annual growth of around 20 per cent during last two decades. In Bangladesh, this industry has immense potentialities from the point of view of the economic growth of the country as well as fulfillment of basic needs and to keep the price at a minimum level and ensuring food especially animal protein for the human being.

The chicken (*Gallus gallus domesticus*) is a domesticated fowl, a subspecies of the red junglefowl, as one of the most common and widespread domestic animals, with a population of more than 24 billion in 2003.Two species of chicken are available in Bangladesh: indigenous (domestic) and broiler

(hybrid), of them local chicken meat is the famous one among Bangladeshi people. In addition to broiler and local chicken, now a days cross-breed Sonali (Fayoumi x Rhode Island Red) getting popular for meat supply in Bangladesh (Perrins *et al* 2003).

2.0 Taxonomic position: Kingdom: Animalia

Phylum: Chordata

Class: Aves

Order: Galliformes

Family: Phasianidae

Genus: *Gallus*

Species: *Gallus gallus*

Subspecies: *Gallus gallus domesticus*

(Source: Linnaeus 1758.)

Figure 2.1: Domestic Chicken

Figure 2.2 Hybrid Chicken

Identification and characterization of the chicken genetic resources generally requires information on their population, adaptation to a specific environment, possession of traits of current or future value and sociocultural importance, which are crucial inputs to decisions on conservation and utilization (Weigend and Romanov 2001). Indigenous chickens of the tropics are important reservoirs of useful genes and possess a number of adaptive traits (Horst 1989).

2.1 Nutritional contribution of chicken:

2.1.1 A Very Good Source of Protein

Chicken is considered as a very good source of protein, providing 67.6% of the daily value for protein in 4 ounces. Chicken meat, when compared with other meat sources appear to be the one of the highest protein source of the 'traditional' meats though the equal second when compared with fish**.** Studies show (Ensminger *et al* 1986) that some sections of the population, especially older people, have poor protein intake. But protein may be important in reducing bone loss in older people. In one study, the 70 to 90 year-old men and women with the highest protein intakes lost significantly less bone over a four-year period than those who consumed less protein. Animal protein, as well as overall protein intake, was associated with preserving bone. With data from 615 participants in the Framingham (MA) Osteoporosis Study, researchers examined the relationship between protein intakes in 1988-1989 and changes in bone mineral density four years later. They accounted for all factors known to increase risk of bone loss.

Participants who reported the lowest daily protein intakes roughly equivalent to half a chicken breast had lost significantly more bone in the hip and spine four years later than those with the highest intakes - equivalent to about 9 ounces of steak and 1 cup of tuna salad. The group with the next lowest intake - equivalent to about 2 cups of cottage cheese - also lost significantly more bone than the highest protein intake group, but only at the hip (Hannan *et al* 2000).

2.1.2 Chicken's Cancer-Protective Nutrients

Chicken is a very good source of the cancer-protective B vitamin, niacin. Components of DNA require niacin, and a deficiency of niacin (as well as other B-complex vitamins) has been directly linked to genetic (DNA) damage. A four-ounce serving of chicken provides 72.0% of the daily value for niacin. Chicken is also a good source of the trace mineral, selenium. Selenium is of fundamental importance to human health. It is an essential component of several major metabolic pathways, including thyroid hormone metabolism, antioxidant defense systems, and immune function. Accumulated evidence from prospective studies, intervention trials and studies on animal models of cancer has suggested a strong inverse correlation between selenium intake and cancer incidence (Kassie *et al* 2003). Several mechanisms have been suggested to explain the cancer-preventive activities of selenium. Selenium has been shown to induce DNA repair and synthesis in damaged cells, to inhibit the proliferation of cancer cells, and to induce their apoptosis, the self-destruct sequence the body uses to eliminate worn out or abnormal cells. In

addition, selenium is incorporated at the active site of many proteins, including glutathione peroxidase, which may be the most important for cancer protection. One of the body's most powerful antioxidant enzymes, glutathione peroxidase is used in the liver to detoxify a wide range of potentially harmful molecules. When levels of glutathione peroxidase are too low, these toxic molecules are not disarmed and wreak havoc on any cells with which they come in contact, damaging their cellular DNA and promoting the development of cancer cells. Four ounces of chicken supply 40.0% of the daily value for selenium (Vogt *et al* 2003).

2.1.3 Protect against Alzheimer's and Age-related Cognitive Decline

Regular consumption of niacin-rich foods like chicken provides protection against Alzheimer's disease and age-related cognitive decline. Researchers from the Chicago Health and Aging Project interviewed 3,718 Chicago residents aged 65 or older about their diet, then tested their cognitive abilities over the following six years. Those getting the most niacin from foods (22 mg per day) were 70% less likely to have developed Alzheimer's disease than those consuming the least (about 13 mg daily), and their rate of age-related cognitive decline was significantly less (Morris *et al* 2004).

2.1.4 Vitamin B for Energy

Chicken is not only a very good source of niacin, but is also a good source of vitamin B6. This particular mix of B-complex vitamins makes chicken a helpful food in supporting energy metabolism throughout the body, because these B

vitamins are involved as cofactors that help enzymes throughout the body guide metabolic reactions. Both of these B vitamins are important for energy production. In addition to its DNA actions, niacin is essential for the conversion of the body's proteins, fats, and carbohydrates into usable energy. Niacin helps optimize blood sugar regulation via its actions as a component of a molecule called glucose tolerance factor, which optimizes insulin activity. Vitamin B6 is essential for the body's processing of carbohydrate (sugar and starch), especially the breakdown of glycogen, the form in which sugar is stored in muscle cells and to a lesser extent in our liver. A four-ounce serving of chicken supplies 72.0% of the daily value for niacin and 32.0% of the DV for vitamin B6 (Margen 1992).

2.1.5 Vitamin B6 for Cardiovascular Health

In addition to its role in energy metabolism, vitamin B6 plays a pivotal role as a methyl donor in the basic cellular process of methylation, through which methyl groups are transferred from one molecule to another, resulting in the formation of a wide variety of very important active molecules. When levels of B6 are inadequate, the availability of methyl groups is also lessened. One result of the lack of methyl groups is that molecules that would normally be quickly changed into other types of molecules not only do not change, but accumulate. One such molecule, homocysteine, is so damaging to blood vessel walls that high levels are considered a significant risk factor for cardiovascular disease. As noted above, 4 ounces of chicken will supply

about one-third (32.0%) of a person's daily needs for vitamin B6 (Altschul *et al* 1955).

2.2 Chicken oil

Chicken oil is rich in polyunsaturated fatty acid (Van heerden *et al* 2002). Polyunsaturated fatty acids are fatty acids that contain more than one double bond in their backbone. This class includes many important compounds, such as essential fatty acids of which omega-3 fatty acid have potential health benefit (Harris 1989).

2.2.1 N-3 fatty acids

N−3 fatty acids, popularly referred to as ω−3 fatty acids or omega-3 fatty acids) are essential unsaturated fatty acids with a double bond (C=C) starting after the third carbon atom from the end of the carbon chain.

Essential fatty acids are molecules that cannot be synthesized by the human body but are vital for normal metabolism. One of the two families of these essential fatty acids is the omega-3 fatty acids.

The carbon chain has two ends, the acid (COOH) end and the methyl (CH_3) end. The location of the first double bond is counted from the methyl end, which is also known as the omega (ω) end or the *n* end.

Nutritionally important *n*−3 fatty acids include α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), all of which are polyunsaturated.

Mammals cannot synthesize *n*−3 fatty acids, but have a limited ability to form the "long-chain" *n*−3 fatty acids EPA (20-carbon atoms) and DHA (22-carbon atoms) from the "short-chain" eighteen-carbon *n*−3 fatty acid ALA List of some n-3 fatty acid are shown in following table

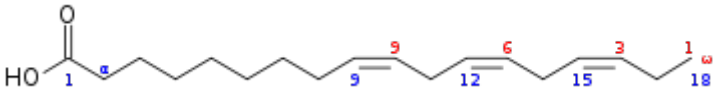
Table 2.1: List of n-3 fatty acid

Common name	Lipid name	Chemical name
Hexadecatrienoic acid (HTA)	16:3 (*n*−3)	*all-cis*-7,10,13-hexadecatrienoic acid
α-Linolenic acid (ALA)	18:3 (*n*−3)	*all-cis*-9,12,15-octadecatrienoic acid
Stearidonic acid (SDA)	18:4 (*n*−3)	*all-cis*-6,9,12,15-octadecatetraenoic acid
Eicosatrienoic acid (ETE)	20:3 (*n*−3)	*all-cis*-11,14,17-eicosatrienoic acid
Eicosatetraenoic acid (ETA)	20:4 (*n*−3)	*all-cis*-8,11,14,17-eicosatetraenoic acid
Eicosapentaenoic acid (EPA)	20:5 (*n*−3)	*all-cis*-5,8,11,14,17-eicosapentaenoic acid
Heneicosapentaenoic acid (HPA)	21:5 (*n*−3)	*all-cis*-6,9,12,15,18-heneicosapentaenoic acid
Docosapentaenoic acid (DPA), Clupanodonic acid	22:5 (*n*−3)	*all-cis*-7,10,13,16,19-docosapentaenoic acid
Docosahexaenoic acid (DHA)	22:6 (*n*−3)	*all-cis*-4,7,10,13,16,19-

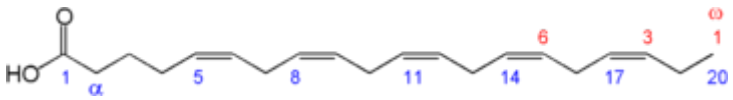
		docosahexaenoic acid
Tetracosapentaenoic acid	24:5 (*n*−3)	*all-cis*-9,12,15,18,21-tetracosapentaenoic acid
Tetracosahexaenoic acid (Nisinic acid)	24:6 (*n*−3)	*all-cis*-6,9,12,15,18,21-tetracosahexaenoic acid

2.2.2 Chemistry of N-3 fatty acid

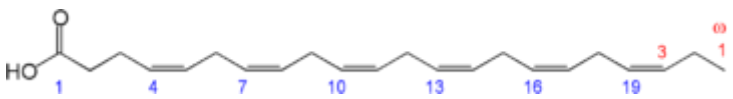
N−3 fatty acids that are important in human physiology are α-linolenic acid (18:3, *n*−3; ALA), eicosapentaenoic acid (20:5, *n*−3; EPA) and docosahexaenoic acid (22:6, *n*−3; DHA). These three polyunsaturates have 3, 5, or 6 double bonds in a carbon chain of 18, 20, or 22 carbon atoms, respectively. As with most naturally-produced fatty acids, all double bonds are in the *cis*-configuration; in other words, the two hydrogen atoms are on the same side of the double bond.



- Chemical structure of alpha-linolenic acid (ALA), an essential *n*−3 fatty acid, (18:3Δ9c,12c,15c, which means a chain of 18 carbons with 3 double bonds on carbons numbered 9, 12, and 15).



- Chemical structure of eicosapentaenoic acid (EPA).



- Chemical structure of docosahexaenoic acid (DHA).

Fig 2.3: Chemical structure of some n-3 fatty acid.

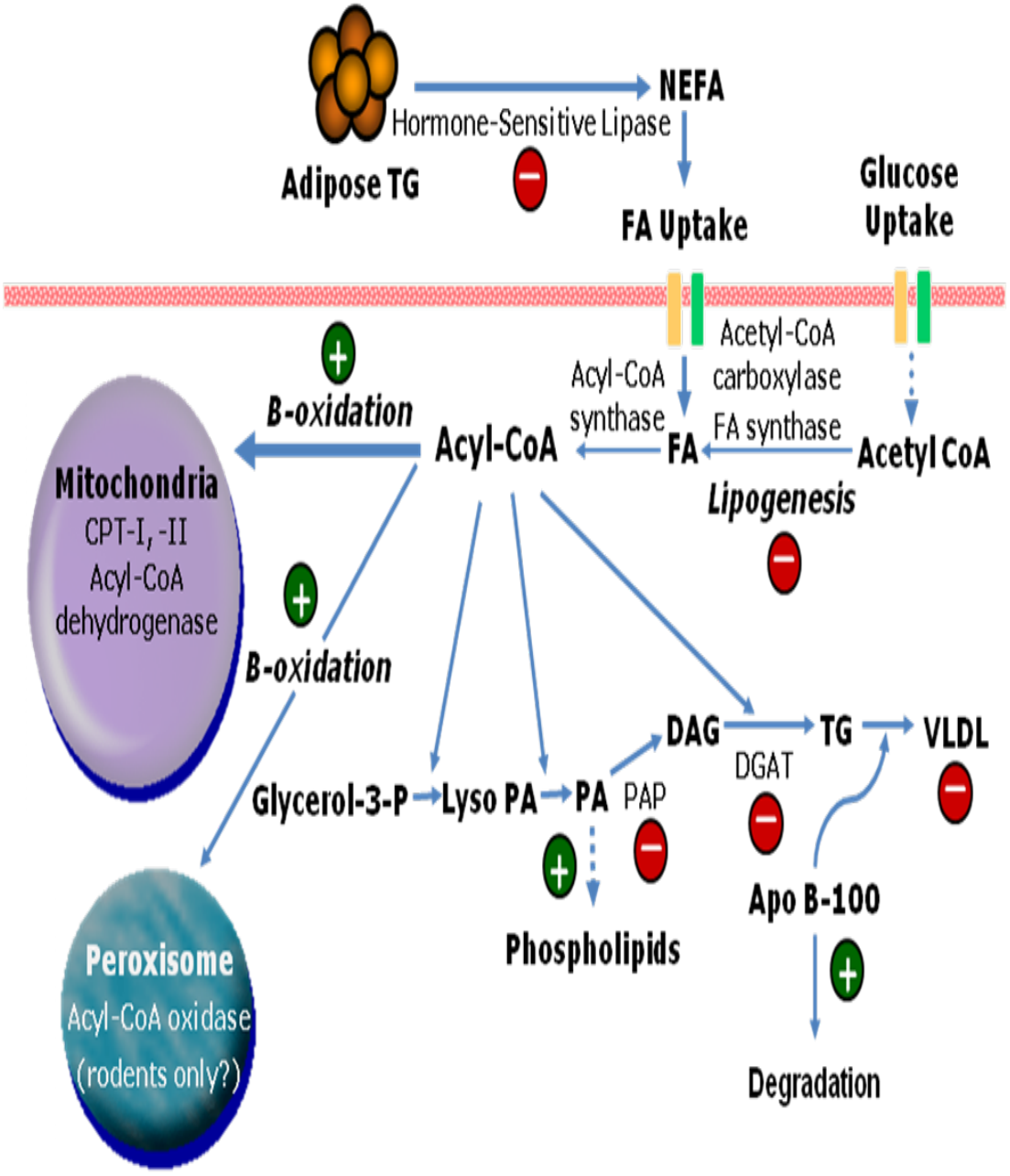


Fig 2.4: Potential triglyceride lowering mechanism of *n*-3 fatty acid

(**Source**: Harris WS and Bulchandani D.*Curr Opin Lipido*l2006; 17:387-393)

2.2.3 Cardiac benefit

Fatty acids can be divided into four general categories: saturated, monounsaturated, polyunsaturated, and trans fats. Saturated fatty acids and trans fats are associated with an increased risk of coronary heart disease. Monounsaturated fatty acids and polyunsaturated fatty acids are associated with a decreased risk of coronary heart disease (White 2009).

Consumption of n-3 fatty acid is inversely associated with fatal ischemic heart disease (IHD) and arrhythmic death, consistent with prevention of fatal arrhythmias by long-chain n-3 polyunsaturated fatty acids (PUFAs) in chicken (Bang *et al* 1980, Siscovick *et al* 1995).

The American Heart Association (AHA) has reviewed the benefits of regular consumption of n-3 fatty acid. The review concludes that n-3 fatty acid help prevent cardiovascular disease including fatal and non-fatal heart attacks, strokes, sudden cardiac death, and coronary artery disease (angina). The reviewers believe that the mechanisms by which n-3 fatty acid exert their protective effect include (Etherton *et al* 2002)

- Decrease in platelet aggregation
- Reduction in triglyceride levels
- Retardation of atherosclerosis
- Anti-inflammatory effects.

Scientists aren't completely certain why omega-3 fatty acids, which are found in chicken oil and other sources, lower blood lipid levels, but the research is clear, chicken oil is good for the heart (Bourre 2005).

Omega-3 enriched chicken oil has shown to reduce other risk factors such as lowering serum triglyceride levels and total cholesterol levels which are both heart attack risk factors. A study showed that postmenopausal women can reduce their risk of heart attack by 27% when taking omega-3 chicken oil (Balk *et al* 2006).

N-3 fatty acid consumption may help to normalize the prethrombotic state and reduce arterial disease. It has been attributed to a reduction in platelet activation, a lowering of plasma triglycerides and (vitamin K–dependent) coagulation factors and/or a decrease in vascular tone. Most intervention studies have shown only moderate effects of (n-3) PUFA on these homeostatic variables.

There is increasing evidence that lipoprotein-associated phospholipase A2 (LPA2) is an independent risk factor for cardiovascular disease. About 80% of LPA2 is bound to low-density cholesterol (LDL), 15-20% is bound to high-density cholesterol (HDL), and the remainder can be found in very low-density cholesterol (VLDL). A group of Danish and German researchers recently conducted a study to determine if the level of LPA2 in blood plasma correlates with the severity of coronary artery disease (CAD) and if there is any

correlation between LPA2 level and the concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fat (adipose) tissue.

The researchers observed a significant correlation between LPA2 concentration and severity of CAD with patients with 3-vessel disease (50% or greater narrowing [stenosis] of 3 major coronary arteries) having the highest LPA2 level. They also found that a high LPA2 level was significantly associated with a low level of EPA. The researchers speculate that the mechanism(s) by which EPA+DHA protect against CAD may include an interaction with LPA2. More specifically, n-3 fatty acid may reduce the concentration of small dense LDL particles, which are the primary carriers of LPA2. n-3 fatty acid also have proven antiinflammatory effects which, through an inhibitory effect of monocyte and macrophage reactivity, may reduce circulating levels of LPA2 (Seikikawa *et al* 2008).

In 1964, it was discovered that enzymes found in sheep tissues convert *n*−6 arachidonic acid into the inflammatory agent called prostaglandin E (Bergstrom 1964), which both causes the sensation of pain and expedites healing and immune response in traumatized and infected tissues. By 1979, more of what are now known as eicosanoids was discovered: thromboxanes, prostacyclins, and the leukotrienes. The eicosanoids, which have important biological functions, typically have a short active lifetime in the body, starting with synthesis from fatty acids and ending with metabolism by enzymes. However, if the rate of synthesis exceeds the rate of metabolism, the excess

eicosanoids may have deleterious effects. Researchers found that certain *n*−3 fatty acids are also converted into eicosanoids, but at a much slower rate. Eicosanoids made from *n*−3 fatty acids are often referred to as anti-inflammatory, but in fact they are just less inflammatory than those made from *n*−6 fats. If both *n*−3 and *n*−6 fatty acids are present, they will "compete" to be transformed, so the ratio of long-chain *n*−3:*n*−6 fatty acids directly affect the type of eicosanoids that are produce. This competition was recognized as important when it was found that thromboxane is a factor in the clumping of platelets, which can both cause death by thrombosis and cause death by bleeding. Likewise, the leukotrienes were found to be important in immune/inflammatory-system response, and therefore relevant to arthritis, lupus, asthma, and recovery from infections. These discoveries led to greater interest in finding ways to control the synthesis of *n*−6 eicosanoids. The simplest way would be by consuming more *n*−3 and fewer *n*−6 fatty acid (Lands and William 1992).

When administered as the ethyl ester, the omega-3 fatty acid EPA appears to form potent anti-inflammatory molecules, called resolvins and omega-3-oxylipins (Shearer *et al* 2009) which may partly explain the positive effects of chicken oil.

The n-3 fatty acids DHA and EPA may act as direct ligands to a cell surface G-protein receptor, affecting anti-inflammatory and insulin sensitization in mice (Young *et al* 2010).

There was a strong correlation between the extent of the reduction in platelet-monocyte aggregation and the level of EPA, DHA and total omega-3 fatty acids in plasma phospholipids.

2.2.4 Potential Mechanisms by Which Omega-3 Fatty Acids May Reduce Risk for Cardiovascular Disease (Oomen *et al* 2000)

- ➤ Reduce susceptibility of the heart to ventricular arrhythmia
- ➤Reduce adhesion molecule expression
- ➤Reduce platelet-derived growth factor
- ➤Promote nitric oxide-induced endothelial relaxation
- ➤Mildly hypotensive

The hypotriglyceridemic effects of omega-3 fatty acids are well established. In a comprehensive review of human studies (Connor *et al* 2000), it has been reported that approximately 4 g/d of omega-3 fatty acid decreased serum triglyceride concentrations by 25% to 30%, with accompanying increases in LDL cholesterol of 5% to 10% and in HDL cholesterol of 1% to 3%. At present, it seems that both EPA and DHA have triglyceride-lowering propertie. Omega-3 fatty acids decrease platelet aggregation resulting in a modest prolongation of bleeding time (Mori *et al* 1997). Some evidence indicates that n-3 fatty acid supplementation may enhance fibrinolysis (Knapp *et al* 1997)

The possibility that omega-3 fatty acids (including linolenic acid) may reduce risk for sudden cardiac death is based on evidence from a prospective cohort

study (Albert *et al* 1998), a case-control study (Siscovick *et al* 1995) and three prospective dietary intervention trials (Johansen *et al* 1999, Lorgeril *et al* 1999, Burr *et al* 1989). Proposed mechanisms to explain these observations center not on lipid or blood pressure lowering or on antithrombotic effects, but on a novel stabilizing effect of omega-3 fatty acids on the myocardium itself. Evidence for a direct effect of these fatty acids on the heart has come from several observations. First, increased heart rate variability in survivors of MI was associated with the consumption of one fish meal per week (Singh *et al* 1997) or 4.3 g/d of omega-3 fatty acids.(Christensen *et al* 1997). EPA and DHA also have been shown to reduce resting heart rate and increase left ventricular filling capacity (Christensen *et al* 1996). A study (Grimsgaard *et al* 1998) showed that acetylcholine-stimulated relaxation of small arteries taken from hypercholesterolemic patients was significantly improved after three months of supplementation with 3 g/d of EPA+DHA. N-3 feeding has also been shown to improve endothelial function (Goode *et al* 1997) and to increase arterial compliance (Chin and Dart 1995). N-3 fatty acid also affects the metabolism of inflammatory mediators like the interleukins and tumor necrosis factors, molecules believed to play a role in atherogenesis and plaque stability.

2.2.5 Chicken Oil Consumption and Reduction of Arterial Disease

High consumption of n-3 PUFA (>5 g daily) was considered to be responsible for the low incidence of arterial diseases (Hornstra 1989). Dietary n-3 PUFA influences the composition of membrane phospholipids, an effect that is

already detectable at low fish oil intake. Chicken oil–derived (n-3) PUFA replace especially arachidonic acid, 20:4 (n-6), in the structural phospholipids of platelets and vascular cells by eicosapentaenoate and docosahexaenoate. Because these PUFA are all cleaved from phospholipids by cytosolic phospholipase A_2, chicken oil lowers the arachidonate production by the phospholipase and, thereby, the substrate level for cyclooxygenase and lipoxygenase. In platelets and vascular cells, dietary chicken oil thereby reduces formation of the (n-6) PUFA-derived prostaglandins and leukotrienes, although this effect is compensated for in part by formation of (n-3) PUFA-derived homologues of these prostanoids (Cicero *et al* 2010). For platelets, early work showed that the reduced formation of prostaglandin H_2/thromboxane A_2 by chicken oil via cyclooxygenase-1 (COX1) is physiologically important because the COX1 product of chicken oil–derived eicosapentaenoate, thromboxane A_3, stimulated platelets in a less effective manner than thromboxane A_2, whereas the platelet-inhibiting effect of endothelial prostaglandin I_3 [derived from (n-3) PUFA] was similar to that of prostaglandin I_2 (derived from arachidonic acid (Bang *et al* 1971). Chicken oil was therefore considered to influence the so-called thromboxane-prostaglandin balance, controlling platelet activation in a favorable, i.e., less platelet-stimulatory way. However, later ex vivo model studies with rats indicated that the (n-3) PUFA-derived prostanoids from the endothelium contributed little to the suppression of platelet activation. Thus, at least in this animal model, it is unlikely that the antithrombotic potential of chicken oil

depends entirely on an altered thromboxane-prostaglandin balance (Needleman *et al* 1979)

2.2.6 Coagulation and Relation to Platelet Activation

In recent diet studies with rats, (n-3) PUFA reduced the levels of the vitamin K–dependent factors- II and X to such a degree that sensitive clotting assay were affected (Oosthuizen *et al* 1994). In particular, the process of tissue factor–induced thrombin generation, which is ntervention with low doses of (n-3) PUFA Fibrinogen, an independent cardiovascular driven by the vitamin K–dependent coagulation factors, was reduced after i risk factor, is a vitamin K–independent protein, that is required for both coagulant activity and platelet function (Leray *et al* 2001).

2.2.7 Relation of chicken oil with cancer

Chicken oil is known to protect from many types of cancers of the colon, liver, breast, prostate and lung (Potter 1992, Armstrong and Doll 1975). Emerging evidences from epidemiological and experimental studies indicate a relationship between dietary fat and the risk of cancer .Especially it has been shown that populations that consume high amounts of omega-3 fatty acids have lower incidence of breast, prostate and colon cancers than those who consume fewer amounts of n-3 fatty acids.

Inflammatory bowel diseases (IBD) increase the risk of developing colorectal cancer. Dietary components that reduced inflammation are associated with

lower cancer risk. The long chain omega-3 fatty acid, docosahexaenoic acid (DHA), is present in chicken oil and has potent anti-inflammatory properties (Terry *et al* 2003).

2.2.8 Chicken oils combat weight loss in cancer patients

Cachexia (abnormal weight loss) is a major problem in many types of cancer especially cancer of the pancreas. Preliminary research has shown that supplementing the diet with 2.2 grams of EPA (eicosapentaenoic acid) and 1.4 grams of DHA (docosahexaenoic acid) daily, will stabilize weight in patients with inoperable pancreatic cancer (Albino *et al* 2000).

METERIALS AND METHODES

3. 1 Study design:

The study followed a multistage hybrid research design in which constituted a descriptive observational and experimental study. The study was divided into two stages:

Stage 1

In this stage the nutritional status (macro and micronutrient content) of the subjects and characterization of oil were observed.

Stage 2

In this stage, comparative biological health effect of extracted oil of the two specific subjects has been carried out on experimental rats.

3.2 Location and time period

The place of study was the Clinical Biochemistry Lab, Dept of Biochemistry & Molecular Biology, University of Rajshahi. The study was carried out for a period of 4 years from Jan 2011 to Dec 2014.

3.3 Materials

3.3.1 Collection of chickens

Both domestic and hybrid chicken, ten of each type were collected from the nearby market at Binodpur, Rajshahi. The collected chickens were cleaned, air-dried, packed in polyethylene bag, sealed and stored at 4^0C for use in subsequent experiments. Only the meat portion of chicken was used for various experimental purposes.

3.3.2 Collection of rats

42 female wister strain rats weighing about 100–150 gm, age about 21-28 days, were collected from Chittagong BCSIR to carry out the experiment. All the animals were kept and maintained under laboratory conditions of temperature (25±7.2ºC), humidity (45.75%) and 12 h day: 12 h night cycle; and were allowed free access to food (standard pellet diet) and water *ad libitum*. The animals were divided into seven groups of six rats in each group and provided with standard diet for 21 days in animal's house.

Figure 3.1: WISTER STRAIN RAT

3.3.3 Grouping of animals

The animals were grouped as follows:

Group:

NCD - normal (non-diabetic rats),

DC- Diabetic Control (Alloxan induced diabetic rat without treatment),

DG - Diabetic + Glibenclamide,

DDO- Diabetic + Domestic chicken oil (1% of total diet),

DHO- Diabetic + Hybrid chicken oil (1% of total diet),

DDF- Diabetic + Domestic chicken flesh (1% of total diet),

DHF- Diabetic + Hybrid chicken flesh (1% of total diet)

Table 3.1: Diet chart of rat which was given during experimental period

No.	Name of ingredients	For control Rat %	For experimental Rat %
1	Wheat	40%	40%
2	Wheat brane	19%	19%
3	Rice polish	4%	4%
4	Til oil cake	10%	10%
5	Mashcolai	6%	6%
6	Flour	14%	14%
7	Skim milk powder	4%	4%
8	Salt	0.5%	0.5%
9	Molasses	0.5%	0.5%
10	Chicken oil	nil	1%
11	Chicken Flesh	nill	1%

3.4 Methods

3.4.1 General methods

3.4.1.1: Nutritional analysis of chicken meat before sun dry :

- Moisture content was determined by conventional procedure (ICOMR, 1971). Appendix-I
- Ash content was determined by A.O.A.C method (Hofelmann & Hartmann, 1985). Appendix- II.
- Lipid content was determined by Blight and Dyer method. (Bligh & Dyer 1959). Appendix- III
- Total sugar was estimated by Anthrone method (Desnuelle 1961). Appendix- IV
- Reducing sugar was estimated by Dinitrisalicylic acid (Miller 1972). Appendix- V
- Glycogen content was determined by Anthrone method(Boel *et al* 1988). Appendix- VI
- Total protein was estimated by micro-kjeldahl method (Ranganna 1986). Appendix- VII
- Mineral content was determined by atomic absorption spectrophotometer (Black 1965). Appendix-VIII

3.4.1.2 Extraction of oil

Oil portion of hybrid and domestic chicken were extracted by suitable solvents under the operating condition. Continuous Soxhlet extraction device was used for the extraction of oil (Bahl and Bahl 2001).

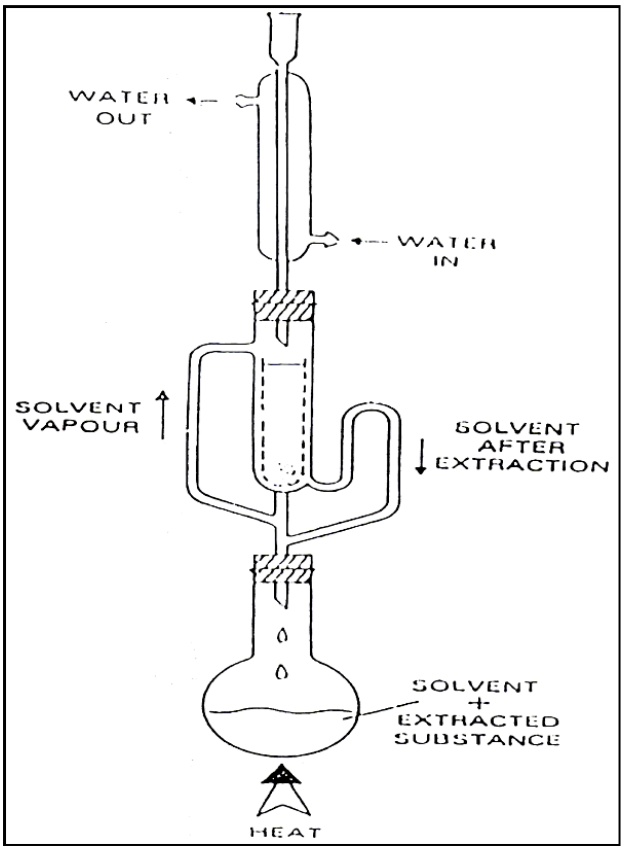


Fig 3.2: A Soxhlet extractor

The following apparatus and chemicals were used for oil extraction through Soxhlet extractor.

Apparatus

Weighing balance ,

Soxhlet apparatus

Rotary evaporator

Flask

Chemicals

N-Hexane (Analytical grade).

Procedure:

Fresh selected species of chickenes were sun dried for complete removal of the moisture and then crushed well to almost pest form by a hand crusher. The chicken oil was extracted using soxhlet extractor and N-Hexane as the solvent. The solid substance or sample was placed in a porous thimble covered with cotton wool and the weight of the sample taken, before it was placed in the inner tube of the apparatus and then fitted to a round bottom flask of appropriate size that contain the solvent. Heat was applied to heat the solvent to its boiling point for 1 hour. As the heating continued, the solvent in the flask started boiling just within 5 minute of heating and the water begins to drop from the top to the sample in the thimble. When the solvent reached the top of the tube, it siphoned over into the flask and thus removes the portion of the oil which has been extracted in the process of refluxing. It was noticed that 18 minutes later, after boiling has started, there was refluxing and this continued at 2 minutes interval. The solvent used was later recovered by applying heat and collected above the round bottom flask into the soxhlet apparatus while the oil extracted was collected and measured. The extract was evaporated under reduced pressure (rotary evaporator) to obtain the oil.

Purification of Crude Oil

Apparatus Required

1) Separating funnel
2) Rotator evaporator

Reagents Required

a) Anhydrous sodium sulfate
b) Diethyl ether: freshly distilled, free from peroxides and residues
c) Saturated solution of sodium chloride

Procedure

About 100 g of oil was taken in a separating funnel followed by the addition of 100 ml of water, 200 ml of ether and 25 ml of saturated sodium chloride solution. The content of the separating funnel was shaken well and allowed to stand for sometimes until two distinct layers were separated. Discarding the aqueous layer, the organic layer was again shaken with 100 ml of distilled water and 25 ml of saturated solution of sodium chloride and was allowed to stand. The ether layer was separated and subjected to similar treatment once more. Finally, the resulting ether extract was taken in a conical flask and dried over anhydrous sodium sulfate. The extract was then evaporated by a rotary evaporator at 40° C to get the purified oil. The flow diagram of extraction and purification of oil using N-hexane from chickenes is shown in Figure 3.3.

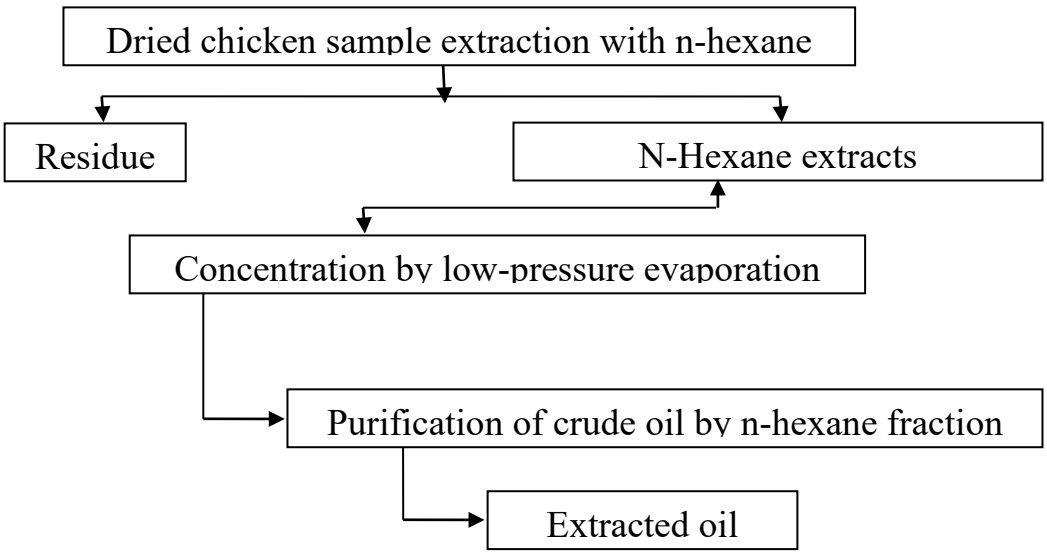


Fig 3.3: Flow diagram summarizing the extraction and purification of lipid using N-hexane.

3.4.1.3 Characterization of extracted oil was performed by following methods:

- The iodine value of oils were measured by Hanus method (Williams 1996, Kalumuddin. *A Test Book of practical physics*). Appendix-IX
- Saponification value, unsaponifiable matter, acid value, peroxide value, % free fatty acids was determined by conventional methods. Appendix-X
- Fatty acid composition of extracted oil was determined by Gas-liquid chromatography (Wilhelm *et al* 1959). GC-2025 gas chromatograph with AOC-20i Auto Injector (Shimadzu Co, Japan) was used for quantitative analysis of chicken oil.

  GC- 2025 Gas chromatography status

  Temperature……..280°C

  Pressure…….175.4KPa

  Total Flow……..165ml/min

  Purge Flow…….3ml/min

  Column Temperature……...270°C

  FIDI

  Temperature……..290°C

Make Up Flow……30ml/min

H_2 Flow ……...40ml/min

Air Flow……400

Injector……..AOC-20i Auto injector(Shimadzu Co, Japan)

Solvent

Methanol, ethanol, hexane of analytical grade produced either from E.MERCK (Germany) or BDH (England) were used.

Chemicals And Reagents

Boron trifluoride-methanol complex and standard methyl esters of fatty acids (Lipid Standard) were produced from Sigma(St Louis, MO, USA). Gas chromatography was conducted with a Gas Chromagraph GC-2025 series (Shimadzu Co, Japan). Each FAME in extract was identified by comparing retention times with those of known standard FAME (Lipid Standard Sigma chemical Co, St Louis, MO, USA).The area of fatty acids was measured with GC solution 2011. The results were expressed as relative percentage of fatty acids. The relative percentage of fatty acids were calculated by the formula:

$$\text{Relative percentage of fatty acid} = \frac{\text{Area of fatty acid } \times 100}{\text{Total area of detected fatty acids}}$$

3.4.1.4 Induction of diabetes in rats:

Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared Alloxan Hydrate (55 mg/kg bw) in 0.9% saline solution. Diabetes was developed and stabilized in these alloxan treated rats over a period of 48 hours. After three days of alloxan administration, plasma glucose levels of each rat were determined. Rats with a fasting plasma glucose range of 11.5 mmol/L were considered diabetic and included in the study (Siddiqul *et al* 2014).

3.4.1.5 Treatment schedule

In experiment, 30 rats (6 normal; 24 Alloxan-diabetic surviving rats) were used. The rats were divided into 5 groups of 6 rats in each group. Group I – normal rats treated with Normal diet (NDC-group); Group II– alloxan-induced diabetic rats were controlled for 21 days with normal diet (DC-group). Group III, IV and V– alloxan induced diabetes affected rats were treated with glibenclamide (0.6 mg/kg body weight) (DG-group), domestic chicken oil (1% of total diet) (DDO-group) and hybrid chicken oil (1% of total diet) (DHO-group) treatment respectively.

3.5 Experimental methods

3.5.1 Sample collection and assessment of serum

In a 15 days experiment, bloods were collected every week from the eyes of rats with highly sterilized capillary tube. Blood (3-4ml) samples were kept in capped, air tight, plastic test tubes The serum was separated by centrifugation of blood samples at 4000 rpm for 15 min and the serum obtained which was used to estimate serum glucose, serum total cholesterol (TC), serum triglyceride (TG) and serum HDL levels and stored at -80°C until the experiments were performed.

3.5.2 Laboratory examination

The semi autoanalyzer (microlab 300) were used for the measurement of serum indices by using commercially available kits according to the manufacture's protocol. All serum samples were analyzed in duplicate and then mean values were taken.

3.5.3 Laboratory analysis

- Serum glucose of the study subjects was measured by Glucose-Oxidase (GOD-PAP) method, commercial kit. Appendix-XI
- Serum total cholesterol was assessed by Enzymatic-Colorimetric (Cholesterol Oxidase/ Peroxidase) method commercial kit. Appendix-XII
- Serum triglyceride was measured by Enzymatic-Colorimetric (GPT-PAP) method, commercial kit. Appendix-XIII
- Serum HDL cholesterol was estimated by Enzymatic-Colorimetric (Cholesterol CHOD-PAP) method, commercial kit. Appendix-XIV
- LDL cholesterol was calculated by using Friedewald's formula. Appendix-XV
- SGPT and SGOT were measured by using colorimetric method. Appendix-XVI,
- Urea, uric acid and creatinine was also measured by enzymatic colorimetric method. Appendix-XVII, XVIII, XIX.
- GC pattern of standard-Appendix XX
- GC pattern of domestic chicken oil- Appendix XXI
- GC pattern of hybrid chicken oil- Appendix XXII

3.5.4 Statistical analysis: All the results were expressed as mean± SD. Difference between the groups were tested statistically by using independent t-test at 95% confident interval. $P<0.05$ was considered as significant.

RESULTS

4.1 Nutritional analysis of Domestic and Hybrid Chicken

The macro and micronutrient contents of domestic and hybrid chicken were compared. Macronutrient content of these two species is presented in Table-4.1. Figure 4.1 represents the macronutrient content of domestic chicken and Figure 4.2 represents the macronutrient content of hybrid chicken. The results showed that, the moisture and ash contents of domestic chicken were 73.89% and 2.25% respectively. On the other hand moisture and ash content of hybrid chicken were 75.22% and 2.4% respectively. Again total lipid content of domestic and hybrid chicken were 2.82% and 4.78% respectively.

Chicken meat is a very good source of protein. Result showed that protein content of domestic chicken was 12.84% whereas that of hybrid chicken was 13.75%. Glycogen, free sugar and reducing sugar content of domestic chicken were 0.039%, 0.1%, 0.002% whereas that of hybrid chicken were 0.041%, 0.13% and 0.002% respectively. So, all macronutrients such as ash, moisture, total protein, total carbohydrate and total lipid were found to be high for the hybrid chicken. Lipid content was significantly higher (around 40%) in hybrid chicken than that of domestic chicken.

Table 4.1: The Macronutrients content of Domestic and Hybrid Chicken species (gm %)

Parameters	Domestic chicken	Hybrid chicken	% deviation
Moisture	73.89±0.57	75.22±0.71	±2
Ash	2.25±0.73	2.406±0.67	±9
Total lipid	2.83±0.83	4.783±0.94	±40
Total protein	12.84± 0.53	13.75± 0.52	±6.6
Glycogen	0.039±0.003	0.041±0.004	±25
Total sugar	0.1± 0.184	0.13±0.321	±23
Reducing sugar	0.002± 0.04	0.003± 0.03	±22

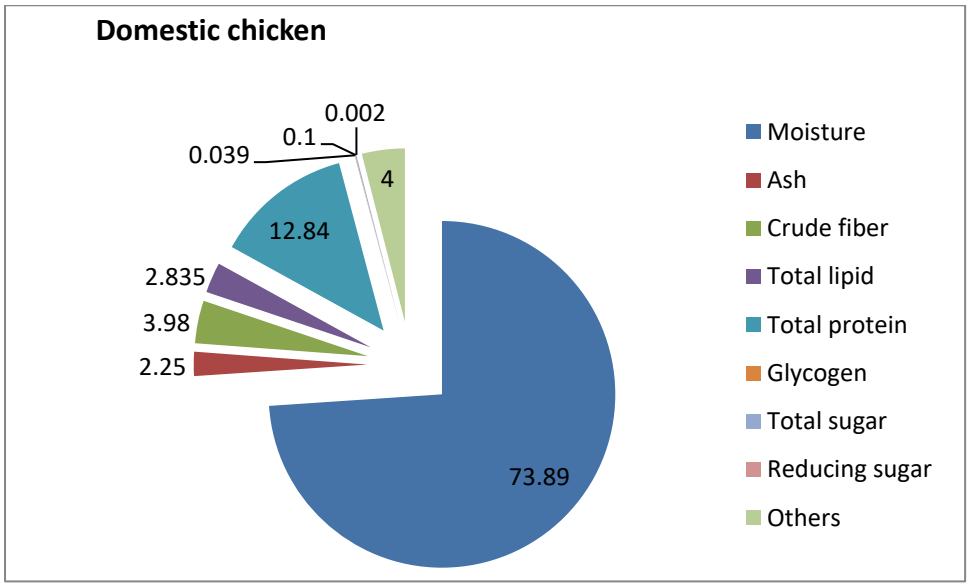


Figure 4.1: Macronutrient content of Domestic Chicken

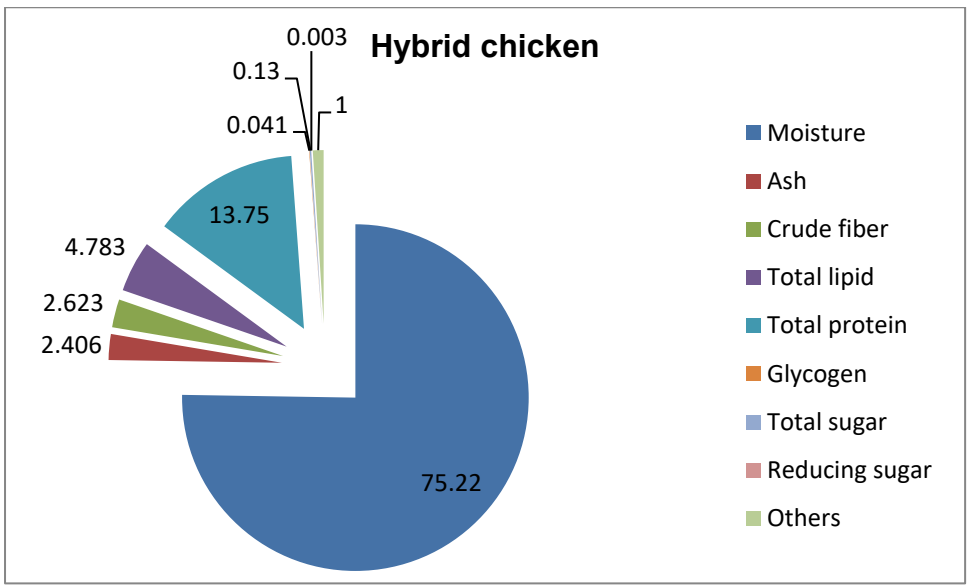


Figure 4.2: Macronutrient content of Hybrid Chicken

Minerals are inorganic substances required by the organism in very small amounts for their growth and maintenance of functional activities. Food and vegetables are the important sources of mineral for human beings and exist in food as organic and inorganic combination.

Micronutrients content of domestic and hybrid chicken are presented in Table-4.2. Figure 4.3 and Figure 4.4 represent the micronutrient contents of domestic and hybrid chicken respectively. Iron, calcium, zinc and lead content of domestic chicken were found to be high that of hybrid chicken. Iron, calcium, zinc and lead content of domestic chicken were 32, 105.2, 17.84 and 38.28 µg/kg whereas, that of hybrid chicken were 27.0, 97.5, 9.11 and 37.17 µg/kg respectively. Again potassium and manganese which were found to be high in hybrid chicken (127.8 µg/kg and 9.74 µg/kg respectively) than that of domestic chicken (86.83µg/kg and 6.83 µg/kg respectively). Zinc content of hybrid chicken was significantly lower (around 50%) than that of domestic chicken.

Table 4.2: The Micronutrients content of Domestic and Hybrid Chicken species (µg/kg)

Name of minerals	Domestic chicken	Hybridchicken	% deviation
Iron	32	27	±20
Calcium	105.2	97.5	±9
Potassium	86.83	127.8	±34
Manganese	6.83	9.74	±28
Zinc	17.84	9.11	±50
Lead	38.28	37.17	±3

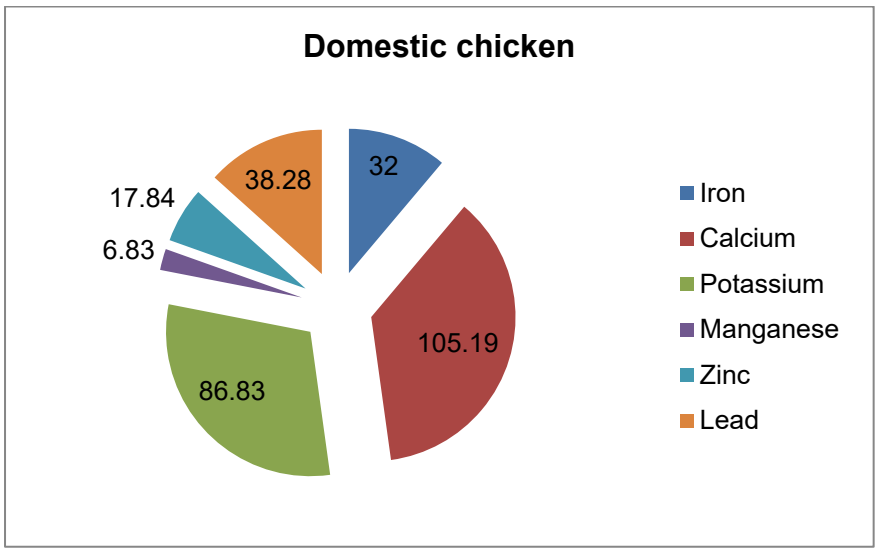


Figure 4.3: Micronutrient content of Domestic Chicken

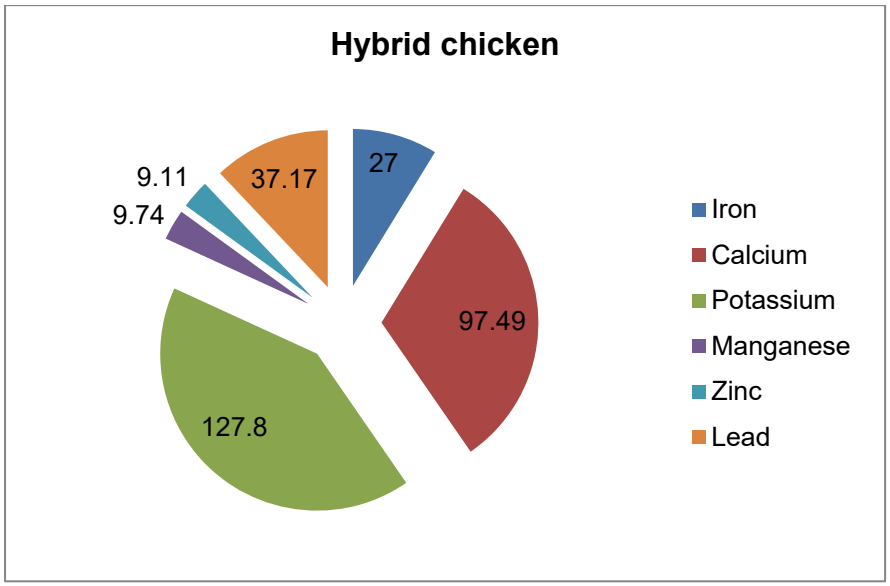


Figure 4.4: Micronutrient content of Hybrid Chicken

4.2 Nutrients content of Hybrid Chicken feed

Feed for the hybrid chicken was collected from CP-Bangladesh farm, a famous industry for the production of poultry chicken feed in Bangladesh, and analysis of nutrient content was done by investigating various parameters such as moisture, total protein, fat, total sugar, calcium and phosphorus. Feed of hybrid chickens contain 12% moisture, 17% total protein, 5% fat, 0.34% total sugar, 1mg /kg calcium and 0.70mg/kg phosphorus which are shown in Table 4.3.

Table 4.3: Nutrients content of Hybrid Chicken feed

Name of parameters	Amount(gm/100gm)
Moisture	12g
Total protein	17g
Fat	5g
Total sugar	0.34g
Calcium	1mg/kg
Phosphorus	0.70mg/kg

4.3 Characterization of chicken oil

Chemical properties of domestic and hybrid chicken oil are shown in Table 4.4. Figure 4.5 and Figure 4.6 represents the chemical properties of domestic and hybrid chicken respectively. The average amount of oil content of two species were extracted with soxhlet apparatus using n-hexane as an extracting solvent and it was found to be 3.54% for domestic chicken and 4.84% for hybrid chicken. Chemical characteristics of the extracted oil were investigated by analyzing various parameters such as iodine value, saponification value, acid value, peroxide value and percentage free fatty acid. Iodine value and unsaponifiable matter were higher for hybrid chicken (77.92 and 11.14 respectively) than those of domestic chicken (60.56 and 3.5 respectively). Again saponification value, acid value, peroxide value and percentage free fatty acid were higher for domestic chicken than hybrid chicken by 22%, 15%, 14% and 15% respectively

Table 4.4: Chemical characteristics of the Domestic and Hybrid Chicken oil

Parameters	Domestic chicken	Hybrid chicken	% deviation
Oil content	3.54	4.84	±26
Iodine value	60.56	77.92	±23
Acid value	32.47	28.24	±15
Saponification value	303.15	248.6	±22
Unsaponifiable matter	3.5	11.14	±67
Peroxide value	81.62	71.87	±14
Percents of free fatty acid	16.25	14.55	±15

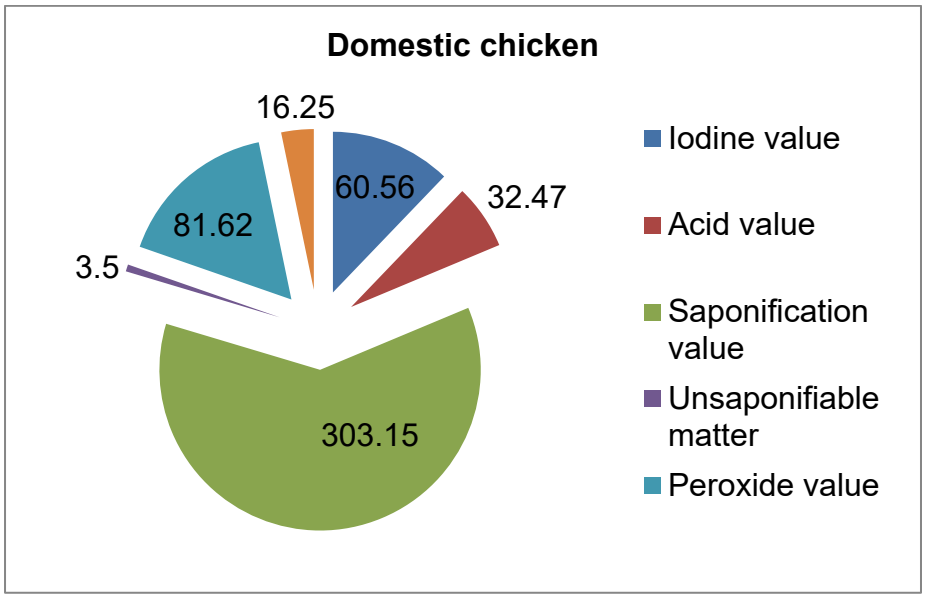


Figure 4.5: Chemical characteristics of Domestic Chicken oil

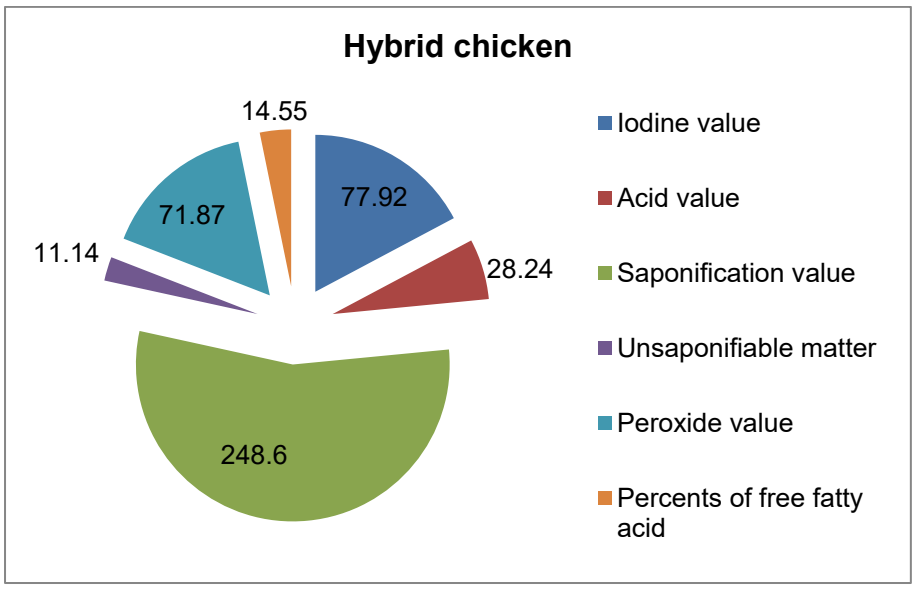


Figure 4.6: Chemical characteristics of Hybrid Chicken oil

4.4 Nutritional comparison of Domestic and Hybrid chickens among breast meat, Drum stick, Thigh meat and meat of Wings

Table 4.5 and Figure 4.7 represent the protein content of domestic and hybrid chicken. Protein content was found to be higher in breast meat than other parts (Thigh, Drum Stick and Wings) of both domestic and hybrid chicken. Lowest amount of protein content was found for thigh meat in both type of chicken.

Table 4.5: Protein content of skinless meat of Domestic and Hybrid chickens

Parameters	Domestic Chicken	Hybrid Chicken
Skinless Breast	16.54±1.5	19.27±1.3
Skinless Drumstick	14.63±0.85	17.36±1.8
Skinless Thigh	13.5±1.1	15.17±1.2
Skinless Wings	15.44±1.6	16.52±1.5

The results are expressed as mean±SD.

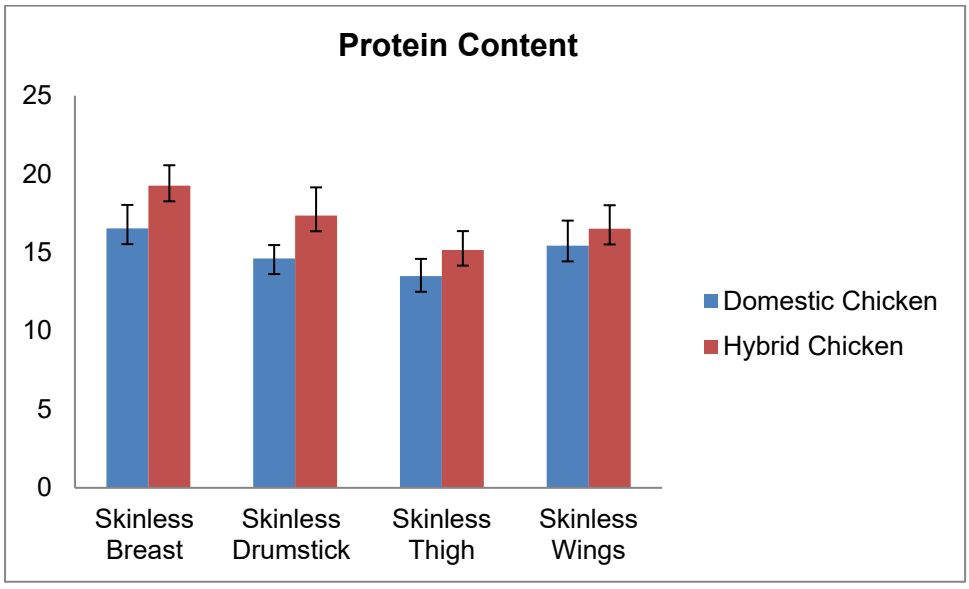


Figure 4.7: Protein content of various parts of meat of Domestic and Hybrid chickens

Higher amount of lipid content was found for thigh meat in contrast with other parts (Breast, Drum Stick and Wings) is shown in Table 4.6 and Figure 4.8.

Table 4.6: Lipid content of skinless meat of Domestic and Hybrid chickens

Parameters	Domestic Chicken	Hybrid Chicken
Skinless Breast	2.11±0.63	3.26±0.35
Skinless Drumstick	2.87±0.46	4.32±0.61
Skinless Thigh	3.65±0.57	5.43±0.9
Skinless Wings	2.68±0.67	3.75±0.54

The results are expressed as mean±SD.

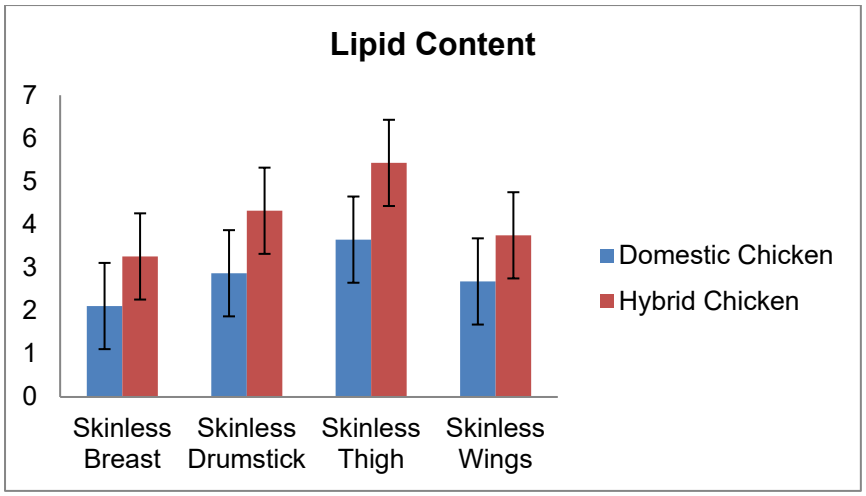


Figure 4.8: Lipid content of various parts of meat of Domestic and Hybrid chickens

Iodine value, saponification value and acid value were observed for different body parts of domestic and hybrid chickens' meat. Hybrid chicken contents higher amount of iodine value than that of domestic chicken. Iodine value was higher for thigh meat in both types of chicken which indicates the presence of

higher amount of unsaturated fatty acid in thigh meat for hybrid chicken (Table 4.7 and Figure 4.9).

Table 4.7: Iodine Value of domestic and hybrid chickens

Parameters	Domestic Chicken	Hybrid Chicken
Skinless Breast	65.76	72.64
Skinless Drumstic	68.44	79.57
Skinless Thigh	74.37	85.38
Skinless Wings	71.28	79.63

The results are expressed as mean±SD.

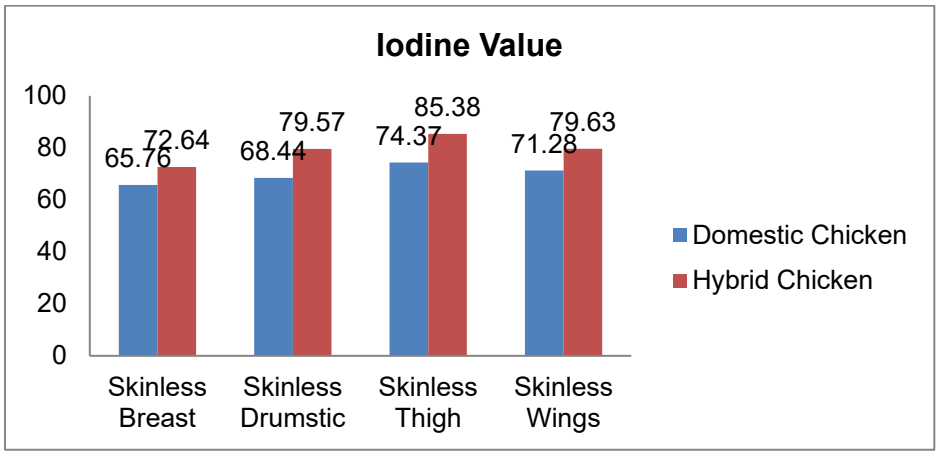


Figure 4.9: Iodine value of various parts of meat of Domestic and Hybrid chickens

Saponification value was higher for domestic chicken than that of hybrid chicken. Specially thigh meat portion of domestic chicken has higher value. On the other hand thigh meat of hybrid chicken contains lower amount of saponification value which indicates the presence of long chain fatty acids (Table 4.8 and Figure 4.10).

Table 4.8: Saponification Value of domestic and hybrid chickens

Parameters	Domestic Chicken	Hybrid Chicken
Skinless Breast	309.09	235
Skinless Drumstick	304.36	241
Skinless Thigh	316.24	240
Skinless Wings	312.43	238

The results are expressed as mean±SD.

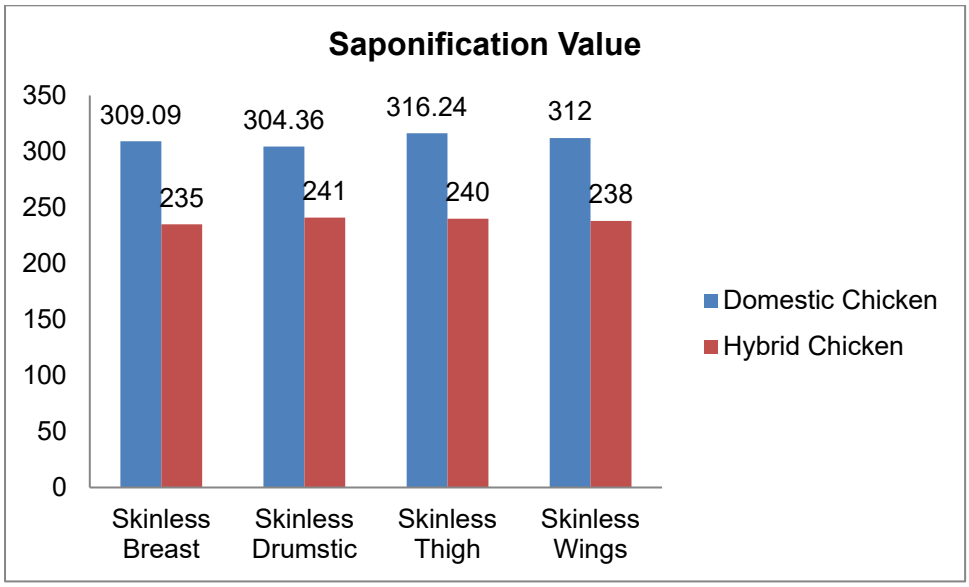


Figure 4.10: Saponification value of various parts of meat of Domestic and Hybrid chickens

Acid Value was found to be higher for domestic chicken oil. Thigh meat portion of domestic has a greater acid value than other parts of the chicken meat. Higher acid value indicates the meat has a tendency to become more rancid (Table 4.9 and Figure 4.11).

Table 4.9: Acid Value of different parts of domestic and hybrid chicken

Parameters	Domestic Chicken	Hybrid Chicken
Skinless Breast	28.86	25.33
Skinless Drumstick	31.55	29.32
Skinless Thigh	35.64	30.42
Skinless Wings	30.47	27.53

The results are expressed as mean±SD.

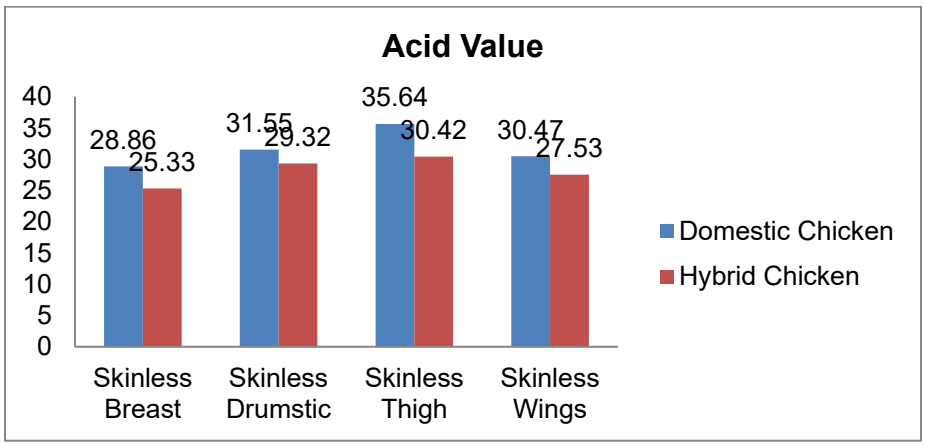


Figure 4.11: Acid value of various parts of meat of Domestic and Hybrid chickens

4.5 Fatty acid composition of Domestic and Hybrid Chicken oil

Fatty acids content was shown in Table 4.10. Both the oils contain myristic acid behenic acid in a very small amount. These two represent a minor saturated constituent of the oil. Bulk of the saturated fatty acids is presented as C16:0 or palmitic acid and C18:0 or stearic acid, however C16:0 or palmitic acid was the major saturated fatty acid of the total fatty acids. The monoenoic acids oleic acid (C18:1) had the highest proportion of the total oil. Palmitoleic acid (C16:1) was the next monoenoic acid in the hybrid chicken oil but domestic chicken contain no palmitoleic acid. Both, domestic and hybrid chicken oil contains arachidonic acid in a small quantity. Figure 4.12 & 4.13,

shows that, among saturated fatty acids, palmitic acid was higher for domestic chicken and stearic acid was higher for hybrid chicken. On the other hand, that among unsaturated fatty acids oleic acid and arachidonic acid was higher for domestic chicken. Palmitoleic fatty acid, a very important mono unsaturated fatty acid was only found in hybrid chicken.

Table 4.10: Fatty acid composition of Domestic and Hybrid Chicken oil

Name of fatty acid	Domestic chicken (Rel%)	Hybrid chicken (Rel%)
Myristic Acid	0.24	0.34
Palmitic Acid	19.62	17.55
Oleic Acid	75.75	48.4
Stearic Acid	1.84	4.67
Arachidonic Acid	1.67	0.49
Behenic Acid	0.85	0.2
Palmitolic Acid	-	28.31
Others	0.03	0.04

*GC patterns of domestic and hybrid chicken oil are shown in Chapter-8: Appendix. Appendix XX: GC pattern of Standard. Appendix XXI: GC pattern of Domestic Chicken. Appendix XXII: GC pattern of Hybrid Chicken

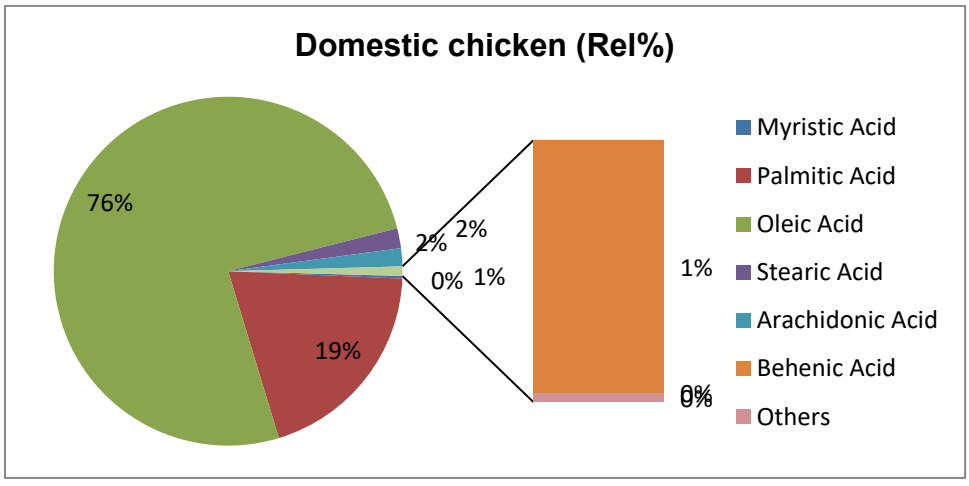


Figure 4.12: Fatty acid composition of Domestic chicken oil

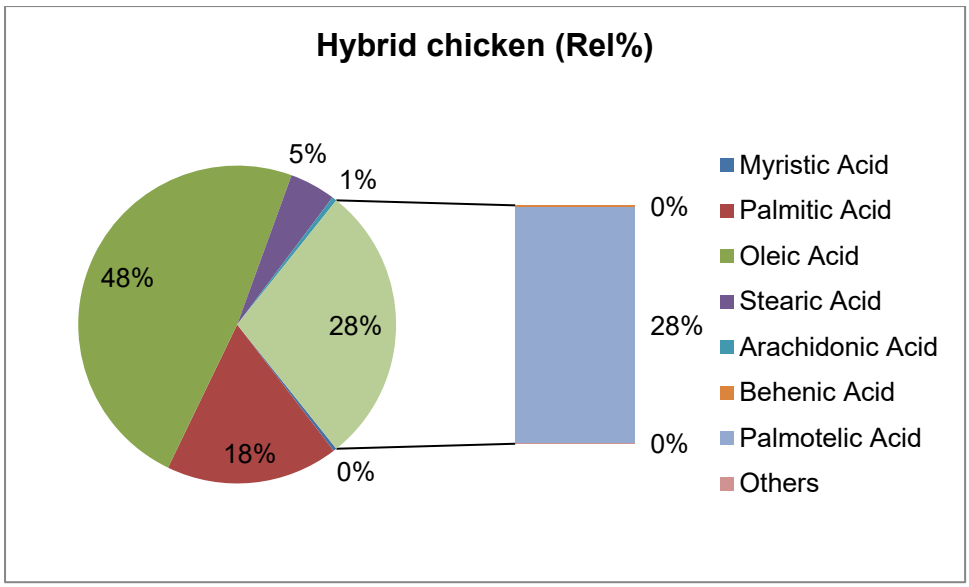


Figure 4.13: Fatty acid composition of Hybrid chicken oil

4.6 Comparative effects of chicken oil and flesh on blood parameters of experimental rats

4.6.1 Effects of chicken oil and flesh on rat's body weight

The results of body weight are shown in the Table 4.11. The body weight of control rats was increased after 2nd week. Initially diabetic rats decrease their body weight but after 2nd week of chicken oil and chicken flesh treatment, there was an increase in body weight. Figure 4.14 and Figure 4.15 shows that after the experimental period domestic and hybrid chicken oil Increased body weight by 81% and 77% respectively. On the other hand domestic and hybrid chicken flesh feeding rats increased body weight around 80% and 83%, which areas near as the glibenclamide treatment group, it was 89%.

Table 4.11: Comparative effect of Domestic and Hybrid Chicken oil and flesh on the body weight of experimental rats, 21 days study (n= 6)

Treatment Group	7 Days	14 Days	21 Days
NDC	92.58±1.24	130.37±0.64	122.83±0.37
DC	103.88±1.94	88.69±0.65	74.61±0.52
DG	94.54±0.54	109.12±0.46	117.94±0.63
DDO	94.49±2,98	105.3±2.58	113.52±2.64*
DHO	95.72±3.10	104.07±O.588	111.05±4.59
DDF	92.27±2.43	101.63±3.17	112.38±1.75
DHF	94.16±1.63	98.42±1.35	114.56±2.54*

*Indicate significance change compared with normal control group (P<0.05). The results are expressed as mean±SD. Group: NCD - normal (non-diabetic rats), DC- Diabetic Control (Alloxan induced diabetic rat without treatment), DG- Diabetic + Glibenclemide, DDO- Diabetic + Domestic chicken oil(1% of total diet), DHO- Diabetic + Hybrid chicken oil(1% of total diet), DDF- Diabetic + Domestic chicken flesh(1% of total diet), DHF- Diabetic + Hybrid chicken flesh (1% of total diet)

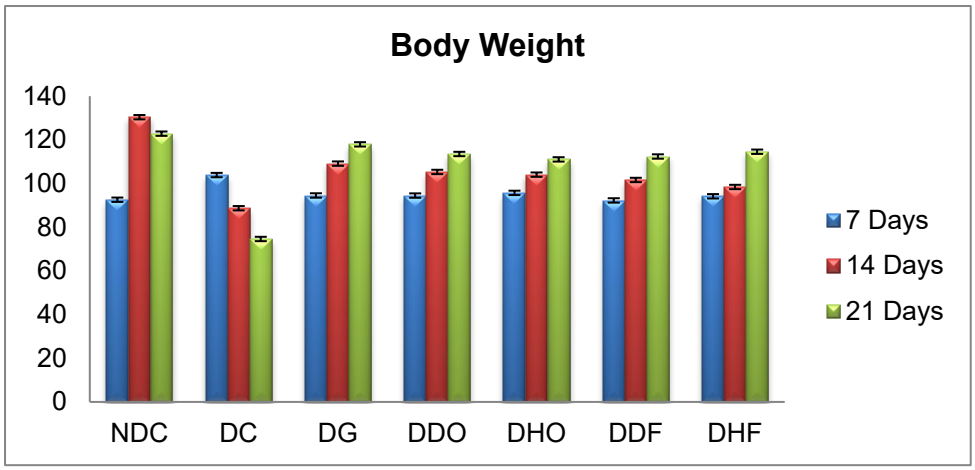


Figure 4.14: Effect of Chicken oil and flesh on the body weight of experimental rats

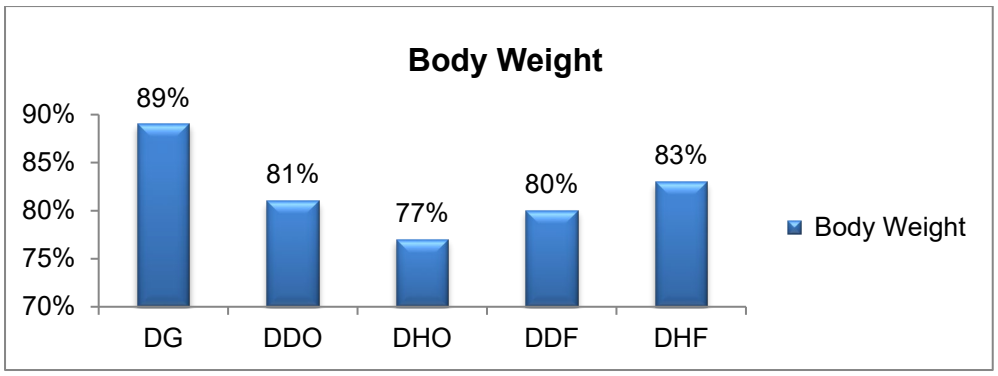


Figure 4.15: Comparative effect of Chicken oil and flesh on the body weight of experimental rats

4.6.2 Effect on blood glucose level of experimental rats

Effect of Domestic and Hybrid chicken oil & flesh are reported in Table 4.12. After first week of chicken oil treatment the domestic and hybrid chicken oil and chicken flesh, had no significant effect on blood glucose level of rat, But after 3rd week of treatment there was a significant hypoglycemic effect had seen, comparing the blood glucose level in alloxan induced rats. Figure 4.16 and Figure 4.17 shows that the blood glucose levels were decreased by 67% and 62% for domestic and hybrid chicken oil administered subject whereas 60% and 52% for domestic and hybrid chicken flesh administered subject which is as near as glibenclamide administered subject (72%).

Table 4.12: Comparative effect of Domestic and Hybrid Chicken oil and flesh on fasting blood glucose level of experimental rats, 21 days study, (n= 6)

Treatment Group	7 Days	14 Days	21 Days
NDC	5.7±0.4	5.6±0.46	5.5±0.57
DC	17.7±1.5	21.2±0.96	25.0±1.4
DG	16.0±1.12	13.7±0.82	10.9±0.81
DDO	15.6±1.09	14.5±1.29	11.9±0.7*
DHO	16.11±0.71	14.13±0.77	12.8±0.56*
DDF	16.4±0.46	15.1±0.45	13.1±0.46
DHF	16.7±0.6	14.8.±0.7	13.9 ±0.51

*Indicate significance change compared with normal control group (P<0.05). The results are expressed as mean±SD. Group: NCD - normal (non-diabetic rats), DC- Diabetic Control (Alloxan induced diabetic rat without treatment), DG- Diabetic + Glibenclemide, DDO- Diabetic + Domestic chicken oil(1% of total diet), DHO- Diabetic + Hybrid chicken oil(1% of total diet), DDF- Diabetic + Domestic chicken flesh(1% of total diet), DHF- Diabetic + Hybrid chicken flesh (1% of total diet)

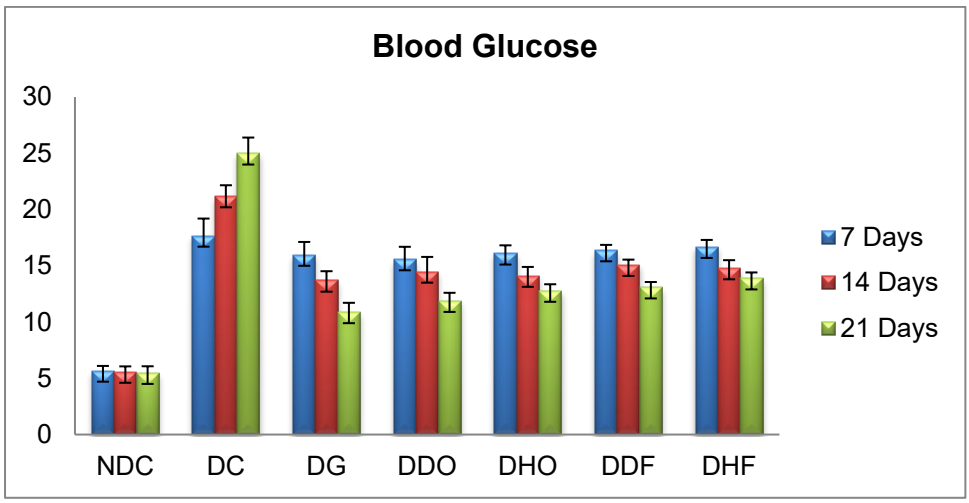


Figure 4.16: Effect of Chicken oil and flesh on fasting blood glucose level of experimental rats

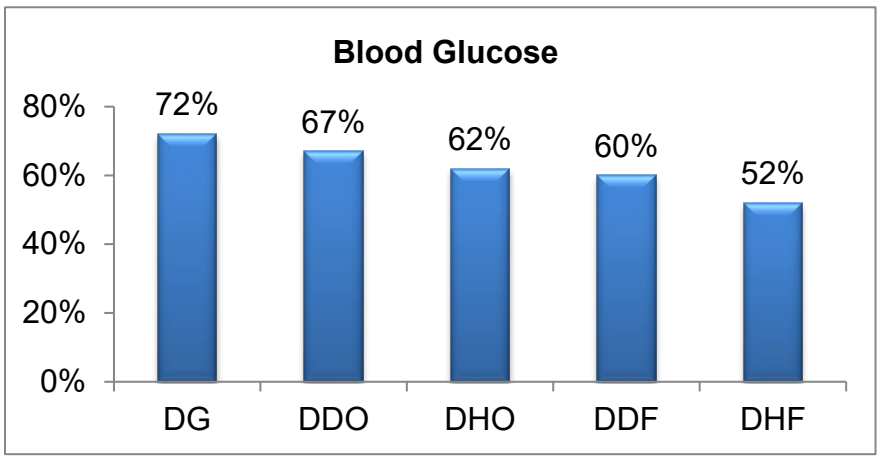


Figure 4.17: Comparative effect of Chicken oil and flesh on fasting blood glucose level of experimental rats

4.6.3 Effect on lipid profile (total cholesterol, TG, HDL, LDL) of experimental rats

The results of the lipid profile of non diabetic and diabetic rats are shown in Table 4.13 and Figure 4.18. 69% and 82% reduction of cholesterol level were observed for domestic and hybrid chickens oil treatment respectively in diabetic rats whereas 44% and 62% reduction were seen for domestic and hybrid chicken flesh. In case of glibenclemide, it was 89% (Figure 4.19).

LDL level was reduced (P<0.001) for domestic chicken oil, hybrid chicken oil, domestic chicken flesh, hybrid chicken flesh and glibenclimide at 65%, 71%, 48%, 57% and 77% (Figure 4.22) whereas HDL level was increased significantly 58%, 70%, 50%, 62% and 79% respectively (Figure 4.21). A little bit different scenario was observed for triglyceride level. Triglyceride reduced the triglyceride level more significantly than chicken oil and flesh. Triglyceride level reduced for domestic chicken oil, hybrid chicken oil, and domestic chicken flesh, hybrid chicken flesh by around 26%, 30%, 22% and 20% respectively. Whereas Triglyceride level was reduced by 86% (Figure 4.20).

Table 4.13: Comparative effect of Domestic and Hybrid Chicken oil and flesh on blood lipid profile of rats, 21 days study, (n= 6)

Treatment Group	21 Days study			
	Cholesterol	**triglceride**	**HDL**	**LDL**
NDC	69.44±0.68	60.07±0.27	69.83±0.50	85.9 ± 0.513
DC	97.97±0.46	110.22±0.36	45.085±0.15	121.10±0.314
DG	72.82±0.33	67.05±0.17	64.98±0.16	94.04±0.215
DDO	78.045±0.18*	97.98±0.18	59.98±0.17	97.99±0.137*
DHO	74.08±0.25*	95.07±0.17*	62.165±0.28*	96.08±0.220*
DDF	85.41±1.15	99.35±1.58	57.11±1.57	104.99±1.76
DHF	80.33±0.74	100.21±2.43	60.31±1.82*	101.08±1.17

*Indicate significance change compared with normal control group (P<0.05). The results are expressed as mean±SD. Group: NCD - normal (non-diabetic rats), DC- Diabetic Control (Alloxan induced diabetic rat without treatment), DG- Diabetic + Glibenclemide, DDO- Diabetic + Domestic chicken oil(1% of total diet), DHO- Diabetic + Hybrid chicken oil(1% of total diet), DDF- Diabetic + Domestic chicken flesh(1% of total diet), DHF- Diabetic + Hybrid chicken flesh (1% of total diet)

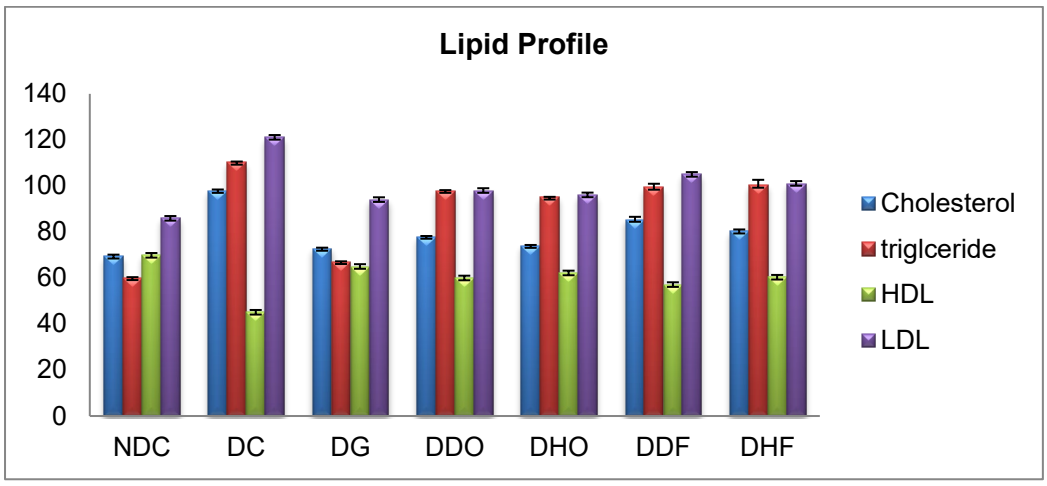


Figure 4.18: Effect of Chicken oil and flesh on blood lipid profile of rats

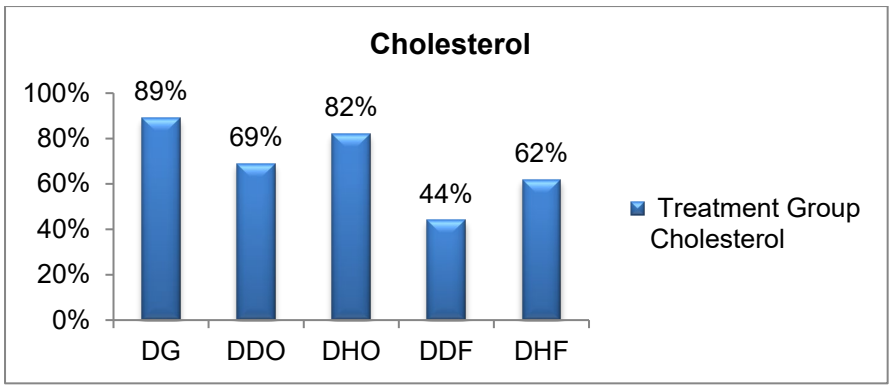


Figure 4.19: Comparative effect of Chicken oil and flesh on serum cholesterol of rats

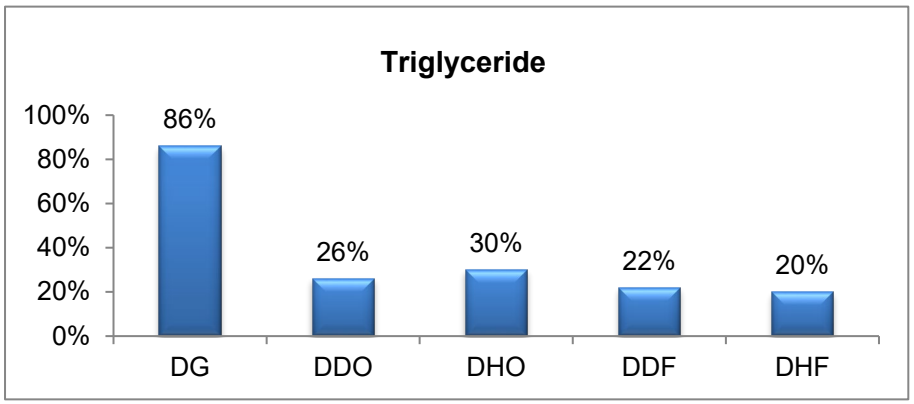


Figure 4.20: Comparative effect of Chicken oil and flesh on serum triglyceride of rats

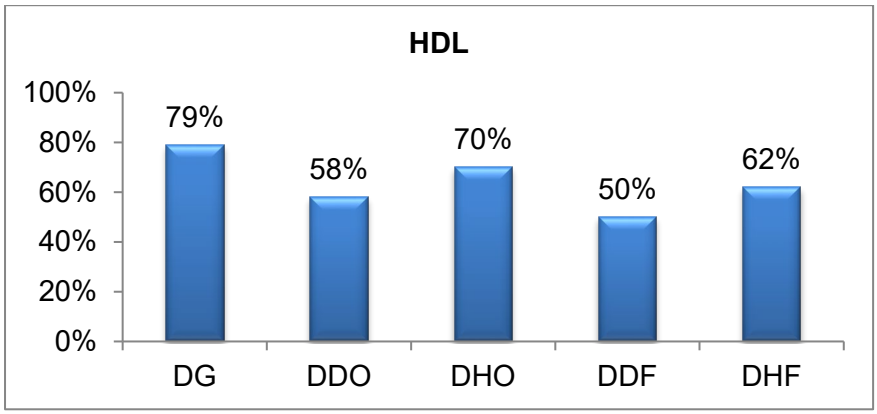


Figure 4.21: Comparative effect of Chicken oil and flesh on serum HDL of rats

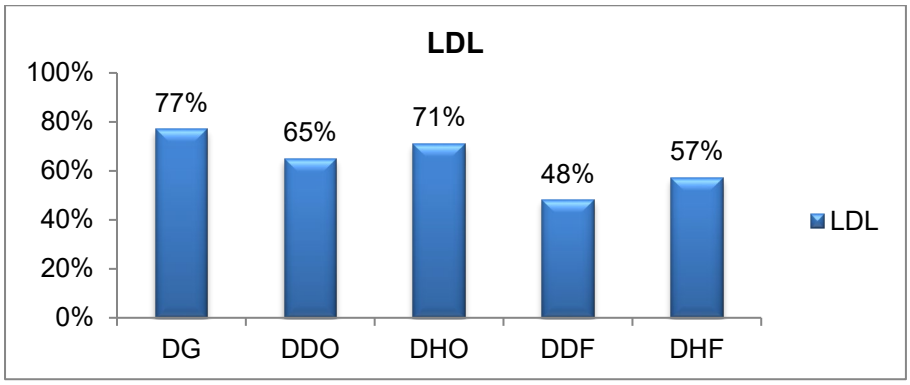


Figure 4.22: Comparative effect of Chicken oil and flesh on serum LDL of rats

4.6.4 Effect of Domestic and Hybrid chicken oils and flesh on serum creatinine, urea, and uric acid level of experimental rats

Effect of chicken oil and flesh were shown in Table 4.14. Results showed that after induction of diabetic, serum uric acid, urea and creatinine level were found to be high in diabetic rats but a moderate decrease was found after treatment with chicken oil and flesh. Uric acid level were 44%, 56%, 21%, 23% and 66% for domestic chicken oil, hybrid chicken oil, domestic chicken flesh and hybrid chicken flesh and glybenclamide treated group respectively compared with control group (Figure 4.23 and Figure 4.26).

Serum urea levels were reduced by 45%, 54%, 33%, 41% and 87% for domestic chicken oil, hybrid chicken oil, domestic chicken flesh and hybrid chicken flesh and glybenclamide treated group respectively compared with control group (Figure 4.24 and Figure 4.27).

Creatinine levels reduction were observed by 41%, 62%, 12%, 35% and 79% for domestic chicken oil, hybrid chicken oil, domestic chicken flesh and hybridchicken flesh and glybenclamide treated group respectively compared with control group (Figure 4.25 and Figure 4.28).

Table 4.14: Comparative effect of Domestic and Hybrid chicken oils and flesh on serum creatinine, urea, uric acid level of experimental rats, 21-days study, n= 6

Treatment Group	Uric acid(mg/dl)	urea(mg/dl)	Creatinine (mg/dl)
NDC	7.871±0.28	32.41±0.43	1.24±0.17
DC	16.00±0.29	56.47±0.45	2.72±0.16
DG	10.03±0.44	35.47±0.45	1.54±0.14
DDO	12.05±0.32	45.98±o.42*	2.11±0.15*
DHO	10.89±0.37*	43.08±0.26*	1.79±0.31*
DDF	14.05±1.26	48. 08 ±1.36	2.53±1.33
DHF	13.89±1.07	46.98±1.15	2.19±1.03

*Indicate significance change compared with normal control group (P<0.05). The results are expressed as mean±SD. Group: NCD - normal (non-diabetic rats), DC- Diabetic Control (Alloxan induced diabetic rat without treatment), DG- Diabetic + Glibenclemide, DDO- Diabetic + Domestic chicken oil(1% of total diet), DHO- Diabetic + Hybrid chicken oil(1% of total diet), DDF- Diabetic + Domestic chicken flesh(1% of total diet), DHF- Diabetic + Hybrid chicken flesh (1% of total diet)

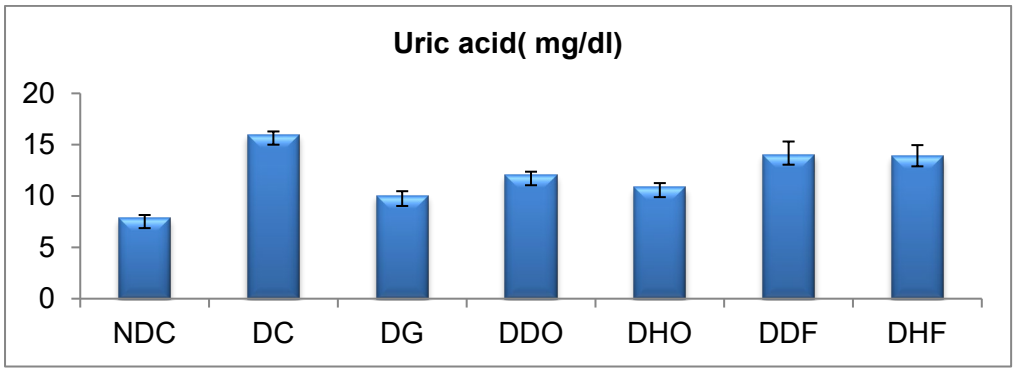


Figure 4.23: E**ffect of Chicken oil and flesh on blood uric acid level**

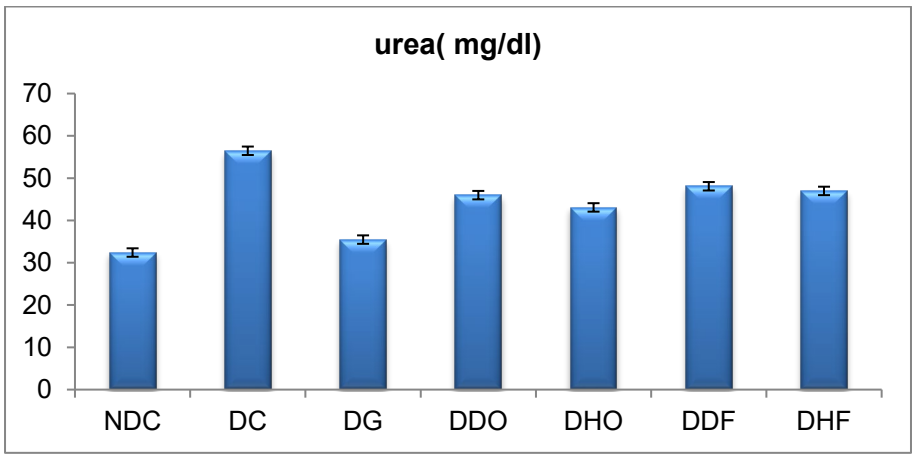


Figure 4.24: Effect of Chicken oil and flesh on blood urea level on experimental rats

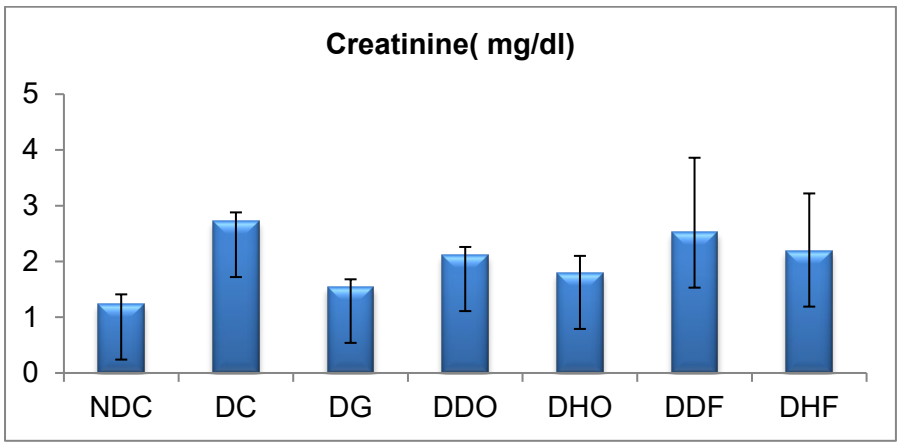


Figure 4.25: Effect of oil and flesh on serum creatinine level on experimental rats

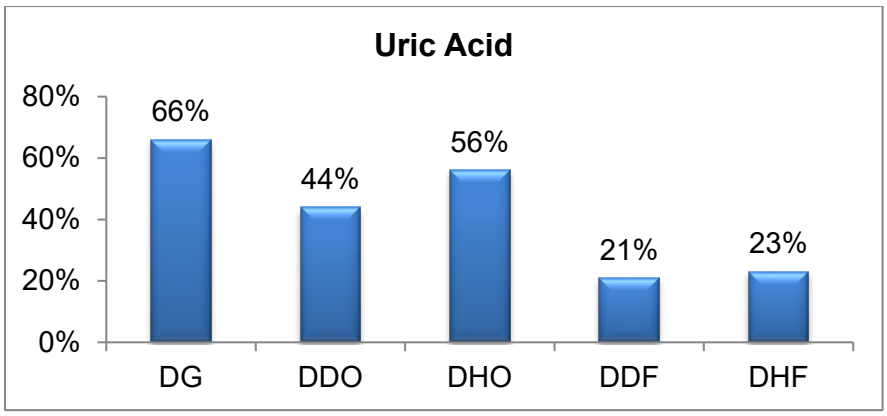


Table 4.26: Comparative effect of Chicken oils and flesh on serum uric acid level of experimental rats

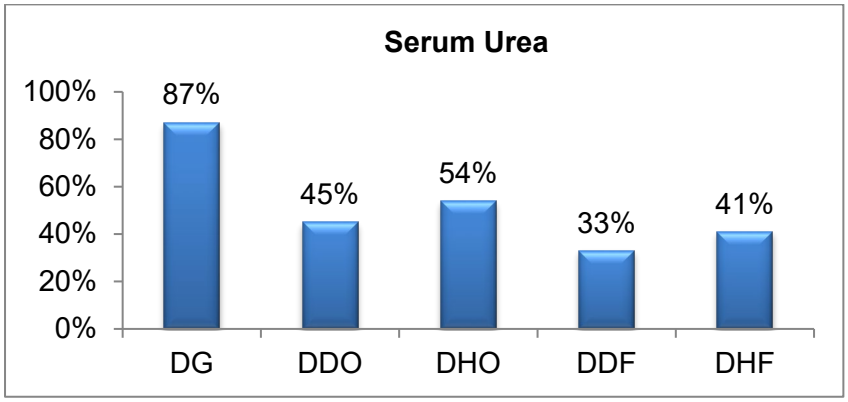


Figure 4.27: Comparative effect of Chicken oils and flesh on serum urea level of experimental rats

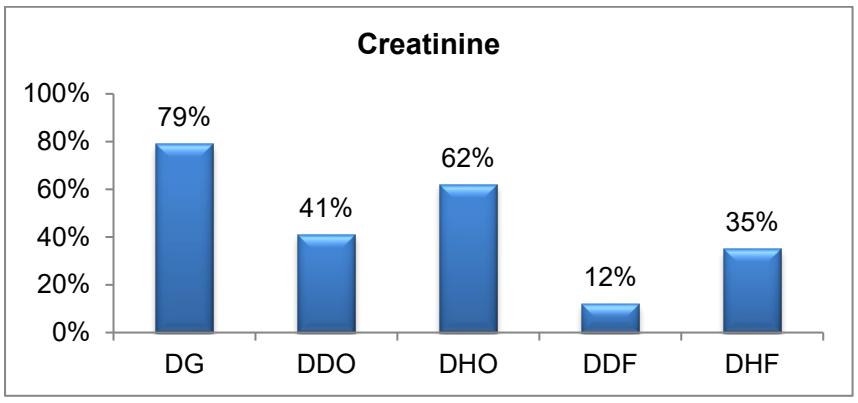


Figure 4.28: Comparative effect of Chicken oils and flesh on serum creatinine level of experimental rats

4.6.5 Effect of domestic and hybrid chicken oils and flesh on SGPT and SGOT level of experimental rats

The effects of chicken oil and chicken flesh on serum SGPT and SGOT level of alloxen induced diabetic rats are shown in Table 4.15 and Figure 4.29. SGPT levels were found to be reduced by 41%, 60%, 45%, 51% and 69% for domestic chicken oil, hybrid chicken oil, domestic chicken flesh and hybrid chicken flesh and glybenclamide treated group respectively compared with control group (Figure 4.30). Similar effect had seen for SGOT level. The level was decreased by 35%, 43%, 28%, 42%and 50% for domestic chicken oil, hybrid chicken oil, domestic chicken flesh and hybrid chicken flesh and glybenclamide treated group respectively compared with control group (Figure 4.31).

Table 4.15: Comparative effect of domestic and hybrid chicken oils and flesh on serum SGPT and SGOT level of experimental rats, 21-days study, n= 6

Treatment Group	SGPT	SGOT
NDC	33.83±3.488	29.16±3.31
DC	67.16±2.875	43.5±2.88
DG	44.33±3.14	35.83±1.169
DDO	53.5±3.39	38.16±1.633
DHO	47.16±3.60*	37.5±1.378*
DDF	51.5±3.39	39.66±1.633
DHF	50.16±3.60*	36.86±1.378*

*Indicate significance change compared with normal control group (P<0.05). The results are expressed as mean±SD. Group: NCD - normal (non-diabetic rats), DC- Diabetic Control (Alloxan induced diabetic rat without treatment), DG- Diabetic + Glibenclemide, DDO- Diabetic + Domestic chicken oil(1% of total diet), DHO- Diabetic + Hybrid chicken oil(1% of total diet), DDF- Diabetic + Domestic chicken flesh(1% of total diet), DHF- Diabetic + Hybrid chicken flesh (1% of total diet)

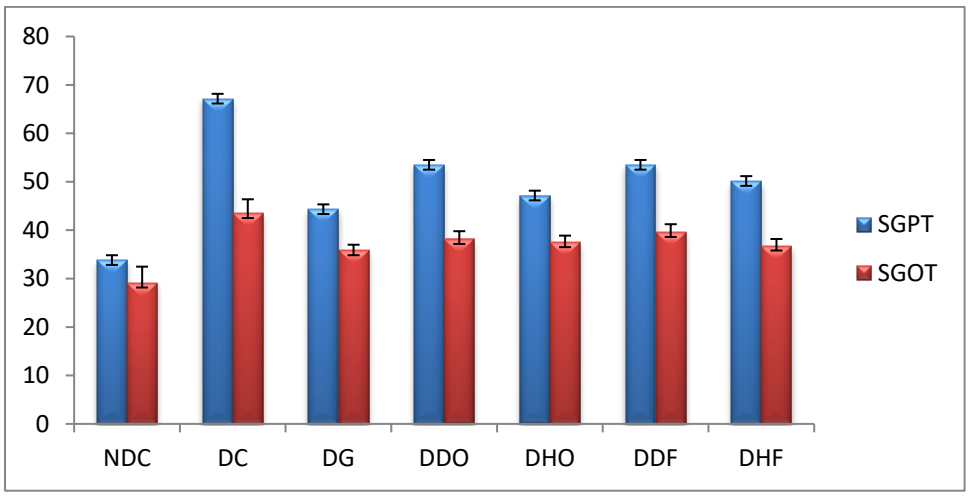


Figure 4.29: Effect of Chicken oil and flesh on serum SGPT level of experimental rats

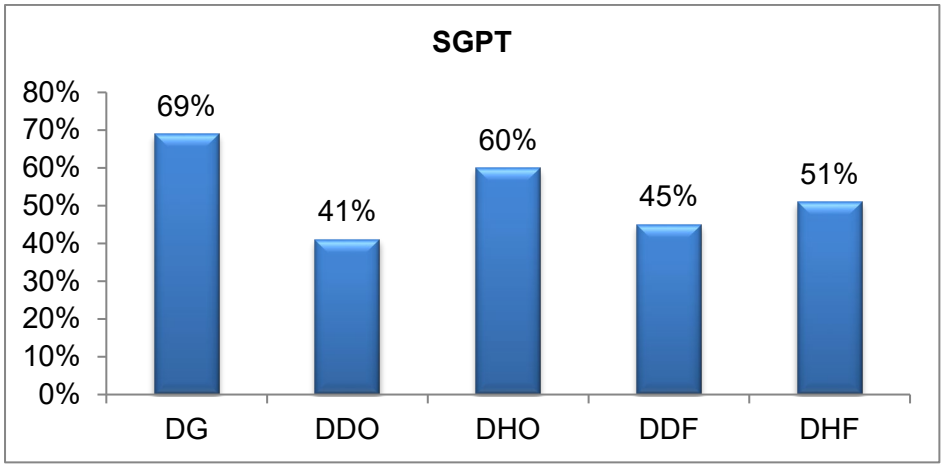


Figure 4.30: Comparative effect of Chicken oils and flesh on SGPT level of experimental rats

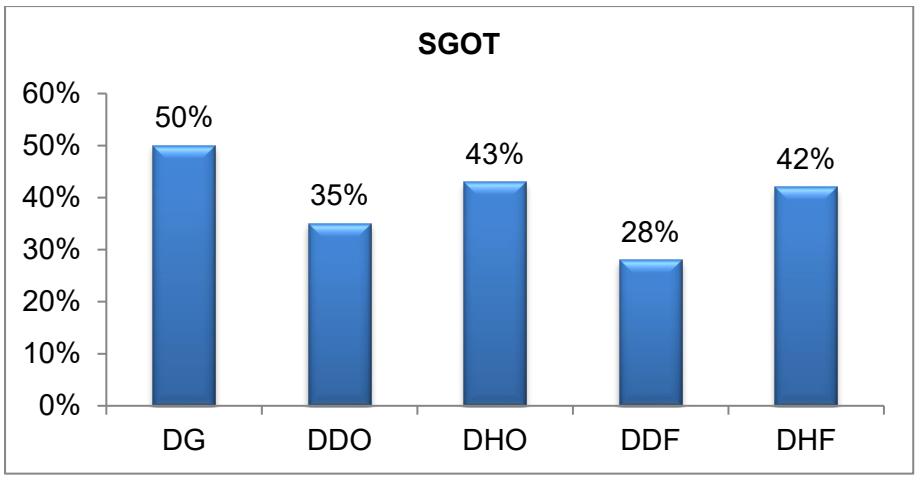


Figure 4.31: Comparative effect of Chicken oils and flesh on SGOT level of experimental rats

DISCUSSION

Non-communicable Diseases (NCDs), of which Diabetes and Cardiovascular Diseases (CVDs) are major components, are increasing at an epidemic rate and these have now outbalanced communicable diseases (CDs) as the cause of death even in developing countries like Bangladesh. Insulin resistance and subclinical inflammation are associated with almost all NCDs and this, in turn, has a direct relation with the quantity and quality of fat intake in the diet. Accordingly, people are increasingly being advised to avoid red meats (which contain more harmful lipids) and switch to lean ones like chicken. People themselves, through educational inputs from various media, are becoming increasingly conscious about the relative importance of chicken meat in their diet.

The euphoria about chicken meat, however, has overlooked one of the possible transitions regarding the quality of the meat in relation to health. Growing demand of chickens has led to large number of initiatives (research and industrial points of views) in enhancing the production of the species both by traditional genetic manipulation as well as by biotechnological means. Increasing urbanization and industrialization coupled with cost considerations have, in a way, forced people to consume more and more firmed hybrid chickens in comparison to the natural domestic ones (although later are tasty and popular to most of the people). However, the health impacts of these switching has not yet been properly investigated and advices are now given blindly based on facts which were mostly generated from analysis/experiments with natural chickens. The present work is one of the first studies in this direction.

5.1 Nutritional Composition of Chicken Meat

5.1.1 Moisture

The results showed that hybrid chicken contained 75.22% moisture. On the other hand domestic chicken contained 73.89% moisture (Table 4.1, Figure 4.1 & 4.2). Moisture plays an important part in the growth and metabolism of animals. Water is indispensable for the absorption and transport of food, to carry out metabolize materials and to regulate fluidity in living systems. The high moisture content is a disadvantage in that in increases the susceptibility to microbial spoilage, oxidative degradation of polyunsaturated fatty acid and consequent decreases in the quality of fleshes for longer preservation time (Omolara *et al* 2009). It contributes as much as to the essential properties of life as do the other constituents like protein, carbohydrate, etc. Moisture is also essential for most of the physiological reactions animal tissues. Without its presence, life does not exist.

5.1.2 Ash

Ash is one of the component in the proximate analysis of biological materials, consisting mainly of salty, inorganic constituents. It includes metal salts which are important for processes requiring ions such as Na^+ (Sodium), K^+ (Potassium), and Ca^{2+} (Calcium). It also includes trace minerals which are required for unique molecules, such as chlorophyll and hemoglobin (IUPAC 2006). Most of the inorganic constituents or minerals are present in ash. The hybrid chicken was found to contain 2.4% of ash. On the other hand the domestic chicken was found to contain 2.25% (Table 4.1, Figure 4.1 & 4.2).

5.1.3 Lipid content

Human can synthesize saturated and monounsaturated fatty acids but cannot synthesize polyunsaturated omega-3 and omega-6 fatty acids de novo. This is because humans like other animals, lack the desaturase enzymes required to produce the simplest members of these families (ALA and LA, respectively). Thus ALA and LA are considered “essential fatty acids” (EFAs) that need to

be included in the diet. EFAs act as precursors for the synthesis of more highly unsaturated and longer-chain omega-3 and omega-6 fatty acids (Tapiero *et al* 2002). Omega-3 and ω-6 fatty acids are essential components of cell-membrane phospholipids and they have several other functional roles (Hardman 2004).

Total lipid content of hybrid and domestic chickens were 4.78% and 2.83% respectively. Lipid content is higher in hybrid chicken by 40% than domestic chickens (Table 4.1, Figure 4.1 & 4.2). Hybrid chicken contains substantially higher amount of lipid in comparison with domestic chicken.

5. 1.4 Total protein

Proteins are essential nutrients for the human body. They are one of the building blocks of body tissue, and can also serve as a fuel source. Proteins are polymer chains made of amino acids linked together by peptide bonds. During human digestion, proteins are broken down in the stomach to smaller polypeptide chains via hydrochloric acid and protease actions. This is crucial for the synthesis of the essential amino acids that cannot be biosynthesized by the body (Genton *et al* 2010). Protein is a nutrient needed by the human body for growth and maintenance. Aside from water, proteins are the most abundant kind of molecules in the body. Protein can be found in all cells of the body and is the major structural component of all cells in the body, especially muscle. This also includes body organs, hair and skin. Proteins are also used in membranes, such as glycoproteins. When broken down into amino acids, they are used as precursors to nucleic acid, co-enzymes, hormones, immune response, cellular repair, and other molecules essential for life. Additionally, protein is needed to form blood cells (Food and Nutrition Board 2005).

Total proteins of hybrid and domestic chickens were determined to be 13.7%, and 12.84% respectively (Table 4.1, Figure 4.1 & 4.2). The protein constituents are primary importance not only as component of nuclear and

cytoplasmic structures but also as complement of enzymes involved in metabolism during growth, development and maturation.

5.1.5 Total soluble sugar

Carbohydrate plays an important role on the physiological activities of both in animal and plant. Glucose and glycogen serves important sources of energy for vital activities. Some carbohydrates have highly specific functions. The experimental data for the hybrid and domestic chickens were presented in the Table 4.1, Figure 4.1 & 4.2. From the Table it was found that the sugar content of domestic and hybrid chickens were 0.1% and 0.13% respectively.

5.1.6 Glycogen content

Glycogen is storage carbohydrate in animal body. Glycogen serves as reserve nutrient. Glycogen content of both selected hybrid and domestic chicken species have been determined and the data were presented in the Table 4.1, Figure 4.1 & 4.2. From the Table it was found that the quantity of glycogen presented in domestic and hybrid chickens meat were 0.003% and 0.004%.

5.1.7 Reducing sugar

Reducing sugar is another kind of carbohydrate. The data in the Table 4.1, Figure 4.1 & 4.2 showed that the value of reducing sugar of domestic and hybrid chickens were 0.002% and 0.003% respectively.

Data from this study show that hybridization does not affect the moisturisation of the birds (75% in Hybrid vs 73% in Domestic). This means the proportion of the total solid content of the birds also remain almost similar (25% and 27%). However, there are remarkable changes in proportions of some of the solid components in the hybrid chickens. Notably, the protein content is 6% higher, the total lipid content 40% higher, sugar 23% higher and glycogen is 25% higher for hybrid than domestic chicken. The overall increase in the macronutrient content may seem to be beneficial, but looked from the NCD

points of views, further detailed analysis of the individual macronutrient is necessary for getting towards any conclusion on their impacts on health. In this thesis, an attempt has been made to partly analyze the lipids (particularly in relation to the health related fatty acids) and also investigate the effect of chicken oils on diabetes (an important NCD) in rats. However, before going to the discussion on the lipid issues, the possible health impacts of the changed micronutrient contents in the hybrid chickens needs proper attention.

5.1.8 Micronutrient content

Minerals are inorganic substances required by the organism in very small amounts for their growth and maintenance of functional activities. Food and vegetables are the important sources of minerals for human beings and exist in food as organic and inorganic combination. In food mineral elements are present as salt. They combined with organic compound e.g. iron in hemoglobin. Minerals are required for the teeth and bone formation. Minute amount of mineral elements are constituents of various regulatory compounds such as vitamins, enzymes and hormones. Some enzymes require calcium for their activities such as lipase and succinate dehydrogenases. Iron is required for the enzymatic activities of several enzymes such as ferredoxin catalase, indophenol oxidase, aldehyde oxidase etc. Minerals also present in both extra cellular and intra cellular spaces. Mineral content of domestic and hybrid chicken meat are reported to Table 4.2, Figure 4.3 & 4.4.

Among the micronutrients manganese and potassium contents are remarkable increased (28% and 34% respectively) and reverse phenomena happens with iron (20%) and zinc (50%) lower for hybrid chicken.

The higher levels of Manganese and Potassium in hybrid chickens may be beneficial depending on the balance between specific RDAs and intake from other dietary sources. Excessive intake of the elements may be harmful and, in case of potassium, it may be life-threatening for some patients. Usually chicken soups are one of the most prescribed diets for critically ill patients or for those in the recovery phase. Maintenance of appropriate serum potassium

levels in such situation is a central clinical challenge and overdose through dietary potassium may have a devastating consequence on cardiac function. Thus utmost care should be taken to calculate potassium input by considering whether the soup is prepared from hybrid or domestic chicken. Potassium helps to regulate body fluid and mineral balance in and out of body cells. It is involved in maintaining blood pressure, transmitting nerve impulses and helping muscles and heart to contract. Specially, potassium has been noted to reduce both systolic and diastolic blood pressure in people with high blood pressure (Whelton *et al* 1997, Dyer *et al* 1994). Again manganese is a component of many enzymes and is needed for good metabolism, for the growth of bones and tendon formation. Calcium and lead were found to be high for domestic chicken by 9% and 3% respectively than that of Hybrid chicken. It is one of the keys to develop healthy bones and teeth. Another important consideration in this respect is the lower contents of iron and zinc in the chicken meats. Again, the ultimate beneficial or harmful effects of this lower content depend on the specific RDAs and dietary intake from other sources. Iron is an essential part of hemoglobin, which transport oxygen. Iron is widely distributed in the body. It is found in the blood, liver, spleen and bone marrow. Moreover excessive iron can increase oxidative stress of pancreatic beta cell resulting beta cell apoptosis. Both low and high intake of dietary iron is harmful due to the effects on anemia and subclinical inflammation respectively. Thus, careful calculation and attention are required by the physicians, dieticians and nurses when hybrid chickens are included as a principal component of the diet. The lower zinc content needs equally, if not more, attention, as zinc is highly required at the growing stage and many children are absolute fond of chicken. Zinc is involved in the several enzyme systems and is a constituent of insulin. It is also essential for growth, reproduction and support immunity. Low zinc content of hybrid chicken meat may be a threat because zinc plays a key role in the synthesis, secretion and action of insulin. Zinc acts as a cellular second messenger in the control of insulin signaling and glucose homeostasis (Stephen *et al* 2012).

5.1.9 Nutrients content of Hybrid chicken feed

Hybrid chicken feed contains 12% moisture, 17% total protein, 5% fat, 0.34% total sugar, 1mg /kg calcium and 0.70mg/kg phosphorus which are shown in Table 4.3. Here we can see the moisture content was only 12%, in this sense it seems that processed feed of hybrid chicken contain high amount of macro and micronutrient (Table 4.3).

5.2 Characterization of oil

5.2.1 Oil content of domestic chicken and hybrid chicken oil

The average amount oil of two chicken species were extracted with soxlet apparatus using n-hexane as an extracting solvent and it was found to be higher for hybrid chicken by 26% shown in Table 4.4.

5.2.2 Iodine value and Saponification Value

Hybrid chicken is rich in unsaturated fatty acid which is contributed mostly by long chain fatty acid because iodine value was higher for hybrid chicken than domestic chicken by 23% which was indication of the presence of higher amount of unsaturated fat in hybrid chicken. Iodine values give an estimation of the amount of unsaturated fatty acids in the triglyceride molecules of fat and oil. Moreover hybrid chicken contain higher amount of long chain fatty acids than domestic chicken as saponification value of hybrid chicken was lower by 22% than domestic chicken (Table 4.4 and Figure 4.5 & 4.6). Saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids in fat or oil. Fat and oil consisting largely of C18 fatty acids generally have saponification value around 290.80, indicating the presence of appreciable quantity of long chain fatty acids (Rahman *et al* 1997). Unsaturated fat is generally more health friendly, but not without further qualification.

5.2.3 Acid value, Peroxide value, Percentage Free Fatty acids

Acid value, per oxide value and percentage free fatty were higher for domestic chicken than hybrid chicken by 15%, 14% and 15% respectively (Table 4.4 and Figure 4.5 & 4.6). Lipids readily undergo oxidation to produce peroxides and aldehydes. The oxidative stability of unsaturated lipids decreases as their degree of unsaturation increases. There is an inverse relationship between the number of instaurations of a fatty acid and their half-life. Thus, the higher the amount of unsaturated fatty acid present, the greater the risk of oxidation, causing rancidity and color deterioration (Eder *et al* 2005, Rymer and Givens 2005).

5.2.4 Unsaponifiable matter

The unsaponifiable matter is a fraction of fat or oil that remains insoluble after saponification of the sample by alkali. The unsaponifiable matter of 0.5-2.0% in an oil represents the presence of a mixture of several lipid classes viz, sterols, tocopherols, hydrocarbon, higher aliphatic fatty acids and terpinoids alcohols (Jacobs 1962). Unsaponifiable matter found to be higher for hybrid chicken than that of domestic chicken (Table 4.4 and Figure 4.5 & 4.6).

5.2.5 Nutritional comparison of Domestic and Hybrid chickens among Breast meat, Drum stick, Thigh meat and meat of Wings

Skinless meat of different body part (Breast, Drum Stick, Thigh, and wings) of both types of chickens was isolated. Any kind of animal meat is rich in protein. From the Table 4.5 and Figure 4.7, it has been seen that whether it was domestic or hybrid chicken, breast meat contents highest amount of protein. The recommended intake of protein is 46 grams a day for women and 56 grams a day for men. Protein is important for immune function, cell re growth and muscle building. In these cases breast meat may be a very good source of protein.

Total lipid content of different body parts of domestic chicken and hybrid chickens is presented by Table 4.6 and Figure 4.8. Lipid content was higher for the both, domestic and hybrid chickens in their thigh meat portion.

Iodine value, saponification value and acid value of extracted oil were observed for breast meat, drumsticks, thigh meat and meat of wings. Iodine value, saponification value and acid value were shown in Table 4.7 and Figure 4.9, Table 4.8 and Figure 4.10, and Table 4.9 and Figure 4.11 respectively. Iodine value and acid value were found to be higher for thigh meat in both of the chickens. Again saponification value was lower for thigh meat portion for both chickens.

The values indicate that whether it was domestic or hybrid species, thigh meat contents higher amount of unsaturated fatty acids. This portion of meat also contents long chain fatty acids as its saponification value was found to be lower than other meat portion of both chickens. The values are supported by the data obtained from National Chicken Council, 2012 (**SOURCE: USDA National Nutrient Database for Standard Reference (http://www.nal.usda.gov/fnic/foodcomp/search/.)**

5.2.6 Fatty acid composition of Domestic and Hybrid chicken

One of the major focuses of the study is the fatty acid profiling of the two varieties of chicken. A deeper reflection on data regarding individual fatty acids shows that the two varieties of oils have both good and bad fatty acid profiles. Regarding fatty acids comparison domestic chicken are much more beneficial as it contains higher amount of oleic and arachidonic acid than hybrid chickens (Table 4.10, Figure 4.12 & 4.13). Which are, generally antiatherogenic. A study (Eduardo and Huertas 2010) also reported that substitution of dietary saturated fat by oleic acid and polyunsaturated fatty acids (PUFA) has been described to reduce the cardiovascular risk by reducing blood lipids, mainly cholesterol. Oleic acid is a common monounsaturated fat in human diet. Monounsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol. However, its

ability to raise HDL is still debated. Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects. Adverse effects also have been documented, however, since both oleic and monounsaturated fatty acid levels in the membranes of red blood cells have been associated with increased risk of breast cancer, although the consumption of oleate in olive oil has been associated with a decreased risk of breast cancer (Martin *et al* 1994). On the other hand, Arachidonic acid is a polyunsaturatedomega-6 fatty acid 20:4(ω-6). It is the counterpart to the saturated arachidic acid. Arachidonic acid is not one of the essential fatty acids. However, it does become essential if there is a deficiency in linoleic acid or if there is an inability to convert linoleic acid to arachidonic acid, which is required by most mammals. Some mammals lack the ability to-or have a very limited capacity to-convert linoleic acid into arachidonic acid, making it an essential part of their diets (MacDonald *et al* 1984, Rivers *et al* 1975). Arachidonic acid is also act as a precursor in the production of eicosanoids (Baynes *et al* 2005).

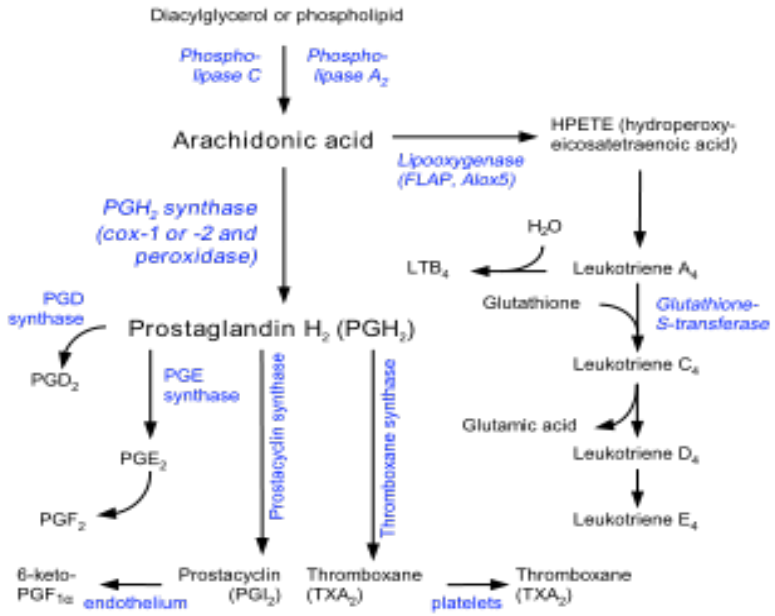


Figure 5.1: Eicosanoids synthesis from arachidonic acid

However, at the same time, domestic chicken contains around 19% of palmitic acid which has cholesterol raising property. Domestic chicken contains no

palmitoleic acid whereas hybrid chicken contains a large portion of palmitoleic acid around 28%. Palmitoleic acid is an n-7 monounsaturated fatty which can reduce serum cholesterol, triglyceride and LDL and it can also increase serum HDL cone. So palmitoleic acid has a beneficial effect on blood lipids. A study (Adam *et al* 2014) reported that adults with dyslipidemia and evidence of mild systemic inflammation (high-sensitivity C-reactive protein (hs-CRP) between 2 and 5 mg/L were randomly allocated to receive 220.5 mg of *cis*-palmitoleic acid (n = 30) once per day for 30 days. Participants were asked to maintain their current diet. Serum lipids and hs-CRP were drawn at baseline and study completion. At 30 days, there were significant mean (95% confidence interval [CI]) reductions in CRP (−1.9 mg/L), triglyceride (−30.2 [−40.2 mg/dl), and low-density lipoprotein (LDL) (−8.9 mg/dl), and a significant increase in high-density lipoprotein (HDL) (2.4 mg/dl) in the intervention group compared with control. These changes equated to 44%, 15%, and 8% reductions in CRP, triglyceride, and LDL respectively, and a 5% increase in HDL compared with control. So, palmitoleic acid may be useful in the treatment of hypertriglyceridemia with the beneficial added effects of decreasing LDL and hs-CRP and raising HDL.

So, chicken oil contains MUFA and PUFA which are known to have hypoglycemic and hypolipidemic properties. A study (Yang *et al* 2011) reported that Saury oil contains 18% n-3 polyunsaturated fatty acids (n-3 PUFA) and 35% monounsaturated fatty acids (MUFA). Diabetic KKAy mice were fed a 10% soybean oil diet (control) or a 10% saury oil diet for 4 weeks, and diet-induced obese C57BL/6J mice were fed a high-fat diet containing 32% lard (control) or 22% lard plus 10% saury oil for 6 weeks. After the intervention periods, the levels of glucose, insulin and lipids in plasma had decreased significantly for the saury oil diet group, and insulin sensitivity had improved.

Domestic and hybrid chickens also contain stearic acid which is a 18 carbon long chain fatty acid.It differs from other saturated fatty acid as it lowers blood LDL and cholesterol/HDL ratio. Stearic acid has a neutral impact on HDL

concentration of blood. So it is favorable to cardiovascular health. In contrast to the predominant long-chain SFAs in the diet that raise blood total and LDL cholesterol levels (i.e. lauric, myristic, and palmitic acids), studies consistently show that stearic acid has a neutral effect on these lipid levels in humans (Etherton-Kris 2002). A meta-analysis of 35 controlled trials found that when stearic acid replaced carbohydrate in the diet it had a neutral effect on blood lipid and lipoprotein levels (Mensink *et al* 2003.). Researchers in the Netherlands reported that when intakes of stearic acid, oleic acid, or linoleic acid (7% of energy) were consumed by healthy adults for five weeks, there were no significant differences between the diets in serum lipid and lipoprotein levels (i.e., serum LDL and HDL cholesterol levels, very low density lipoprotein particle sizes, and lipoprotein subclasses) (Thijssen and Mensink 2005). A study (Aro *et al* 1997) reported on serum lipoproteins of stearic acid, trans fatty acids, and dairy fat. 80 healthy subjects consumed a dairy fat-based (baseline) diet for 5 week, then an experimental diet high in either trans fatty acids (8.7% of energy; n = 40) or stearic acid (9.3% of energy; n = 40) for another 5 week. All diets provided 32.2-33.9% of energy as fat, 14.6-15.8% as saturated plus trans fatty acids, 11.4-12.5% as cis-monounsaturated fatty acids, 2.9-3.5% as polyunsaturated fatty acids, and 200-221 mg cholesterol/10 MJ. Compared with the dairy fat diet, stearic acid and trans fatty acids decreased serum total cholesterol concentrations similarly (by 13% and 12%, respectively, $P < 0.001$). Stearic acid but not trans fatty acids reduced concentrations of LDL cholesterol and apo B significantly ($P< 0.001$).

5.3 Comparative effects of chicken oil and flesh on blood parameters of experimental rats

5.3.1 Effect on body weight

Obesity is a health care problem today and its prevalence has increased greatly in recent decades in almost every country and all age group. The major cause in the recent obesity epidemic is a changing environment that promotes excessive calorie intake and discourages physical activity, causing

an energy imbalance. According to WHOs latest projections approximately 1.6 billion adults (age 15+) were overweight worldwide in 2015 and at least 400 million adults were obese. WHO further estimates that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese (WHO 2006).

After induction of diabetes alloxan induced diabetic rats became thin and their weight were reduced (Table 4.11). For normal rats, their weights were increased substantially after 2nd and 3rd week. Initially diabetic rats decrease their body weight but after 2nd week of chicken oil and chicken flesh treatment, there was an increase in body weight. Domestic and hybrid chicken oil Increase of body weight by 81% and 77% respectively. On the other hand Domestic and hybrid chicken flesh feeding rats, increase body weight around 80% and 83%, which areas near as the glibenclamide treatment group, it was 89%. So the results indicate that chicken meat had a significant effect on body weight reduction. From some studies, it has been shown that animal fat consumption increases body weight (Figure 4.14 and Figure 4.15).

A study (Ahmad 2007) reported that an investigation on Long Evans male rats fed with different edible fats and oils was conducted to determine and to compare the effect of feeds on body weight gain. The study revealed that animal oil consumption can increase body weight. In our study, we also observed that chicken oil consumption increases the body weight significantly in diabetic rats.

5.3.2 Effect on blood glucose level

The result showed that hybrid chicken lowers the blood glucose level of rats and maintains it at standard level more significantly than domestic chicken (Table 4.12, Figure 4.16 & 4.17). The plasma glucose lowering activity was compared with glibenclamide, a standard hypoglycemic drug. Glibenclamide has been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic β cells (Eidi and Darzi 2009).The blood glucose levels were decreased by 67% and 62% for domestic and hybrid chicken oil administered

subject whereas 60% and 52% for domestic and hybrid chicken flesh administered subject which is as near as glibenclamide administered subject (72%). Mr Kwoon (kwoon *et al* 2009) showed that liquid commercial chicken meat extract, administered orally, exerted significant hypoglycemic effect after 6 weeks and 8 week of treatment in diabetic KKAy mice and GK rats. It is supported to our result. We can say that the result indicates that domestic and hybrid chicken oil administered group is more significant effect on reduction of blood glucose level.

5.3.3 Effect on serum lipid profile

Chicken oil and flesh also has hypolidemic effect shown in Table 4.13 and Figure 4.18. 69% and 82% reduction of cholesterol level were observed for domestic and hybrid chickens oil treatment respectively in diabetic rats whereas 44% and 62% reduction were seen for domestic and hybrid chicken flesh (Figure 4.19). In case of glibenclamide, it was 89%. LDL level was reduced for domestic chicken oil, hybrid chicken oil, domestic chicken flesh, hybrid chicken flesh and glibenclamide at 65%, 71%, 48%, 57% and 77% (Figure 4.22), whereas HDL level was increased significantly 58%, 70%, 50%, 62% and 79% respectively (Figure 4.21). Triglyceride level reduced for domestic chicken oil, hybrid chicken oil, domestic chicken flesh and hybrid chicken flesh by around 26%, 30%, 22% and 20% respectively (Figure 4.20). Whereas Triglyceride level was reduced by 86% for glibenclamide treatment. Chicken consumption also exerts a potential effect in reducing cholesterol, triglyceride, LDL level and increase HDL level. In this case, hybrid chicken plays more important role than domestic chicken.

A study (Jorge *et al* 2002) repoted that a randomized, crossover, controlled trial was conducted with 28 patients with type 2 diabetes, 15 patients were normoalbuminuric (UAER 20 g/min) and 13 patients were microalbuminuric (UAER 20–200 g/min). A chicken-based diet (red meat replaced with chicken) and a low protein diet were compared with the patients' usual diet. Patients followed each diet for 4 weeks with a 4-week washout period between. GFR, apolipoprotein B, total cholesterol, LDL cholesterol, HDL cholesterol, and

triglycerides were measured after each diet. GFR values in normoalbuminuric patients were lower after the Low protein diet (LPD) and the Chicken diet (CD) than after the Usual diet (UD). In normoalbuminuric patients, the diets did not affect the lipid profile. In microalbuminuric patients, apolipoprotein B and total cholesterol were lower after the LPD and the CD than after the UD.

Another study (George 2007) on linseed oil (a ingredient of hybrid chicken feed) was shown that the effect of Linseed oil (LO) rich in α-linolenic acid (ALA, C18 : 3 n-3), on lipid profile and some growth parameters in rats fed high saturated fat diet (HSF) were observed. Result showed that LO supplementation significantly prevent the marked increase body and liver weight gain, hepatic lipids as well as it caused limitation of the negative effect on lipoprotein parameters caused by HSF supplementation. These data suggest that LO participate in the normal regulation of plasma lipid and cholesterol levels in liver, demonstrating that LO may be developed as useful popular and commercial oil for protection against hyperlipidemia

5.3.4 Effect on Kidney and Liver function test

The uric acid test of blood is used to detect high level of this compound in the blood in order to help diagnose gout. The uric acid urine test is used to help diagnose the cause of recurrent kidney stone and to monitor people with gout for stone information. Elevated level of uric acid in blood has been reported to be a risk factor for cardiovascular disease, metabolic syndrome and diabetes mellitus (Bruce *et al* 1999, Costa *et al* 2002).

Uric acid level were 44%, 56%, 21%, 23% and 66% for domestic chicken oil, hybrid chicken oil, domestic chicken flesh and hybrid chicken flesh and glibenclamide treated group respectively compared with control group (Table 4.14, Figure 4.23 & 4.26). Serum urea levels were reduced by 45%, 54%, 33%, 41% and 87% for domestic chicken oil, hybrid chicken oil, domestic chicken flesh and hybrid chicken flesh and glibenclamide treated group respectively compared with control group (Table 4.14, Figure 4.24 & 4.27).

Creatinine is formed from creatine. Muscle contains 98% of total body creatinine. Creatinine leaves muscles and enters blood, from where it is removed by kidney. If the kidneys are failing, serum creatinine levels increase. The use of serum creatinine, as a marker of GFR originated from the work of Rehberg, in 1926 (Rehberg 1926). Creatinine levels reduction were observed by 41%, 62%, 12%, 35% and 79% for domestic chicken oil, hybrid chicken oil, domestic chicken flesh and hybrid chicken flesh and glibenclamide treated group respectively compared with control group (Table 4.14, Figure 4.25 & 4.28). Reduction level of uric acid, Urea and Creatinine level were higher for Oil or flesh of hybrid chicken. A similar study (Moneim *et al* 2014) was conducted on fish oil. The study reported that essential fatty acids which found in fish oil have been widely recognized due to their beneficial effects on health, and are considered as essential supplements in human food. The results showed that the liver function parameters were affected by hyperlipidemia. Additionally, serum creatinine and uric acid concentrations were increased significantly. As previously showed that chicken meat contains large amount of MUFAs and PUFAs, so the administration of chicken oil content feed supplement might be improved the function of kidney and liver significantly.

SGPT and SGOT are typically used to detect liver injury. It is often ordered in conjunction with SGOT. These two tests are considered as a most important test to detect injury. SGPT and SGOT are known to be associated to the liver and the red blood cells. The destruction of the liver and red blood cells triggers the release of these enzymes into circulation. The elevation of the enzymes indicates rapture of hepatocytes and R.B.C membranes leading to hepatic dysfunction and hyperbilirubinaemia (Maegraith *et al 1981, Gregorakos et al* 1999). SGPT levels were found to be reduced by 41%, 60%, 45%, 51% and 69% for domestic chicken oil, hybrid chicken oil, domestic chicken flesh and hybrid chicken flesh and glibenclamide treated group respectively compared with control group (Table 4.15, Figure 4.29 & 4.30). Similar effect had seen for SGOT level. The level was decreased by 35%,

43%, 28%, 42% and 50% for domestic chicken oil, hybrid chicken oil, domestic chicken flesh and hybrid chicken flesh and glibenclamide treated group respectively compared with control group (Table 4.15, Figure 4.29 & 4.31). Whether, it was oil or flesh, hybrid chicken oil and flesh reduced serum SGPT and SGOT level more significant way.

So, chicken lipid may be good for diabetes because it has hypoglycemic effect and hypolipidemic effect. The high fat content in red meats causes elevated levels of cholesterol which can lead to blockages in the arteries resulting in coronary heart disease. Diabetics are at a higher risk of developing coronary heart disease and should replace red meat in their diets with lean white meats like chicken. This has a beneficial effect on both the heart and the kidneys, two organs that are at greater risk in diabetics.

Domestic and hybrid chickens, both two types of chickens are popular in our country. Actually fatty acid composition of domestic chicken is build up in a natural way as they are outdoor rearing and they eat natural food. So these types of chickens are tasted good and health friendly. On the other hand, hybrid chickens are indoor rearing and they were fed almost processed food like rapeseed, flaxseed, chia seed and chia meal. These feed ingredients can increase the ω-3 fatty acid and other important nutrient like ω-7 & ω-9 content of meat because fatty acid composition does not depend only their biosynthesis of the body but also the ingested lipid by the body. The fatty acid composition can be varied for hybrid chicken if their feed quality varies. So, production of quality meat can be possible for hybrid chicken to get a maximum health benefit if feed quality is analyzed and increased.

CONCLUSION

Both domestic and hybrid chicken meats are very popular in our country. But meat of domestic chicken is still now considered as more health friendly in comparison to hybrid chicken meat, though the production rate of hybrid chicken is increasing day by day due to its increased rate of consumption. The study aimed to investigate the meat composition of both type of chickens mentioned above. From the study, it may be concluded that:

- The protein and lipid contents of the hybrid chicken were higher by 40% than the domestic chicken. All other macronutrient contents were also found to be high in the hybrid chicken as they were fed with highly nutritious artificial feeds.

- Except potassium and manganese, all the other micronutrients were found to be high in domestic chicken. On the other hand, zinc content of hybrid chicken was found to be lower by 50% than that of domestic chicken.

- The oil analysis showed that iodine value was higher for hybrid chicken oil than domestic chicken oil which was an indication of the presence of higher amount of unsaturated fat in hybrid chicken. On the other hand, acid value and peroxide value were higher for domestic chicken oil than hybrid chicken oil which indicated that domestic chicken oil has a higher tendency to become rancid.

- Moreover, hybrid chicken oil contained higher amount of long chain fatty acid than domestic chicken oil as the saponification value for the hybrid chicken oil was found to be lower than domestic chicken oil. Percentage of free fatty acid was also found to be higher for the domestic chicken oil.

- Fatty acid composition of the oil showed that domestic chicken contains oleic acid and arachidonic acid in greater amount, which have very beneficial health effect.

- On the other hand, presence of a large amount of plamoteleic acid is noticed only in hybrid chicken. It is a ω-7 monounsaturated fatty acid which has also cholesterol, TG and LDL lowering property.
- Comparative nutritional analysis of various parts of chicken showed that, whether it was domestic or hybrid, breast meat contains higher amount of protein and thigh meat contains higher amount of lipid than that of other body parts.
- Iodine Value of thigh meat portion was found to be higher than other part of chicken meat (breast meat, drumstick and meat of wings). It indicates that thigh meat contains higher amount of unsaturated fatty acids than that of other meat portions of chicken.
- Comparing the blood sugar level in alloxan induced rats, domestic chicken oil and hybrid chicken oil administered subject shows significant reduction of blood sugar by 67% and 62% respectively, which is as near as glibenclemide administered subject (72%).
- Domestic chicken oil and Hybrid chicken flesh Increase body weight significantly by 81% and 83% respectively which was as near as the glibenclemide treatment (89%) .
- A significant reduction, by 69% and 82% of cholesterol level were observed for domestic and hybrid chicken oil treatment respectively in diabetic rats whereas in case of glibenclemide, it was 89%. LDL level was reduced significantly for domestic chicken oil, hybrid chicken oil and glibenclemide by 65%, 71% and 77% respectively, whereas HDL level was increased significantly by 70%, 62% and 79% for hybrid chicken oil, hybrid chicken flesh and glibenclamide respectively. A different scenario was observed for triglyceride level. Significant reduction was seen for only hybrid chicken oil by 30%, for glibenclamide it was 86%.

- Urea, uric acid and creatinine level were increased after diabetic induction in experimental rats. A significant reduction (56%) was seen in uric acid for hybrid chicken oil treatment. Domestic chicken oil and hybrid chicken oil also have significant effect on urea and creatinine level.

- There was a massive increase in the SGPT level and also a little increase in SGOT level after diabetes induction which was compensated by hybrid chicken oil and hybrid chicken flesh significantly. The reduction of SGPT by hybrid chicken oil and hybrid chicken flesh was 60% and 51% respectively, whereas 69% for glibenclemide.

- Domestic and Hybrid chicken oil contains predominantly antiatherogeic fatty acids and it seems to have a beneficial effect on hyperglycemia and hyperlipidemia as well as it seems to have a beneficial impact on weight loss in alloxan induced diabetic rats.

The information obtained from this investigation would be useful to help to the customer choosing chicken based diet. Moreover quality meat production can be possible from hybrid chicken as its meat quality depends on their feed type, environment and genetic manipulation. So hybrid chicken may be a good source of meat. Further study is needed to elucidate the mechanisms behind the process of the effects of fatty acids found in both type of chicken meat on blood parameters in rats.

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APPENDIX-I

Determination of moisture content of chicken meat:

Moisture content was determined by the conventional procedure (ICOMR, 1971).

Materials:

a) Porcelain crucible
b) Electrical balance
c) Oven
d) Desiccators

Procedure:

5-6 grams of chicken meat were weighed in a porcelain crucible (which was previously cleaned, heated to about 100°C, cooled and weighed). The crucible with the sample was heated in an electrical oven for about six hours at 100°C. It is was then cooled in desiccators and weighed again.

Calculation:

Percent of moisture content (gm per 100 g of chicken meat)

$$= \frac{\text{Weight of the moisture}}{\text{Weight of the chicken meat}} \times 100$$

APPENDIX-II

Determination of ash content of chicken meat:

Ash content was determined by the following method of A.O.A.C (Hofelmann and Hartmann,1985)

Materials:

a) Porcelain crucible
b) Muffle furnace
c) Electrical balance
d) Desiccator

Procedure:

5-6 gm of chicken meat was weighed in a porcelain crucible (which was previously cleaned, heated to about 100C°, cooled and weighed). The crucible with its content was placed in a muffle furnace for about four hours at about 600°C. It was then cooled in desiccators and weighed again. To ensure the completion of ashing, the crucible was again heated in the muffle furnace for half an hour, cooled and weighed again. This was repeated till two consecutive weights became same and the ash was almost white in color.

Calculation:

Percent of ash content (g per 100 g of chicken meat)

$$= \frac{\text{Weight of ash obtained}}{\text{weight of chicken meat}} \times 100$$

APPENDIX-III

Determination of total lipid contents of Chicken meat.

Lipid content of chicken meat were determined by the method of Bligh and Dyer (Blight & Dyer 1989).

Reagent

A mixture of chloroform and ethanol (2:1V/V).

Procedure

About 5 g of chicken meat were first grinded in a mortar and pestle with about 10 ml of distill water. The grinded flesh was transferred to a separating funnel and 30 ml of chloroform-ethanol mixture was added. The mixture was mixed well. It was then kept overnight at room temperature in the dark. At the end of this period 20 ml of chloroform and 20 ml of water were further added and mixed. Generally three layers were seen. A clear lower layer of chloroform containing the entire lipid, a colored aqueous layer of ethanol with all water soluble materials and a thick pasty interphase were seen.

The chloroform layer was carefully collected in a pre-weighed beaker (50 ml) and then placed on a steam bath for evaporation. After evaporation of the chloroform, the weight of the beaker was determined again. The difference in weight gives the amount of the lipid.

Calculation

Percent of lipid content (g per 100 g of Chicken meat).

$$= \frac{\text{Amount of lipid obtained}}{\text{weight of chicken meat}} \times 100$$

APPENDIX-IV

Determination of free /total sugar content chicken meat:

The sugar content of chicken meat were determined calorimetrically by the anthrone method (Desnuelle 1961).

Reagents:

a. Anthrone reagent: 0.2% in Conc. H_2SO_4.
b. Standard glucose solution: 10mg/ 100 ml distilled water.

(a) Extraction of sugar from chicken meat:

Extraction of sugar from chicken meat were done by the following method as described by Loomis.

4-6 g of chicken meat were plunged into boiling ethyl alcohol and allowed to boil for 5-10 minutes (5 to 10 ml of alcohol was used for each g of chicken meat). The extract was cooled and crushed thoroughly in a mortar with a pestle. Then the extract was filtered through two layers of muslin cloth and re-extracted the ground fish for three minutes in hot 80 percent alcohol, using 2 to 3 ml of alcohol for each g of fish sample. This second extraction ensured complete removal of alcohol soluble substances. The extract was cooled and passed through muslin cloth. Both the extracts were filtered through Whatmann no-41 filter paper. The volume of the extract was evaporated to about $^1/_4$ of the volume over a steam bath and cooled.

This reduced volume of the extract was then transferred to a 100 ml volumetric flask and made up to the mark with distilled water. Then 1 ml of

diluted solution was taken into another 100 ml volumetric flask and made up to the mark with distilled water (working standard).

Procedure:

Aliquot of 1 ml of the chicken meat extract from each part was pipetted into different test tubes and 4 ml of the anthrone reagent was added to each of this solution and mixed well. Glass marbles were placed on the top of each to prevent loss of water bath and then cooled. A reagent blank was prepared by taking 1 ml of water and 4 ml of anthrone reagent in a tube and treated similarly. The absorbance of the blue-green solution was measured at 680 nm in a colorimeter. A standard curve of glucose was prepared by taking 0.0, 0.1, 0.2, 0.3, 0.4, 0.5 0.6, 0.8 and 1 ml of standard glucose solution in different test tubes containing 0.0, 0.01 mg, 0.02 mg, 0.03 mg, 0.04 mg, 0.05, 0.06, 0.08 and 0.1 mg of glucose respectively and made the volume up to 1 ml with distilled water. Then 4 ml of anthrone reagent was added to each test tube and mixed well. All these solutions were treated similarly as described above. The absorbance was measured at 680 nm using the blank containing 1 ml of water 4 ml of anthrone reagent. The amount of free sugar was calculated from the standard curve of glucose (Fig-1.3). Finally, the percentage of free sugar present in the chicken meat was determined using the formula given below:

Percentage of free sugar (g per 100 gm of chicken meat).

$$= \frac{\text{Amount of sugar}}{\text{Weight of chicken meat}} \times 100\%$$

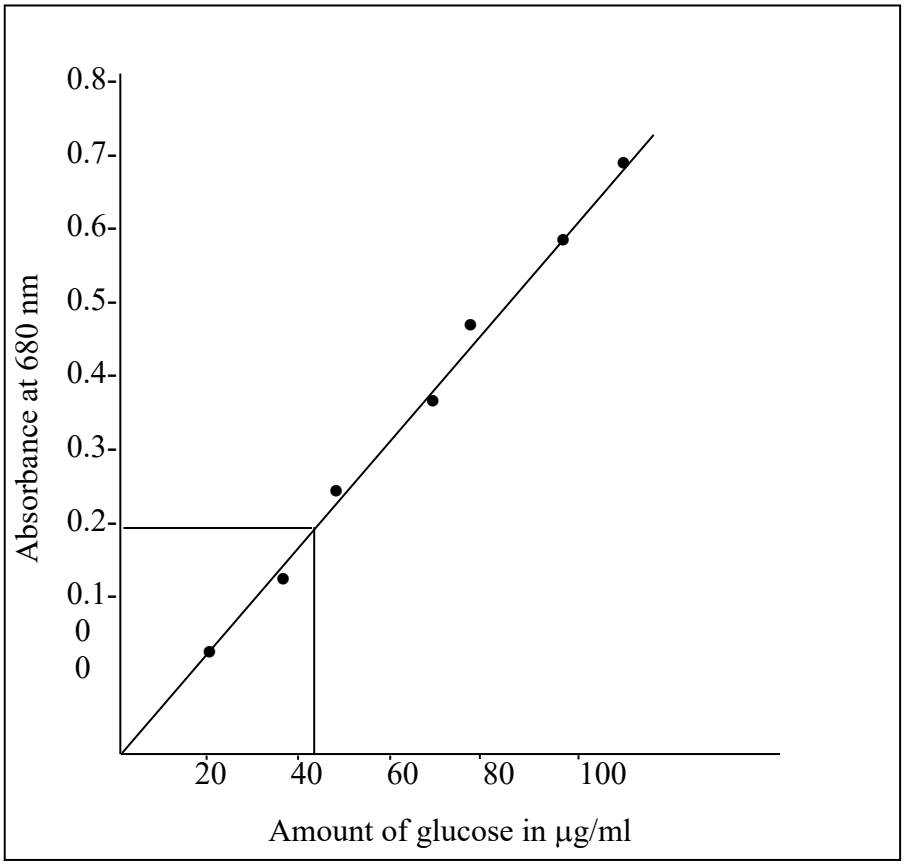


Fig-8.1: Curve for the determination of free sugar

APPENDIX-V

Determination of reducing sugar of chicken meat:

Reducing sugar content of the chicken meat was determined by dinitrisalicylic acid (Miller 1972).

Reagents

(a) Dinitrosalicylic acid (DNS) reagent: Simultaneously 1gm of DNS, 200 mg of crystalline phenol and 50 mg of sodium sulphate were placed in a beaker and mixed with 100 ml of 1% NaOH solution by stirring (if it is needed to store then sodium sulphite must be added just before use)

(b) 40% solution of Rochelle salt.

Extraction of sugar extract from chicken. Sugar was extracted from chicken by the method as described in section (2.3.4. a)

Procedure

Aliquot of 3 ml of the extract was pipetted into test tubes and 3ml of DNS reagent added to each of the solutions and mixed well. The test tubes were heated for 5 minutes in a boiling water bath. After the color has developed, 1 ml of 40% Rochelle salt was added when the contents of the tubes were still warm. The test tubes were then cooled under a running tap water. A reagent blank was prepared by taking 3ml of water and 3 ml of DNS reagent in a tube and treated similarly. The absorbance of the solutions were measured at 575 nm in a colorimeter.The amount of reducing sugar was calculated from the standard curve of glucose fig-(1.4).

Calculation

The percent of reducing sugar (gm per 100 gm of chicken meat)

$$= \frac{\text{Amount of sugar}}{\text{Weight of Catla and Mrigel fishes}} \times 100.$$

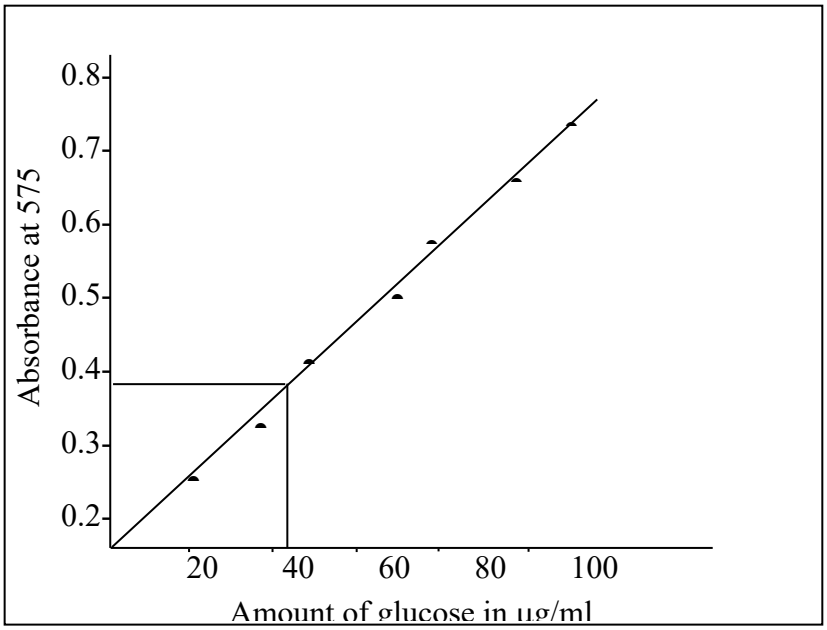


Fig 8.2: Standard curve of glucose for estimation of reducing sugar.

APPENDIX-VI

Determination of glycogen content of chicken meat**:**

The glycogen content of chicken meat were determined by the Anthrone method (Boel *et al* 1988).

Reagents:

(i) Anthrone regent (0.2% in concentrated H_2SO_4)

(ii) Standard glucose solution (10 mg / 100 ml distilled water)

(iii) 1M HC1

Procedure:

5-6 gm of chicken meat was homogenized well with 10 ml of water. It was then filtered through double layer of muslin cloth. To the filtrate twice the volume of ethanol was added to precipitate the polysaccharide, mainly glycogen. After kept it over night in cold the precipitate was collected by centrifugation in a clinical centrifuge at 3000 r.p.m. for 15 minutes. The precipitate was then dried over a steam bath. Then 40 ml of 1M hydrochloric acid was added to the dried precipitate and heated to about 70°C. It was then transferred to a volumetric flask and diluted to 100 ml with 1M HC1. Then 2 ml of diluted solution was taken in another 100 ml volumetric flask and diluted to 100 ml with 1M HC1.

Aliquot of 1 ml of the extract of each part was pipetted into different test tubes and 4 ml of anthrone reagent was added to the solution of each tube and mixed well. Glass marbles were placed on top of each tube to prevent loss of water by evaporation. The tubes were placed in a boiling water bath for 10

minutes, then removed and cooled. A reagent black was prepared by taking 1 ml of water 4 ml of anthrone reagent in test tube at 680 nm in a colorimeter. The amount of glycogen present in chicken meat were calculated from standard curve of glucose. (Fig. 1.5).

Calculation:

The percentage of glycogen content (g per 100 gm of chicken meat**).**

$$= \frac{\text{Amount of glycogen}}{\text{Weight of Catla and Mrigel fishes}} \times 100\%$$

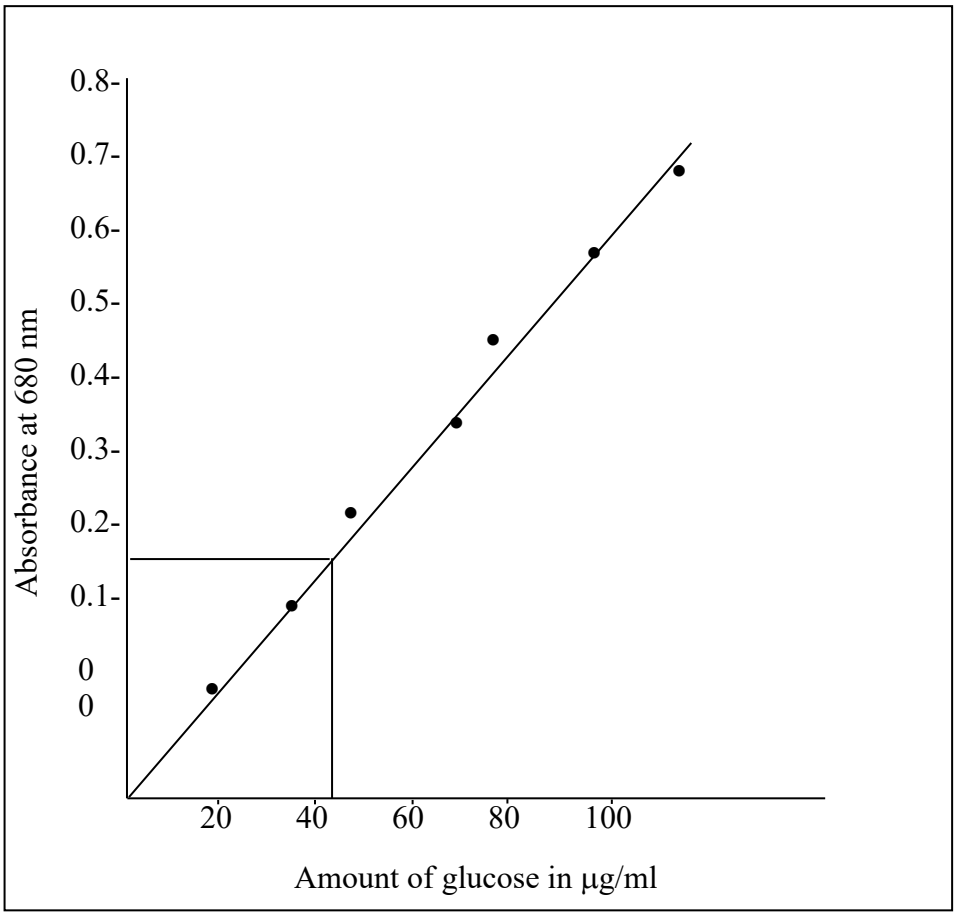


Fig-8.3: Curve for the determination of glycogen

APPENDIX-VII

Determination of Total Protein

Total protein contents of chicken meat were determined by the micro-kjeldahl method (Ranganna 1986) .

Apparatus Required:

a) Kjeldahl digestion flask: 250 ml capacity.
b) Distillation apparatus.
c) 100 ml conical flask.
d) 50 ml beaker.
e) 50 ml burette.
f) 100 ml volumetric flask.

Reagents Required

a) **Mixed indicator:** Prepared 0.1% bromocresol green and 0.1% methyl red indicators in 95% alcohol separately. 10 ml of the bromocresol green was mixed with 2 ml of the methyl red solution in a bottle provided with a dropper, which delivered about 0.05 ml per 4 drops.
b) **2% Boric acid:** 10 g of boric acid (crystals) was dissolved in 500 ml of boiling distilled water. After cooling, the solution was transferred into a glass-stoppered bottle.
c) **30% Sodium hydroxide solution:** 150 g of sodium hydroxide pellets was dissolved in 375 ml of distilled water. The solution was stored in a bottle closed with rubber stopper.

d) **Catalysts for digestion:** 2.5 g of powdered selenium dioxide (SeO_2), 100 g of potassium sulphate (K_2SO_4) and 20 g of copper sulphate ($CuSO_4$, $5H_2O$) were mixed.

e) **0.01N Hydrochloric acid:** The concentration of the solution was checked against pure sodium carbonate.

Procedure

Digestion

The samples were weighed accurately and transferred to a 250 ml Kjeldahl flask. 1 g of catalyst mixture and 25 ml of conc. H_2SO_4 were added to it. The flask was placed in an inclined position on the stand in the digestion chamber. The flask was heated gently over a low flame until the initial frothing was ceased and the mixture was boiled briskly at a moderate rate. During heating the flask was rotated several times. The heating was continued until the color of the digest was pale blue. The digest was cooled and 30 ml of water was added to 5 ml portion with mixing. The digest was transferred to a 100 ml volumetric flask. The flask was then rinsed 2 or 3 times with water and the washings were transferred to the volumetric flask. The solution was made up to volume with water. A blank digestion was carried out without the sample and the digest was made up to 100 ml in total.

Distillation and Titration

The distillation apparatus was set up as a flask was placed under the condenser. The distilled water was boiled in the steam generator using a Bunsen burner. Stopcock and pinck clamp were closed. Cold water was run through the condenser, from which about 5 ml of distillate was collected per minute. The burner was removed; where upon the condensate in the distilling flask was sucked back into the steam trap. Funnel was filled with distilled water, and the stopcock was opened momentarily to drain the water into flask. The burner was then replaced under the steam generator for about 20 seconds and it was removed again. 20 ml of 2% boric acid was pipetted into a clean conical flask and the mixed indicator was added to it. The micro burette was filled with 0.01 N HCI to the zero mark by this time; the distilling flask had become empty. The burner was replaced under the steam generator and pinch clamp was opened to remove liquid from the steam trap. The pinch clamp was left on the glass tubing through which the steam was escaped. The beaker was replaced under the condenser with the conical flask containing boric acid, and the flask was supported in an oblique position, so that the tip of the condenser was completely immersed into the liquid. The stopcock was opened with one hand and with the other hand 10 ml of the digest was pipetted into. The funnel was rinsed twice with about 2 to 3 ml portions of distilled water. Then necessary amount of 30% NaOH was introduced and stopcock was closed. The pinck cock was replaced on the digestion mixture and sodium hydroxide, and the ammonia was liberated which escaped with steam through the condenser into the boric acid solution.

The boric acid was changed from bluish purple to bluish green as soon as it came in contact with ammonia. The change, which was very sharp, took place between 20 to 30 seconds after the pinch clamp was closed. Boric acid had changed color within 5 minutes; the conical flask was lowered so that the condenser tip was 1 cm above the liquid. The end of the condenser was washed with a little distilled water. Distillation was continued until sufficient distillate was collected. The burner was then removed. The distillate was titrated with standard hydrochloric acid until the blue color was disappeared. The titrated was done in daylight. The blank distillation and titration were carried out as in the case of the sample. The percentage of protein in sample was calculated using the following formula:

$$\text{Percentage of nitrogen} = \frac{(V_A - V_B) \times N \times 14 \times V_M \times 100}{A \times W \times 100}$$

Where,

W = Weight of the sample taken

V_A = Volume of HCl in actual titration

V_B = Volume of HCl in blank titration

N = Normality of HCl

V_M = Volume made up of the digest

A = Aliquot of the digest taken

Thus, % Protein = % Nitrogen $\times$ 6.25

APPENDIX-VIII

Determination of Minerals (Black 1965).

Preparation of samples for analysis

Drying: A clean container (dish or beaker) was placed in an oven at 105^0C overnight and allowed the container to cool in desiccators and weighed. The sample was put into the container and weighed the container with the sample. Then the container was placed in the oven at 105^0C for 24 hours and allowed the container to cool in desiccators and weighed it again. Drying was repeated. Cooling and weighing were continued until the weight become constant. The dried sample was stored in an airtight container. The moisture content in the sample was calculated.

Grinding:

If necessary, the dried sample material was cut into small pieces with a knife or scissors. The sample was glinted in a grinder fitted with a suitable screen. If the grinding takes a long time, the sample will absorb moisture and then it is necessary to dry the sample again in the oven at 105^0C overnight.

Determination of Ca, Mn, Fe, Zn, Pb, As and K :

Organic matter was digested and Ca, K. Na, Fe, Cu, P, Pd and As were released by digestion with nitric acid. Ca, Fe, Mn, Zn, K, Pb and As were determined by atomic absorption spectrophotometry,

Reagent:

1. Iron accelerator
2. Copper accelerator
3. Concentrated sulphuric acid
4. Catalyst mixture
5. 33% sodium hydroxide
6. 0.0500 M sodium hydroxide
7. 0.0500 M Hydrochloric acid
8. Methyl red- methyl blue indicator solution
9. 68% Nitric acid
10. 1: 20 diluted HNO_3
11. 1:100 diluted HNO_3
12. 5 M HNO_3
13. $LaCL_3$- solution
14. Acetate buffer solution
15. Azomethine-H reagent
16. Perchloric acid
17. Hydrochloric acid 1:1
18. 0.5 M Barium chloride solution
19. Silver nitrate solution
20. Used two stock solutions and one slandered solution of each mineral at different concentration.

Digestion:

0.500g dried material was weighed into each of 38 nitrogen digestion tubes. The two remaining tubes were blanks. 5 ml 68% nitric acid was added to each of all 40 tubes. The content in each tube was mixed and left the tubes overnight. The tubes in the digester were placed and covered with the exhaust manifold. The temperature was set at 125^0C. The digester was turned on and continues the digestion for 4 hours after boiling was started. It was need to observe that no tubes become dry.

After cooling, the digestion mixture was transferred with distilled water to a 100ml volumetric flask. The flask was made to volume with water and mixed. It was then filtered into a dry bottle, which can be closed with a screw cap. The filtrate was kept in the closed bottle. Ca, Mn, Zn, Fe and K were determined in the filtrate.

Determination of Ca, Mn, Zn, Fe and K

Using a pipette, transfer 20 ml filtrate was transferred to a 100 ml volumetric flask. The flask was made to the required volume with distilled water and was mixed.

Measurement of K

Transfer 10 ml diluted filtrate was transferred into a 50 ml volumetric flask using a pipette. The flask was made to the required volume with water and mixed. The content of K and Na were measured by flame photometer. If the reading is higher than the reading of the highest standard solution, it is need

to a larger dilution, e.g. 5 ml volumetric flask. In this case 0:100 diluted HNO_3 must be added to the volumetric flask to make the total volume of 1:100 diluted HNO_3 and filtrate equal to 10 ml.

Measurement of Ca.

20 ml diluted filtrate was transferred from 50 ml filtrate to a 100 ml volumetric flask. The flask was made up to volume with distilled water and mixed. The content of Ca was measured by atomic absorption spectrometer (AAS). If the reading was higher than the reading of the highest standard solution, larger dilution was required, e.g. 10 ml filtrate into a 50 ml volumetric flask. In this case 1:100 dilution HNO_3 had to be added to the volumetric flask to make the total volume of 1:100 diluted HNO_3 and filtrate equal to 20 ml

Measurement of Fe, Mn, Pb, As and Zn

The content of these elements were measured by atomic absorption spectrometer (AAS) directly in the undiluted filtrate.

Calculations:

Ca, K

mg per kg chicken material = $\frac{a \times 25000}{b \times c}$

Where

a = mg/l Ca or K measured on atomic absorption spectrometer, flame photometer or spectrophotometer, flame photometer or spectrophotometer,

b = ml diluted filtrate transferred into the 50 ml volumetric flask for determination of Ca or K

c = g fish material weighed into the digestion tube.

If an additional dilution is made before the transfer to the 50 ml volumetric flask, the result is multiplied with the dilution factor.

Fe, Mn, Pb, As and Zn

mg per kg chicken material = $\frac{d \times 100}{c}$

Where

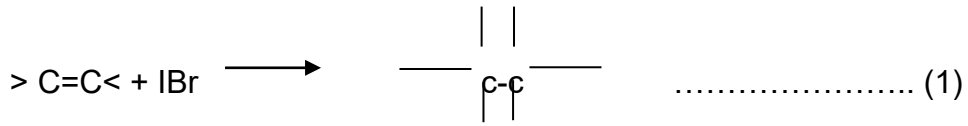
d = mg/l Fe, Mn, Zn, Pb and As measured on atomic absorption spectrometer or spectrophotometer,

c = g material weighed into the digestion tube.

APPENDIX-IX

Determination of Iodine Value:

The iodine value of oils were measured by Hanus method (Williams 1996, Kalumuddin. *A Test Book of practical physics*). The iodine value of fat or oil is the amount of halogen absorbed under specific conditions and is expressed as the number of grams of iodine per 100 grams of fat or oil. It is measured of the proportion of unsaturated constituents present. According to the method, iodine monochloride or monobromide is the agent absorbed as seen in the equation-1. It is of great importance in characterizing individual oil or fat and also for finding their solubility as soap materials.



…….. (1)

Apparatus Required

a) 250 ml wide necked glass bottle, with glass stoppers.

b) 50 ml burette.

Reagent Required

a) Potassium iodide solution (10%): 10 g. of potassium iodide was dissolved in 100 ml distilled water.

b) Sodium thiosulphate solution (0.1 N): 24.82g of sodium thiosulphate crystal was dissolved in 100 ml of distilled water and previously standardized with potassium dichromate (0.1 N).

c) Hanus reagent: 13.2 g of iodine was dissolved in 800 ml of glacial acetic acid.

3 ml of bromine was added to 200 ml of glacial acetic acid.

Then these two solutions were mixed and thus reagent was prepared.

d) Starch solution (1%): Prepared from natural soluble starch.

Procedure

0.250 g of oil was dissolved in 10 ml chloroform in a wide necked glass bottle with ground glass stopper. 30 ml Hanus solution was added to the bottle and the mixture was allowed to stand in the dark for exactly 30 minutes. 15 ml of potassium iodide solution was added to it and the mixture was shaken well. Then 75 ml of distilled water was added and titrated with standardized sodium thiosulphate solution using starch as indicator.

$2\ Na\ S_2\ O_3 + I_2 = Na_2\ S_4\ O_6 + 2NaI$

A blank experiment (without oil) was performed in the same way as described above. The iodine value was calculated using the following expression.

$$\text{Iodine value} = \frac{(V_1 - V_2)\ X\ S\ X\ 0.127\ X\ 100}{W}$$

Where

V_1 = Volume in ml of sodium thiosulphate solution required for blank experiment.

V_2 = Volume in ml of sodium thiosulphate solution used.

S = Normality of sodium thosulphate solution used.

W = Weight of the oil in gram.

Sodium thiosulphate was standardized against a standard posassium dichromate and the strength of the thiosulphate solution was found to be 0.1 N.

APPENDIX-X

Determination of Saponification Value and Saponiafication Equivalent

Saponification Value

Definition

The saponification value of the fat or oil is the number of milligrams of potassium hydroxide required to saponify completely 1 g of fat or oil.

The saponification value is related to the molecular weight of fat or oil and therefore provides information on the mean molecular weight of the combined fatty acids. Saponification value together with iodine value is the most important chemical constant. It gives an estimation of the non-fatty impurities and tells the amount of alkali that would actually required by the fat for its conversion into soap.

Principle

A weighed quantity of the oil is saponified with a known amount of potassium hydroxide, excess of which is determined by titration.

Apparatus required

a) 250 ml round bottomed flasks.

b) Reflux condensers, to fit the round bottomed flasks.

c) 25 ml pipette.

d) 50 ml burette, graduated in 0.1 ml.

e) Heating device.

f) Boiling chips.

Reagent Required

a) Potassium hydroxide: Approximately 0.5 N solutions in 95 percent (v/v) ethanol.

b) Hydrochloric acid: 0.5 N aqueous solution, accurately standardized.

c) Phenolphthalein: 10 g /1 solution in 95 percent (v/v) ethanol.

d) Sodium Carbonate

Procedure

The oils were weighed accurately and taken into a 250 ml round bottomed flask. 25ml of the ethanolic potassium hydroxide solution and some boiling chips were added to it. The flask was then connected to a reflux condenser and boiled on water bath for an hour with occasional shaking.

H_2C-O-CO-R $\longrightarrow$ H_2C-OH

HC-O-CO-R-3KOH HC-OH + 3R-COOK

H_2C-O-CO-R H_2C-OH

Triglyceride Glycerol Soap

Solution of the flask, while was titrated with 0.4578N hydrochloric acid solution using phenolphthalein indicator.

A blank test was carried out under the same conditions and saponification value of the oil was calculated using the following formula.

Saponification Value = $\frac{(V_1 - V_2) \times \mathrm{N} \times 56.1}{W}$

Where

V_1 = Number of ml of standardized hydrochloric acid solution used for blank test.

V_2 = Number of ml of standardized hydrochloric acid solution used for the test with oil

N = Exact normality of standardized hydrochloric acid solution used,

W = Weight of gram of oil sample.

Saponification equivalent

Saponification equivalent of a fat or oil is the number of grams of material saponified by one mole of potassium hydroxide.

It was determined by the following formulae.

$$\text{Saponification Equivalent} = \frac{56100}{\text{Saponification value of the oil}}$$

Determination of Acid Value and Percentage of Free Fatty Acid.

Definition

The acid value of a fat or oil is the number of milligrams of potassium hydroxide required to neutralize 1 g of the fat or oil. It is a measured number of free fatty acid present in it.

Principle

A weighed quantity of material is titrated in a suitable solvent with aqueous sodium hydroxide solution under conditions, which do not sapoinfication of the neutral portion.

Apparatus Required

a) ml conical flask
b) ml burette, graduated in 0.01 ml

Reagents Required

a) Solvent: Mixture of equal parts of 96% ethanol and diethyl ether, which was neutralized immediately before used by titration with 0.1 N sodium hydroxide solution, phenolphthalein being used as indicator.
b) Sodium hydroxide: 0.1 N aqueous solution, accurately standardized.
c) Phenolphthalein indicator: 1% solution in 96% ethanol.

Procedure

The oils were weighed accurately, taken in a 250 ml conical flask and dissolved in 50 ml of the neutral solvent. The solution was titrated, while shaking with 0.1391 N sodium hydroxide solution using phenolphthalein as indicator.

The acid value was calculated using the following formula.

Acid value = **Error! Bookmark not defined.Error! Bookmark not defined.**$\frac{56.1 \times N \times V}{W}$

Where

N = Exact normality of the standard sodium hydroxide solution used.

V = Number of ml of the standard sodium hydroxide solution used for the test with the oil.

W = Weight in gram of oil sample.

Free Fatty Acid

The percentage of free fatty acid was calculated using the following formula.

Percent of free fatty acid = $\frac{\text{Acid value}}{1.99}$

Determination of peroxide value

The peroxide value is defined as the milliequivalents of peroxide oxygen combined in a kilogram of oil.

Reagents used

a) Acetic acid-chloroform solution (3:2 v/v)
b) Saturated potassium iodide solution.
c) 0.01N sodium thiosulphate solution.
d) Starch indicator: Starch (1gm) was dissolved in 50ml hot water and diluted to 100ml with cold water.

Procedure

Peroxide value of the oil was determined as milliequivalent to peroxide oxygen per kg of sample, using the following method. The oil was weighed and taken in a 250 ml glass stoppered flask and 30ml acetic acid-chloroform solution was added to it and mixed uniformly. 0.5ml of saturated potassium iodide solution was added to it. The solution was allowed to stand for 20 minutes with occasional shaking. 30ml distilled water was then added. The solution was titrated with 0.01N sodium thiosulphate solution until the yellow color had almost disappeared. 0.5ml starch solution was added to it and again titrated until the blue color just almost disappeared.

A blank experiment was performed in the same manner without the oil and the peroxide value was calculated using the formula given below:

$$\text{Peroxide value} = \frac{(S-B)\times N\times 1000}{\text{Weight of sample}}$$

Where

S= ml of sodium thiosulphate solution required in the test experiment.

B= ml of sodium thiosulphate solution required in the blank experiment.

N= Normality of sodium thiosulphate solution.

Determination of unsaponifiable matter

The unsaponifiable matter is a fraction of fat or oil that remains insoluble after saponification of the fat sample by alkali.

Reagents used:

a) diethyl ether

b) Acetone

c) Alcoholic solution of potassium hydroxide 1.0N (approximately)

d) Aqueous solution of potassium hydroxide (0.5N)

The amount of unsaponifiable matter present in the oil was determined using a method described earlier.

Procedure

The oil (750 mg) was taken in a round bottomed flask and 1N alcoholic potassium hydroxide (50ml) was added to it. The mixture was then refluxed for 45 minutes on a water bath at about 70◦c to 80◦ with occasional swirling. The solution was transferred into a separator funnel and rinsed with 100 ml water. The solution while still perceptibly warm, extracted four times with ether using 50 ml each time. Each extraction was done by vigorous shaking in the separator funnel and allowed the layers to be separated. The aqueous alcoholic layer at the bottom of the separator funnel was run off and the ethereal solution from the top was poured into another separator funnel

containing 20 ml water. The total ether extracts in the separator funnel was washed thrice using 20ml water each time.

The ethereal layer was then washed three times with 20 ml aqueous potassium hydroxide solution each time. Each alkali wash was followed by a wash with 20 ml water. After the aqueous alkali wash the solution was washed with sufficient amount of water until the washed water did not show alkaline reaction to phenolpthaline solution.

The ether extract was then transferred into a weighed flask and the ether was evaporated. The residue was dried to constant weight at 80° c. Drying was assisted by adding 2-3 ml of acetone to the extract when nearly all the ether was evaporated. The flask with its contents was then weighed. The quantity of unsaponifiable matter present in 100 gms of oil was calculated from the formula.

$$\text{U.M.} = \frac{\text{Weight of unsaponifiable matter}}{\text{Weight of oil taken}} \times 100$$

Where

U.M. = unsaponifiable matter.

APPENDIX-XI

Estimation of serum blood glucose (Bio Systems S.A. Barcelona Spain)

Serum glucose was measured by enzymatic colorimetric (GPO-PAP) method Micro lab, 300 (Semiautoanalyzer). (BioSystems S.A. Barcelona Spain)

Principle

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red violet quinoneimine dye as indicator.

$$\text{Glucose} + H_2O \xrightarrow[Oxidase]{Glucose} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{Peroxidase} \text{Quinoneimine} + 4H_2O$$

Reagents

Contents	Initial concentration of solution
Buffer	
Phosphate Buffer	0.1 mol/l, pH 7.0
Phenol	11 mol/l
GOD-PAP Reagent	
4-aminophenazone	0.77 mmol/l
Glucose oxidase	≥1.5 kU/l
Peroxidase	≥1.5 kU/l
Standard	
Glucose	5.55 mmol/l (100 mg/dl)

Additional Reagent

Uranyl Acetate 0.16% cat NO DP 647 2x 500 ml

Materials required

Microcentrifuge tube

Micropipettes and pipettes with disposable tips

Microlab- 300 (Semiautoanalyzer)

Procedure

Procedure for glucose GOD-PAP assay without deproteinization. The instrument was calibrated before estimation.

Serum and reagent were taken in specific cup. They were arranged serially into the Microlab, 300 (Semi autoanalyzer). The Auto lab was programmed for the estimation of glucose and allowed to run with following procedure:

5 μl sample and 500 μl reagent were mixed and incubated at 370 C for 10 minutes. The reaction occurred in reaction cell or cup. The absorbance of the sample and the standard against the reagent blank were measured at 500 nm within 60 minutes.

Calculation of result

Optical densities or absorbances were fed into a computer and calculation was done using the software program. Values for the unknown samples were calculated by extrapolating the absorbance for the standard using following formula.

Glucose concentration (mmol/l) = $\frac{A_{Sample}}{A_{S\,\tan dard}} \times 5.55$

APPENDIX-XII

Estimation of serum Total Cholesterol (Bio Systems S.A. Barcelona Spain)

Total cholesterol was measured by enzymatic endpoint method (cholesterol Oxidase/Peroxidase) method in Microlab, 300 (Semiautoanalyzer) using reagent of BioSystems S.A. Barcelona Spain.

Principle

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantiphyrine in the presence of phenol and peroxidase.

Cholesterol ester + H2O $\xrightarrow{Cholesterol\ Esterase}$ Cholesterol + fatty acids

Cholesterol + O2 $\xrightarrow{Cholesterol\ Oxidase}$ Cholestene- 3-one + H2 O2

2H2O2+phenol+4-aminoantiphyrine $\xrightarrow{Peroxidase}$ quinoneimine+H2O

Reagent composition

Contents	Initial Concentration of Solution
Reagent	
4-Aminoantipyrine	0.30 mmol/l
Phenol	6 mmol/l
Peroxidase	≥ 0.5 U/ml
Cholesterol esterase	≥ 0.15 U/ml
Cholesterol oxides	≥ 0.1 U/ml

Pipes Buffer	80 mmol/l; pH 6.8
Standard	5.17 mmol/l (200 mg/dl)

Materials

Microcentrifuge tube

Micropipettes and pipettes

Disposable tips

Micro lab, 300 (Semiautoanalyzer)

Procedure

Serum and reagents were taken in specific cup or cell. They were arranged serially. Then ID number for each test was entered in the MICROLAB. 5 μl sample and 500 μl reagent were mixed and incubated at 370C for 5 minutes within the Microlab. The reaction occurred in reaction cell or cup. The absorbance of the sample and the standard against the reagent blank were measured at 500 nm within 60 minutes.

Calculation of result

Concentration of cholesterol in sample was calculated by using software program with the following formula.

Cholesterol concentration (mg/dl) = $\frac{A_{Sample}}{A_{S\,\mathrm{tan}\,dard}} \times$ concentration of standard.

APPENDIX-XIII

Estimation of serum Triglycerides

Serum triglyceride was measured by enzymatic colorimetric (GPO-PAP) method Micro lab, 300 (Semiautoanalyzer). (BioSystems S.A. Barcelona Spain)

Principle

The triglyceride is determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen- peroxide, 4- aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triglyceride} + H_2O \xrightarrow{Lipase} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow[Kinase]{Glycerol} \text{glycerol-3-phosphate} + ADP$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow[Oxidase]{Glycerol\ Phosphate} \text{dihydroxy acetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{4- chlorophenol} \xrightarrow{Peroxidase} \text{quinoneimine} + HCl + 4\,H_2O$$

Reagents

Contents	Concentrations in the Test
Buffer	
Pipes Buffer	40 mmol/l, pH 7.6
4-choloro-phenol	5.5 mmol/l
Magnesium-ions	17.5 mmol/l
2. Enzyme Reagent	
4-aminophenazone	mmol/l
ATP	1.0 mmol/l
Lipases	>150 U/ml
Glycerol-3-phosphate oxidase	1.5 U/ml

Peroxidase	0.5 U/ml
3. Standard	2.29 mmol/l (200 mg/dl)

Materials

Micropipettes and pipettes

Disposable tips

Micro lab, 300 (Semiautoanalyzer)

Procedure

Serum and reagents were taken in specific cup or cell. They were arranged serially. Then ID number for each test was entered in the MICROLAB. 5 μl sample and 500 μl reagent were mixed and incubated at 370C for 5 minutes within the MICROLAB.

The reaction occurred in reaction cell. The absorbance of the sample and the standard against the reagent blank were measured at 500 nm within 60 minutes.

Calculation of result

Triglyceride concentration was calculated by using software program in MICROLAB with the following formula.

Triglyceride concentration (mg/dl) = $\frac{A_{Sample}}{A_{S\,\mathrm{tan}\,dard}} \times$ Concentration of the standard.

APPENDIX-XIV

Estimation of Serum High Density Lipoprotein (HDL)

Serum High density lipoprotein (HDL) was measured by enzymatic colorimetric (cholesterol CHOD-PAP) method in Micro lab, 300 (Semiautoanalyzer) Barcelona (Spain).

Principle

HDL (High Density Lipoproteins) are separated from chylomicrons, VLDL (very low density lipoproteins) and LDL (Low density lipoproteins) by the addition of a precipitating reagent (phosphotungstic acid-magnesium chloride) to serum or serum. After centrifugation, the cholesterol contents of HDL fraction, which remains in the supernatant, are determined by the enzymatic colorimetric method using CHOD- PAP.

Reagent composition

Contents

Buffer

Enzymes

Standard 50 mg/dl (1.29 mmol/l)

Materials

Microcentrifuge tube, Micropipettes and pipettes

Disposable tips

Micro lab, 300 (Semiautoanalyzer).

Procedure

Samples (200 ml) and precipitating reagents (500 μl) were taken in a microcentrifuge tube. Then it was mixed and allowed to sit for 10 minutes at room temperature. Then it was centrifuged at 4000 rpm for 10 minutes.

The supernatant was used as sample for determination of cholesterol content by the CHOD-PAP method. The sample and reagents were taken in specific cup or cell. They were arranged serially then ID number for test was entered in the MICROLAB. Then 5 μl sample and 500 μl reagent were mixed and incubated at 37ºC for 5 minutes within the MICROLAB. The reaction occurred in reaction cell. The absorbance of the sample and the standard against the reagent blank were measured at 500 nm within 60 minutes.

Calculation of result

Concentration was calculated by using software program.

APPENDIX-XV

Estimation of serum LDL cholesterol:

LDL cholesterol was determined from total cholesterol by the following formula:

$$\text{Total cholesterol} = \text{HDL} + \text{LDL} + \left(\frac{\text{Triglyceride}}{5}\right)$$

APPENDIX-XVI

Estimation of SGPT and SGOT by colorometric method.(Linear Chemicals)

Principle

Aspartate aminotransferase (GOT) catalyzes the transfer of the amino group from aspartate to oxoglutarate with the fomation of glutamate and oxalacetate.

L-Aspartate + 2-Oxoglutarate $\xrightarrow{GOT}$ L-Glutamate + Oxalacetate

Alanine aminotransferase (GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate.

L-Aianine + 2-Oxoglutarate $\xrightarrow{GOT}$ Glutamate + Pyruvate

The transaminase activity is proportional to the amount of oxalacetate or pyruvate formed over a definite period of time and is measured by the reaction with 2,4-dinitrophenylhydrazine (DNPH) and measurement of the color formed in an alkaline solution.

Reagent Composition

R1a **GOT substrate.** Phosphate buffer **100** mrriol/L pH **7.4,** L-aspartate **200** mmol/L, ketoglutarate **2** mmol/L.

R1b **GPT substrate.** Phosphate buffer **150** mmol/L pH 7.4, L-alanine **200** mmol/L, ketoglutarate 2 mmol/L.

R2 **DNPH.** 2,4-Dinitrophenylhydrazine 1 mmol/L. Color developer. Color developer. C R:34/35

R3 **4N NaOH** (10x). Sodium hydroxide 4 mol/L. C R:34/35

CAL Pyruvic standard. 1.8 mmol/L. Secondary standard.

PROCEDURE

1. Bring reagents and samples aTroom temperature.
2. Pipette into labelled tubes:

TUBES	Blank	GOT	GPT
GOT substrate	-	0.5 mL	-
GPT substrate	-	-	0.5 mL

Warm to 37°C into the bath for 5 min.
Add:

Serum	-	100 μL	100 μL

Mix. Return to bath at 37°C for. Add: 60 min 30min.

DNPH	-	0.5 mL	0.5 mL

Mix. Stand for 20 min. at room temperature.
Add:

NaOH 0.4 N	5.0 mL	5.0 mL	5.0 mL

Invert to mix. Stand for 5 min. at room temperature.

3. Read the absorbances (A) of the sample4s against a water blank (Note 1)

The colour is stable for at least 1 hour.

Calculations

From absorbances, read units of GOT or GPT from the corresponding curves. For activities higher than 200 WU (GOT) or 100 WU (GPT) repeat the test diluting the sample 1:1-\0 with saline and assayed again. Multiply the results by 10 (Note 2).

Units

The conversion of colorimetric units into UV units obtained by a kinetic optimized method (IFCC, 1985) cannot take place by the use of a factor as in the classic UV Karmen procedure (1995).

References Values

Serum

GOT/AST	8-40 WU/L
GPT/ALT	5-30 WU/L

APPENDIX-XVII

Estimation of serum urea by enzymatic colorimetric method.

Micro lab, 300 (Semiautoanalyzer). (BioSystems S.A. Barcelona Spain)

Principle of the method

Urea in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measurpd by spectrophotometry.

$$Urea + H_2O \xrightarrow{urease} 2NH_2^+ + CO_2$$

$$NH_4^+ + Salicylate + NaClO \xrightarrow{nitroprusside} Indophenol$$

Contents

COD 11536 COD 11537

A1.	Reagent	2 x 48 mL	1 x 240 mL
A2.	Reagent	2x2 mL	1 x 10 mL
B.	Reagent	2 x 50 mL	1 x 250 mL
I S.	Standard	1 x5 mL	1 x5mL

Composition

A1. Reagent: Sodium salicylate 62 mmol/L, sodium nitroprusside 3.4 mmol/L, phosphate buffer 20 mmol/L, pH 6.9.

A2. Reagent: Urease > 500 U/mL

B. Reagent Sodium hypochlorite 7 mmol/L, sodium hydroxide 150 mmol/L. *Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.*

S. Glucose/Urea/Creatinine Standard. Glucose 100 mg/dL, urea 50 mg/dL (8.3 mmol/L, BUN 23.3 mg/dL), creatinine 2 mg/dL. Aqueous primary standard.

Procedure

1. Bring the Reagents to room temperature.
2. Pipette into labelled test tubes:

	Blank	Standard	Sample
Urea Standard (S)	—	10 ML	
Sample	—	—	10 |iL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.
4. Pipette:

Reagent (B)	1.0 mL	1.0 mL	1.0 mL

5. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25“C) or for 5 minutes at 37°C.
6. Read the absorbance (A) of the Standard and the Sample at 600 nm against the Blank. The colour is stable for at least 2 hours,

Calculations

The urea concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{standard} \times \text{Sample dilution factor} = C_{Sample}$$

If the Urea Standard provided has Been used to calibrate (Note 3):

	Serum and plasma	Urine
$\frac{A_{Sample}}{A_{Standard}}$	x 50 = mg/dL urea x 23.3 = mg/dL BUN x 8.3 = mmol/L urea	x 2500 = mg/dL urea x 1165 = mg/dL BUN x 415 = mmol/L urea

Reference Values

Serum and plasma[4]:15-39 mg/dL urea = 7-18 mg/dL BUN = 2.5-6.5 mmol/L urea. Concentrations in the neonatal period are lower, and in adults over 60

years of age are higher than in adults. Concentrations also tend to be slightly higher in males than in females.

Urine[4]:26-43 g/24-h urea = 12-20 g/24 h BUN = 428-714 mmol/24-h urea

These ranges are given for orientation only; each laboratory should establish its. own reference ranges.

APPENDIX-XVIII

Estimation of serum uric acid level by enzymatic colorimetric method

Micro lab, 300 (Semiautoanalyzer). (BioSystems S.A. Barcelona Spain)**.**

Principle

Uric acid is oxidized by uricase to allantoine and hydrogen peroxide ($2H_2O_2$), which under the influence of POD, 4-aminophenazone (4- AP) and 2-4 Dichlorophenol sulfonate (DCPS) forms a red quinoneimine compound:

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uncase}} \text{Allantoine} + CO_2 + 2H_2O_2$$

$$2H_22O_2\ 2 + \text{4-AP} + \text{DCPS} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

The intensity of the red color formed is proportional to the uric acid concentration in the sample.

Clinical Significance

Uric acid and its salts are end products of the purine metabolism.

With progressive renal insufficiency, there is retention in blood of urea, creatinine and uric acid.

Elevate uric acid level may be indicative of renal insufficiency and is commonly associated with gout.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Reagents

R 1 Buffer	Phosphate pH 7.4 2-4 Dichlorophenol sulfonate (DCPS)	50 mmol/L 4 mmol/L
R 2 Enzymes	Uricase	60 U/L
	Peroxidase (POD)	660 U/L
	Ascorbate oxidase	200 U/L
	4 - Aminophenazone (4-AP)	1 mmol/L
URIC ACID CAL	Uric acid aqueous primary standard 6 mg/dL	

SAMPLES

- Serum or plasma: Stability 3-5 days at 2-8°C or 6 months at -20°C.

- Urine (24 h): Stability 4 days at 15-25°C, pH>8. Dilute sample 1/50 in distilled water. Mix. Multiply results by 50 (dilution factor);

If urine is cloudy; warm the specimen to 60°C for 10 min to dissolve precipitated urates and uric acid. Do not refrigerate.

PROCEDURE

1. Assay conditions:
   Wavelength:.....520 nm (490-550)
   Cuvette:..... 1 cm light path
   Temperature.....37°C / 15-25°C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard (μL)	-	25	-
Sample (μL)	-	-	25

4. Mix and incubate for 5 min at 37°C or 10 min at 15-25°C.
5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

Serum or plasma

$$\frac{(A)\,\text{Sample}}{(A)\,\text{Standard}} \times \text{mg/dL uric acid in the sample}$$

Urine 24 h

$$\frac{(A)\ \text{Sample}}{(A)\ \text{Standard}} \times 6 \times \text{vol.(dL) urine 24 h} = \text{mg/24 h uric acid}$$

Conversion factor: mg/dL x 59.5= ^mol/L.

REFERENCE VALUES

Serum or plasma:

Women	2.5 - 6.8 mg/dL	≅	149-405 μmol/L
Men	3.6-7.7 mg/dL	≅	214-458 μmol/L
Urine:	250 - 750 mg/24	≅	1.49 - 4.5 mmol/24 h

These values are for orientation purpose; each laboratory should establish its own reference range.

APPENDIX-XIX

Estimation of serum creatinine level by enzymatic colorimetric method
Micro lab, 300 (Semiautoanalyzer). (BioSystems S.A. Barcelona Spain).

Principle

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Sample Collection And Preparation

Serum, Heparinized or EDTA plasma. Stable for 7 days at +2 to + 8°C
Urine coiiected without additives. Urine samples should be diluted 1+49 with distilled water. Stable for 4 days at +2 to +8°C.

Reagent Composition

Contents	Initial Concentrations of Solutions
CAL. Standard	See lot specific insert
R1a. Picric acid	35 mmol/l
R1b. Sodium hydroxide	0.32 mo l/l

Stability and preparation of reagents

CAL. Standard

Supplied ready to use. Stable to expiry date when stored at +2 to +25°C.

R1a. Picric Acid

Supplied ready to use. Stable to expiry date when stored at +15 to +25°C.

R1b. Sodium Hydroxide

Supplied ready to use. Stable to expiry date when stored at +15 to +25°C.

Procedure Notes

Reaction rate and absorbance of the reaction product are very sensitive to temperature. The specified temperature must therefore be maintained.

Procedure

Wavelength:	492 (490-510 nm)
Cuvette:	1 cm light path
Temperature:	25/30/37°C
Measurement:	against air

Pipette into cuvette:

	Standard		Sample	
	Macro	Semi Micro	Macro	Semi Micro
Working reagent	2.0 ml	1.0 ml	2.0 ml	1.0 ml
Standard solution	0.2 ml	0.1 ml	-	-
Sample	-	-	0.2 ml	0.1 ml

Mix and after 30 seconds read the absorbance of the standard and sample.

Exactly 2 minutes later, read absorbance A_2 of standard and sample.

CALCULATION

$A_2 - A_1 - \Delta A_{sample}$ or $\Delta A_{standard}$

Concentration of creatinine in serum or plasma.

$$\frac{\Delta Asample}{\Delta Astandard} \times \frac{\text{Standard}}{\text{conc. (µ/mol/l)}} = \text{µmol/l}$$

$$\frac{\Delta Asample}{\Delta Astandard} \times \frac{\text{Standard}}{\text{conc. (mg/dll)}} = \text{mg/dl}$$

Concentration of creatinine in urine.

$$\frac{\Delta Asample}{\Delta Astandard} \times 8.85 = \text{mmol/l}$$

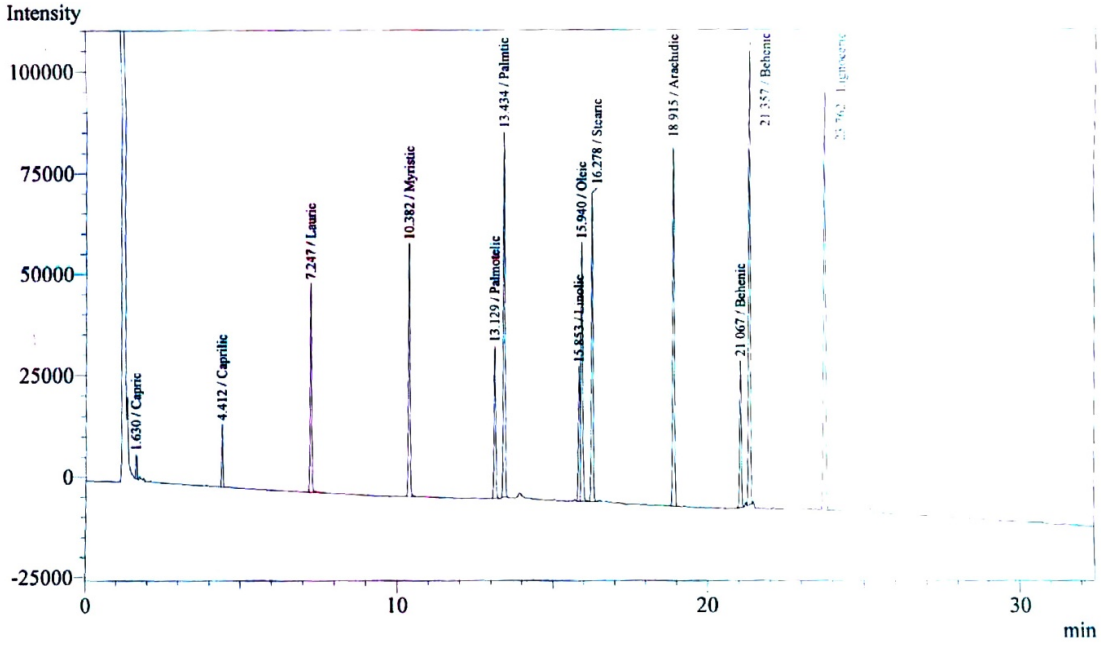
$$\frac{\Delta \text{Asample}}{\Delta \text{Astandard}} \times 100 = \text{mg/dl}$$

$$\textbf{Creatinine Clearance} \quad \frac{\text{mg creatinine/dl urine} \times \text{ml urine 24 hrs}}{\text{mg creatinine/dl serum} \times 1440} \ [\text{ml/min}]$$

Appendix-XX

GC pattern of Standard

Analysis Date & Time : 9/12/2011 10:11:44 AM Admin 1
Sample Name: STANDARD
Sample ID: STANDARD
Sample Type: Unknown
Injection Volume: 1.00
Data Name: D:\Data\Standard_fatty acids\STANDARD 12-09-1 lgcd.gcd

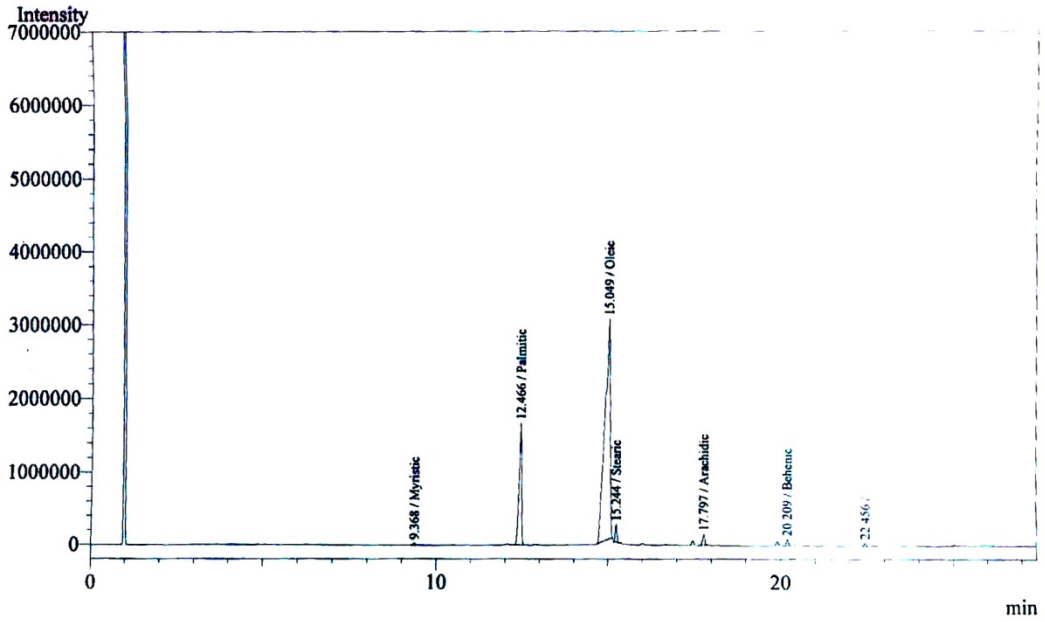


	Ret.Time	Area	Height	Cone.	Unit	Mark	ID#	Cmpd
1	1.630	16583	5952	0.000	ppm		1	Capric
2	4.412	40969	15530	0.000	ppm		2	Capri lie
3	7.247	163574	51323	0.000	ppm		3	Laurie
4	10.382	213895	62291	0.000	ppm	SV	4	Myristic
5	13.129	131640	37430	0.000	ppm	V	5	Palmotelic
6	13.434	317825	90119	0.000	ppm		6	Palmtic
7	15.853	119025	32964	0.000	ppm		7	Linolic
8	15.940	247111	63772	0.000	ppm	SV	8	Oleic
9	16.278	268943	75494	0.000	ppm		9	Stearic
10	18.915	325662	89580	0.000	ppm		10	Arachidic
11	21.067	133387	36327	0.000	ppm		11	Behenic
12	21.357	408824	113919	0.000	ppm		11	Behenic
13	23.762	457675	104361	0.000	ppm		12	Lignoceric

Appendix-XXI

GC pattern of Domestic Chicken oil

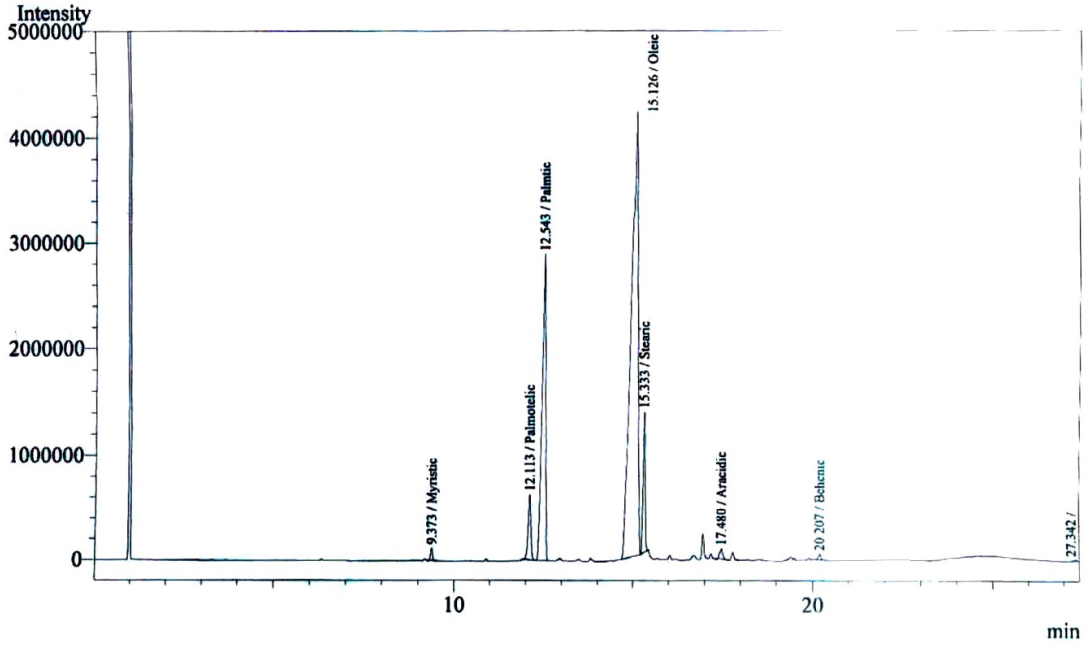
Analysis Date & Time: 12/2/2014 1:00:07 PM Admin 1
Sample Name: Domestic Chicken Oil
Sample ID: Sample No 1
Sample Type: Unknown
Injection Volume: 2.00
Date Name: C:\GCsolution\Sample\Sam--l 2-12-14.gcd



Peak	Ret.Time	Area	Height	Cone.	Unit Mark	ID#
1	9.368	107775	31418	0	ppm	Myristic acid
2	12.466	8584316	1668818	0	ppm	Palmitic acid
3	15.049	33135746	3002080	0	ppm	Oleic Acid
4	15.244	806420	246022	0	ppm	Stearic acid
5	17.797	730492	153576	0	ppm	Arachidonic Acid
6	20.209	374579	91046	0	ppm	Behenic Acid

Appendix-XXII
GC pattern of Hybrid chicken Oil

Analysis Date & Time : 12/2/14,1:34:10 PM
Sample Name: Hybrid Chicken Oil
Sample ID: Smaple No 2
Sample Type: Unknown
Injection Volume: 2.00
Data Name: C:\GCsolution\Sample\Sam--2 2-12-14.gcd



ID#	Ret.Time	Area	Height	Cone. Unit Mark	Cmpd Name
1	9.373	396024	120243	0.000 ppm	Myristic Acid
2	12.113	3292684	626678	0.000 ppm	Palmotelic Acid
3	12.543	20418815	2891257	0.000 ppm	Palmtic Acid
4	15.126	56291616	4189341	0.000 ppm	Oleic Acid
5	15.333	5437563	1336351	0.000 ppm	Stearic Acid
6	17.48	576092	99151	0.000 ppm	Aracidic Acid
7	20.207	235990	53881	0.000 ppm	Behenic Acid