EVALUATION OF FATTY ACID AND CHOLESTEROL CONTENT IN LONG-HORNED ANKOLE CATTLE (*BOS TAURUS INDICUS*) FROM NTUNGAMO AND KIBOGA DISTRICTS, UGANDA

BY

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REG. NO. 21/U/GMCH/14683/PE

A DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN CHEMISTRY OF KYAMBOGO UNIVERSITY

NOVEMBER, 2024

DECLARATION

I, Joseph Wanyama, hereby declare that this work is my own work and that I have not previously in its entirety or in part submitted it at any university for a degree. Where other people's work has been used, this has been properly acknowledged in literature citations.

Signature ……. Date……

Joseph Wanyama

APPROVAL

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DEDICATION

I dedicate this work to my dear parents Raphael Wanyama and Phoebe Nabuteza and to all those who offered unwavering prayers and encouragement throughout the course of my studies.

ACKNOWLEDGMENT

My sincere appreciation is first extended to the Almighty God for His love, guidance, protection and care.

I extend my deepest gratitude to my dear supervisors, Associate Professor Justus Kwetegyeka, Doctor Hannington Twinomuhwezi and Doctor Ivan Kiganda for their invaluable guidance, unwavering support, and boundless patience throughout this journey. Their expertise and encouragement have been instrumental in shaping this work.

Sincere thanks to all my lecturers of the MSc. Chemistry Programme at the Department of Chemistry, Kyambogo University.

I am also indebted to my Course mates in the academic journey for their insightful feedback, moral support and constructive criticism, which have immensely contributed to the refinement of this study.

I am profoundly grateful to my family for their love, encouragement, and understanding during the challenging phases of this endeavor. Their unwavering belief in me has been my greatest source of strength.

Lastly, I would like to express my appreciation to all the individuals who have supported me along this academic journey, including friends, mentors, and colleagues. Special thanks to Julie Akullo, and Edward Katende. Your encouragement and inspiration have been invaluable.

This dissertation would not have been possible without the collective support and encouragement of these individuals, and for that, I am truly grateful.

May the good Lord bless all of you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

AOACS: Association of American Chemistry Society

AR: Analytical grade

CVDs: Cardiovascular diseases

EFA: Essential fatty acids

EU: European Union

FAMEs: Fatty Acid Methyl Esters

FAO: Food and Agriculture Organization of the United Nations.

GC: Gas Chromatography

GDP: Gross Domestic product

GLA: Gamma- Linolenic acid

HDL: High density lipoprotein

HPLC: High-performance liquid chromatography

LA: Linolenic acid

LCFA: Long chain fatty acids

LDL: Low density lipoprotein

MCFA: Medium chain fatty acids

MUFA: Monounsaturated Fatty Acids

NCDs: Non-communicable diseases

NDP: National Development plan

NMR: Nuclear Magnetic Resonance

PUFA: Polyunsaturated Fatty Acids

SCFA: Short chain fatty acids

SFA: Saturated Fatty Acids

VLCFA: Very long chain fatty acids

WHO: World Health Organization

ABSTRACT

This study evaluated the fatty acid and cholesterol content in long-horned Ankole cattle (*Bos taurus indicus*) from Ntungamo and Kiboga districts in Uganda to guide healthier food choices and aid in non-communicable disease prevention. Specifically, the study determined lipid content, fatty acid composition, and cholesterol levels in meat cuts. Meat samples were collected from eight cattle parts: brisket, rib, chuck, round (muscle meat), and liver, kidney, heart, and large intestines (organ meat). Total lipids were measured gravimetrically, fatty acids were analyzed by gas chromatography, cholesterol levels were assessed by high-performance liquid chromatography, and the nutritional quality implications of fatty acids and cholesterol were analyzed using nutritional indices. The liver and kidney exhibited the highest lipid contents (liver: 2.38–3.55%; kidney: 1.21–2.23%), whereas the brisket and round cuts had the lowest (brisket: 0.61–1.22%; round: 0.67–1.63%). Cholesterol was highest in the liver (155.95–187.21 mg/100 g) and kidney (113.40–129.75 mg/100 g), with lower levels in the brisket (24.24–31.84 mg/100 g) and rib (24.76–28.50 mg/100 g). Monounsaturated fatty acids (MUFA) were the most dominant, showing the highest proportions in the kidney (77.94%) and chuck (63.15%), with levels ranging from 30.64% to 77.94%. Saturated fatty acids (SFA) were also abundant, ranging from 32.25% to 55.00%, with palmitic and stearic acids being most prevalent, particularly in the large intestines. Polyunsaturated fatty acids (PUFA) were less common (2.09%–17.71%), being highest in the heart (17.71%) and lowest in the chuck (2.09%). Nutritional quality assessments revealed that the kidney, liver, and heart cuts had relatively favourable profiles, with the kidney showing a beneficial PUFA/SFA ratio of 0.36 and a high health-promoting (HH) ratio of 0.70. The liver's HH index (0.60) and the heart's Unsaturation Index (49.90) further supported their healthier fat profiles, despite the overall nutritional ratios being below recommended dietary guidelines. In contrast, rib, large intestine, and chuck cuts exhibited higher indices of atherogenicity and thrombogenicity, suggesting increased cardiovascular risk. Ntungamo meat cuts exhibited higher lipid and fatty acid levels, while Kiboga cuts had higher cholesterol content, particularly in brisket and kidney. Both districts were dominated by monounsaturated fatty acids (MUFA), followed by saturated (SFA) and polyunsaturated (PUFA) fatty acids, with no significant differences ($p > 0.05$). The PUFA/SFA ratios in both districts (0.04–0.78) were generally below the recommended value of ≥0.4 for optimal cardiovascular health, suggesting limited cardioprotective benefits despite favourable fatty acid balances in organs like the kidney, liver, and heart.

CHAPTER ONE

INTRODUCTION

1.0 Background

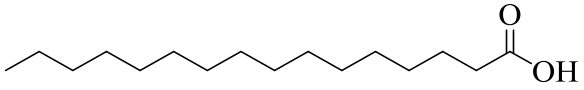
Ruminant meats, such as beef play a crucial role in human nutrition by offering high-quality proteins and fats (Chikwanha et al., 2018; Laskowski et al., 2018). These meats supply essential amino acids and are rich in B vitamins, dietary iron, and zinc. The main constituents of ruminant meats are water, protein, fat, minerals, and minimal carbohydrates. The fat content can vary based on the cut of meat and how it is prepared (Laskowski et al., 2018; Soren and Biswas, 2019)

Different people prefer different organs/ parts of the ruminants' meat, for instance, muscles, liver, kidney, offal and brain. These parts differ in the lipid composition (Biandolino et al., 2021; Abbas et al.,2009; Williams, 2007a) and hence the fatty acid and cholesterol content and this influences health outcomes significantly. Lipids can be defined as biomolecules whose solubility in water is less than that in non-polar solvents. Lipids perform a variety of functions in living system such as structural integrity, energy storage, digestion and communication (Yao et al., 2020). Triglycerides, the main form of stored fat in animal tissues, are composed of three fatty acids attached to a glycerol backbone and serve as the major energy reserve in both animals and humans.

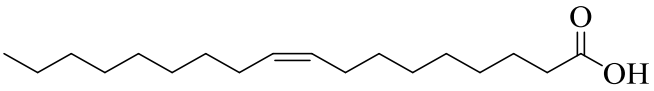
Fatty acids are long chain molecules that have a carboxyl and a methyl end. Fatty acids are long-chain hydrocarbons with a carboxyl group (-COOH) at one end and a methyl group ($-CH_3$) at the other (Ren and Zhang, 2024; Kyselová et al., 2022). They are essential components of lipids, including triglyceride, which play crucial roles in energy storage and cell structure. Fatty acids can be classified into

three main types based on their chemical structure: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA).

Saturated fatty acids have no double bonds between carbon atoms, making them solid at room temperature, and are commonly found in animal fats. Monounsaturated fatty acids contain one double bond, leading to a liquid state at room temperature and are prevalent in olive oil and avocados.



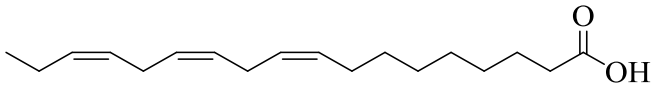
Saturated Fatty acid: Palmitic acid (C16:0)



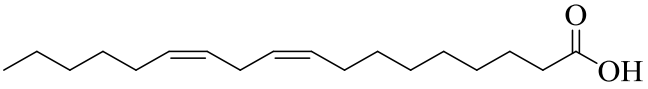
Monounsaturated Fatty acid: Oleic acid (C18:1, n-9)

Figure 1.1: Representative structures of Saturated Fatty acid and Monounsaturated Fatty acid

Polyunsaturated fatty acids, with two or more double bonds, remain liquid even at lower temperatures and include essential omega-3 and omega-6 fatty acids found in fish oils and plant seeds (Mensink, 2016; DiNicolantonio and O'Keefe, 2022; Chen and Liu, 2020d). The chemical structures of representative omega-3 and omega-6 fatty acids are shown in **Figure 1.1**, highlighting the positions of the double bonds along the hydrocarbon chain that define their classification.



Omega-3: α-linolenic acid



Omega-6: Linoleic acid

Figure 1.2: Chemical structures of representative omega-3 and omega-6 fatty acids.

Cholesterol is a 27-carbon sterol present in all animal tissues, serving as a major structural component of cellular membranes (Zhang et al., 2019). Elevated levels of cholesterol in the blood can lead to disorders such as hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia. Cholesterol can be present in the free form or esterified at the hydroxyl group with fatty acids of various chain length and saturation (Oliveira et al., 2012). The chemical structures of free and esterified cholesterol are shown in **Figure 1.2**, illustrating the hydroxyl group in free cholesterol and the ester linkage in cholesterol. Cholesterol is transported in the blood via lipoproteins and is categorized as low-density lipoprotein (LDL) and high-density lipoprotein (HDL). LDL cholesterol, often referred to as "bad" cholesterol, can accumulate in the arteries, leading to atherosclerosis and increased risk of heart disease (Wang et al., 2017), whereas HDL cholesterol helps remove excess cholesterol from the bloodstream, reducing cardiovascular risk. Das and Ingole (2023a) confirmed that maintaining higher levels of HDL and lower levels of LDL is beneficial for heart health.

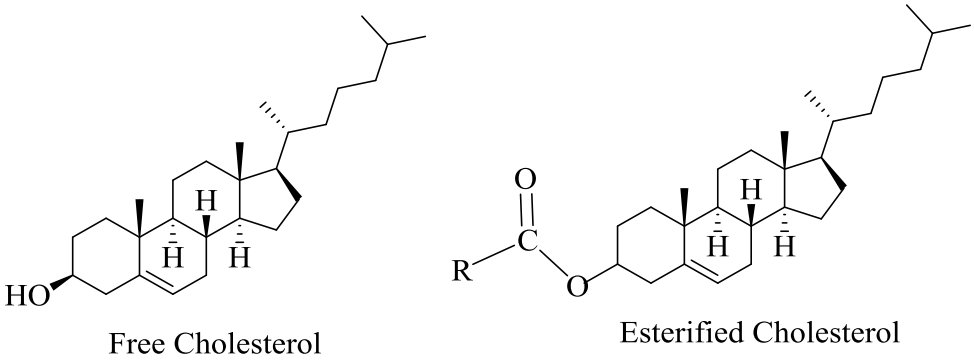


Figure 1.3: Chemical structures of free and esterified cholesterol.

The ratio of fatty acids to cholesterol is an important indicator of the nutritional quality of meat and its potential impact on human health. A higher fatty acid to cholesterol ratio generally indicates a healthier profile, associated with reduced risk of chronic diseases such as cardiovascular disease, obesity, diabetes, cancer, arthritis, and asthma, while a lower ratio may increase susceptibility (Mei et al., 2024)

Specifically, polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) ratios are often reported to assess healthfulness. For instance, a PUFA/SFA ratio of 0.4 or higher is considered favourable for cardiovascular health, whereas lower ratios are linked to higher disease risk (Krauss & Kris-Etherton, 2020). Similarly, a fatty acid to cholesterol ratio of 2:1 or above in meat cuts is generally associated with better lipid metabolism and reduced LDL cholesterol levels in consumers. Monitoring these ratios across different beef cuts, including muscle, liver, kidney, brisket, and heart, provides critical insight into the dietary implications of meat consumption. For instance, saturated fatty acids (SFA) like palmitic acid (C16:0) and stearic acid (C18:0) constitute about 12-15% of depot fat in beef. High consumption of SFAs is linked to increased LDL cholesterol levels, which can contribute to cardiovascular diseases (Feingold, 2000; Baum et al., 2012). In

contrast, monounsaturated fatty acids (MUFA), such as oleic acid (C18:1n9), make up approximately 40% of depot fat in beef. MUFAs are associated with reduced LDL cholesterol and a lower risk of coronary heart disease (CHD) (Virtanen et al., 2014). Polyunsaturated fatty acids (PUFA), including linoleic acid (C18:2n6) and alpha-linolenic acid (C18:3n3), are crucial for health. They contribute to reducing inflammation and lowering the risk of chronic diseases (Johnson, 2014; Dec et al., 2023). The ideal omega-6 to omega-3 ratio for optimal health is between 6:1 and 10:1. A favorable ratio can improve cardiovascular health and reduce the risk of conditions such as type-2 diabetes and cancer (Harris, 2018; Djuricic and Calder, 2021).

The fatty acid profile of ruminant meat varies among different breeds and rearing conditions. For example, pasture-fed cattle generally have a more favorable fatty acid profile compared to grain-fed cattle (Davis et al., 2022; Krusinski et al., 2022). Pasture-fed beef often has higher levels of omega-3 fatty acids and conjugated linoleic acid (CLA). CLA is present in about 0.5-1% of total fatty acids in pasture-fed beef and is associated with reduced cancer risk and improved metabolic health (Jiang, 2011; Hall et al., 2016).

A specific example is the long-horned Ankole cattle from Uganda, which are predominantly fed on natural pastures under traditional grazing systems. These cattle are raised under traditional pastoral systems, grazing on diverse natural pastures (Mulindwa et al., 2009). This results in meat with a higher proportion of unsaturated fatty acids and lower fat content compared to meat from breeds like Angus cattle, which are often fed concentrated grains in intensive farming

systems. Studies have shown that Ankole beef has a more balanced fatty acid profile with increased levels of beneficial unsaturated fats (Solti et al., 2000) .

Modern agricultural practices, including intensive feedlot systems and the use of growth hormones and antibiotics, significantly impact meat quality (Webb and Erasmus, 2013). Intensive systems often result in meat with higher fat content, particularly saturated fats, which are linked to increased health risks. For instance, feedlot cattle can have fat content up to 30% in some cuts, compared to 10-15% in pasture-fed cattle (Elswyk and McNeill, 2014). Conversely, pasture-based systems, which involve grazing on natural forage, generally produce leaner meat with a more favorable fatty acid profile (Daley et al., 2010a). These systems are associated with lower fat content and higher levels of beneficial unsaturated fats.

Dietary trends vary significantly across regions, influenced by cultural practices, economic conditions, and food availability. In many developing countries, meat consumption has increased from around 10 kg per capita in the 1960s to approximately 26 kg in 2000, with projections indicating a rise to 37 kg by 2030 (Font, 2023).This shift often leads to higher intake of saturated fats and cholesterol, contributing to increased rates of chronic diseases such as cardiovascular disease, diabetes, and obesity.

In developed countries, there is a growing awareness of the health risks associated with high meat consumption (Henchion et al., 2014; Stubbs et al., 2018; Kuck and Schnitkey, 2021). This has led to a trend towards consuming leaner meat cuts, plant-based alternatives, and diets emphasizing fruits, vegetables, and whole grains. For instance, the average per capita meat consumption in the United States was about 120 kg in 2020, with significant emphasis on reducing red meat intake

and increasing consumption of poultry and fish (Gu et al., 2023; Vranken et al., 2014). By contrast, in Uganda, meat consumption remains relatively low, averaging around 6 kg per capita per year, which is far below the Food and Agriculture Organization (FAO) and World Health Organization (WHO) recommended level of approximately 50 kg per year (FAO, 2021; Daily Monitor, 2023).

To mitigate the health risks associated with dietary fats, various nutritional guidelines have been established. Global health organizations, such as the World Health Organization (WHO) and the American Heart Association (AHA)**,** have long advocated for the reduction of saturated fat intake to less than 10% of total energy intake and increasing the consumption of unsaturated fatty acids to mitigate cardiovascular risks (Teicholz, 2023; Sacks et al., 2017). Specifically, dietary guidelines suggest that the ratio of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) should be no more than 0.4 to maximize health benefits. The omega-6 to omega-3 ratio should ideally be between 6:1 and 10:1 to achieve optimal health benefits (Krauss and Kris-Etherton, 2020). Maintaining these ratios and following recommended guidelines can significantly reduce the risk of chronic diseases. Incorporating lean cuts of meat, choosing unsaturated cooking oils, and including a variety of plant-based fats can help achieve a balanced and heart-healthy diet (Lichtenstein et al., 2021a).

Moreover, attention to specific indices like the hypocholesterolemic / hypercholesterolemic (HH) ratio and health-promoting index (HPI) is vital for ensuring that dietary fat profiles are conducive to lowering LDL cholesterol and promoting cardiovascular health (Bodnár et al., 2021; Chen and Liu, 2020).

Monitoring the unsaturation Index (UI) and the levels of essential fatty acids like EPA and DHA can further enhance dietary strategies aimed at reducing inflammation and improving overall health (Smolińska et al., 2024; Murariu et al., 2023a). Adhering to these comprehensive guidelines that emphasize reducing saturated fat and increasing unsaturated fats, will support broader public health goals by promoting more informed dietary choices and reducing the prevalence of diet-related chronic diseases (Willett et al., 2006).

Fatty acids and cholesterol play a crucial role in global health discussions due to their significant impact on cardiovascular diseases. Worldwide, there is a strong emphasis on promoting dietary patterns that include healthy fats while reducing harmful ones, reflecting a broad consensus on the need for improved dietary guidelines(Reyes et al., 2021; Kris-Etherton and Krauss, 2020; Liu et al., 2017).

In Uganda, the National Plan for Nutrition and Dietetics III (NPD III) has been central to addressing nutritional concerns related to fatty acids and cholesterol. NPD III aimed to enhance nutritional standards by promoting healthier dietary practices and improving the quality of local food systems (Fowler and Rauschendorfer, 2019; Munro, 2012). By focusing on better agricultural practices and incorporating nutritional education, NPD III aligned with global health recommendations, ensuring that Uganda's strategies effectively addressed both local and international health issues. Building on this foundation, the National Development Plan IV (NDP IV) further strengthens nutrition and health objectives by emphasizing the integration of dietary quality, food security, and non-communicable disease prevention into national development strategies.

NDP IV seeks to continue the progress of NPD III while expanding interventions to include improved monitoring of dietary intakes, promotion of nutrient-rich foods, and targeted public health campaigns aimed at reducing the prevalence of diet-related chronic diseases. Together, these plans reflect Uganda's evolving commitment to enhancing nutrition, promoting healthy diets, and addressing fatty acid and cholesterol-related health risks at both national and community levels.

1.2 Statement of the Problem

In Uganda, the middle class faces a growing health crisis characterized by non-communicable diseases such as cardiovascular ailments, obesity, diabetes, metabolic syndrome, cancer, arthritis, and asthma. These health issues are intricately linked to the consumption of animal fat from different parts of ruminants. Meat cuts sourced from muscles, liver, kidney, heart, and intestines contribute to elevated cholesterol levels, insulin resistance, inflammation, hormonal imbalances, and the development of atherosclerosis. Despite the clear correlation between these health challenges and the consumption of ruminant fat, there has been a notable lack of attention given to the specific composition of fatty acids and cholesterol within these tissues in Uganda. This oversight is particularly alarming given that the middle class, a vital contributor to Uganda's GDP, bears a significant burden of these chronic illnesses. The continued prevalence of these health issues not only jeopardizes the productivity and well-being of the middle class but also places considerable strain on healthcare systems and families. This study was aimed at analyzing the fatty acid and cholesterol profiles of various body parts of long-horned Ankole cattle. By providing consumers with thorough information about the nutritional makeup of these meat cuts, it will empower them to make informed dietary choices, thereby enabling them to safeguard themselves against the onset of chronic diseases.

1.3 Objectives of the Study

1.3.1 General Objective

The general objective of the study was to assess the fatty acid composition and cholesterol content in eight meat cuts from long-horned Ankole cattle.

1.3.2 Specific Objectives

The specific objectives of the study were:

(i) To determine the fatty acid composition in selected meat cuts (brisket, round, chuck, rib, liver, kidney, heart, large intestines) from long-horned Ankole cattle in Ntungamo and Kiboga districts of Uganda.

(ii) To determine the cholesterol content in the same meat samples from long-horned Ankole cattle in Ntungamo and Kiboga districts of Uganda.

(iii) To determine the nutritional quality implications of the fatty acid profiles and cholesterol levels based on established dietary guidelines.

1.4 Scope of Study

The study focused on evaluating the fatty acids and cholesterol content in various muscle and organ meat cuts—namely brisket, chuck, round, rib, liver, heart, kidney, and intestines—from long-horned Ankole cattle purchased from main abattoirs in two sub-counties within each of Ntungamo and Kiboga districts in Uganda. In Kiboga district, samples were collected from Bukomero and Lwamata sub-counties, while in Ntungamo district, they were collected from Ntungamo and Rubaare sub-counties. The study lasted from February 2023 to April 2024.

1.5 Significance of the Study

Beef meat is a significant source of essential nutrients, including vitamins, proteins, carbohydrates, and minerals like zinc, iron, selenium, and phosphorus.

It also contains cholesterol and a variety of fatty acids (Ruxton and Gordon, 2024; Ahmad et al., 2018; Boateng et al., 2020).

The findings of this study will help consumers to compare quality of different meat cuts and make choices based on lipid contents in them. This study will also aid government and policy makers to develop safety regulations and dietary guidelines concerning consumption of meat for each individual per day and also data from this study muscle and organ meat of long- horned Ankole cattle.

This study will also help healthy institutions such as hospitals, clinics and organizations like WHO to make reports about health risks associated with consumption of beef and thus inform the public about the effect of fatty acid and cholesterol content on human health. Fatty acid and cholesterol content has been implicated for causing chronic diseases such cardiovascular diseases, diabetes, arthritis, cancer (Zeng et al., 2023).

1.6 Justification of the Study

Fatty acids and cholesterol in foods have become very significant to consumers as they are becoming more aware of their relationships with the incidence of diseases, such as obesity, diabetes, cancer, arthritis, asthma, and cardiovascular and coronary heart disease. The United States Department of Health recommends a reduction in the saturated fatty acids intake and an increase in polyunsaturated fatty acids intake (Kris-Etherton et al., 2007; Mozaffarian et al., 2010). Meat contains significant levels of fatty acids and cholesterol and thus the need for profiling fatty acid composition and cholesterol content. This study profiled the fatty acid and cholesterol content and helped enable consumers to be aware of quality of meat of various body parts and select the meat cuts that are healthier

for human consumption. The results could guide consumers on choices of foods especially as CVD is already a leading cause of mortality in the developing world (World Health Organisation, 2003). According to WHO (2017), risk of premature death from non-communicable diseases (NCDs) in Uganda stands at 22%, with 35% of the total deaths resulting from NCDs. Furthermore, the probability of dying between ages 30 and 70 years from the 4 main NCDs which include cardiovascular diseases, cancers, diabetes and chronic respiratory diseases is 21% (Mumtaz et al., 2020; Riley and Cowan, 2014; Viegi et al., 2020).

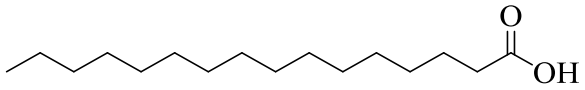
The Uganda government is committed to promoting healthy lifestyles that contribute to prevention or delay of occurrence of non-communicable diseases and therefore producing a healthy and productive population that will effectively contribute to socio-economic growth (NDPII). With information available about the foods that the population consumes like meats, the communities will take greater control of their health by promoting healthy practices and lifestyles. This shift will be anchored on preventive over curative health service delivery approaches. The preventive health system is considerably cheaper to run and hence by far more sustainable (Uganda Vision 2040, 2007).

CHAPTER TWO

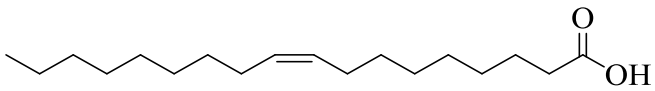
LITERATURE REVIEW

2.1 Lipids

Lipids encompass a wide variety of compounds, including fatty acids and their derivatives, as well as related biosynthetic and functional substances (Vyssotski et al., 2017; Fahy et al., 2005; Asokapandian et al., 2021). This broad category of natural molecules features fatty acids, waxes, sterols, fat-soluble vitamins (like vitamins A, B, C, and D3), glycerides, terpenes, prostaglandins, and cholesterol (Beare-Rogers et al., 2001). The structural configurations of these biomolecules are presented in **Figure 2.1**, highlighting the distinct chemical features of fatty acids, glycerides, sterols, and cholesterol.



Saturated Fatty acid: Palmitic acid (C16:0)



Monounsaturated Fatty acid: Oleic acid (C18:1, n-9)

Figure 2.1 Structural configurations of saturated and monounsaturated fatty acids

Katoch (2011) explains that lipids are primarily non-polar organic molecules, making them mostly insoluble in water but soluble in non-polar organic solvents such as hexane, ether, chloroform, and benzene. Commonly referred to as fats and oils, lipids differ in their physical state; oils are liquid at room temperature, whereas fats are solid (Pike and O'Keefe, 2017; Hashimoto et al., 2018).

Lipids serve several critical functions in the body, including contributing to cell membrane structure, protecting nerve cells, assisting in vitamin absorption, and

supporting hormone production (such as estrogen, testosterone, and cortisol). The molecular structures of these steroid hormones are shown in **Figure 2.2**, demonstrating the characteristic four-ring steroid framework common to lipid-derived hormones. Additionally, they play a role in energy storage and act as fundamental components of cellular structure (Simons and Sampaio, 2011; Ahmed and Ahmed, 2019; Muro et al., 2014).

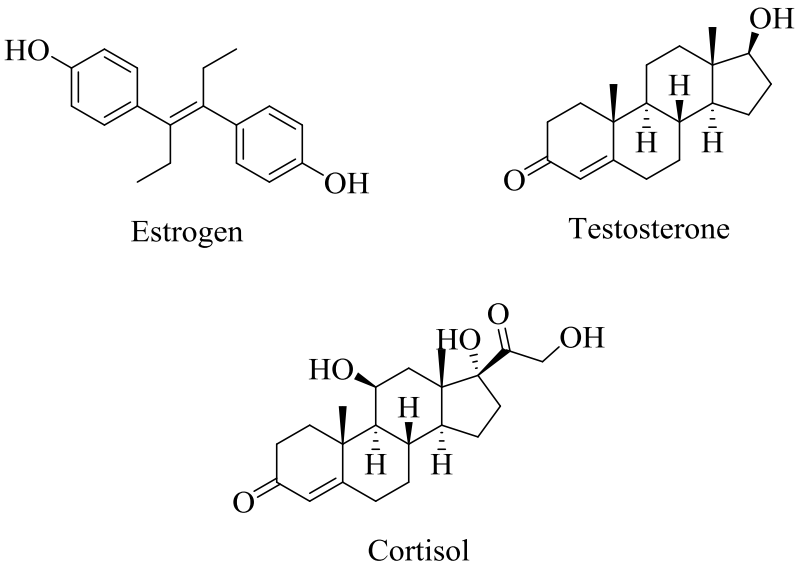


Figure 2.2: Molecular structures of these steroid hormones

2.1.1 Classification of lipids

Fahy et al. (2011) categorize lipids into eight distinct groups: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids (which arise from the condensation of ketoacyl subunits), polyketides, sterol lipids, and prenol lipids (which result from the condensation of isoprene units). In contrast, Asokapandian et al. (2021) classify lipids into two primary categories: simple and complex lipids. Simple lipids, such as triglycerides, sterol esters, and waxy esters, yield at most two different types of entities upon hydrolysis. In comparison,

complex lipids, like glycolipids and phospholipids, produce three or more distinct products when hydrolyzed.

Additionally, Hashimoto et al. (2018) distinguish lipids based on their ability to undergo saponification. Saponifiable lipids are divided into simple types (e.g., waxes and triglycerides) and complex types (e.g., phospholipids and sphingolipids), while non-saponifiable lipids include substances such as steroids and prostaglandins.

2.2 Fatty acids

A fatty acid is defined as a carboxylic acid with an aliphatic chain that may be either saturated or unsaturated (Jiang et al., 2013). Fatty acids from plant, animal, and microbial sources typically feature even numbers of carbon atoms arranged in straight chains, with a carboxyl group at one end and cis double bonds located in specific positions relative to this group (Dhull and Punia, 2020; Kannan et al., 2021).

In animal tissues, common fatty acids range in chain length from 14 to 22 carbons, though they can extend from 2 to 36 carbons or more in some cases. Conversely, higher plants usually have a more restricted range of chain lengths (Selvaraj, 2017). Fatty acids are essential for health and can be obtained from both internal synthesis and dietary sources (Kremmyda et al., 2011; Bessa et al., 2015; Nagy and Tiuca, 2017). The biosynthesis of fatty acids occurs in all organisms, with mammals primarily synthesizing them in adipose tissue, mammary glands, and the liver through the action of a multi-enzyme complex known as fatty acid synthetase (Czumaj and Śledziński, 2020).

2.2.1 Types of fatty acids

Fatty acids are classified in many ways by length, by saturation versus unsaturation, by even versus odd carbon content and by linear versus branched, the essential versus non-essential in both human and animals. Other categories include oxygenated and cyclic fatty acids. (Lobb and Chow, 2007; Shah et al., 2022)

2.2.1.1 Saturated fatty acids

Saturated fatty acids contain only carbon - carbon single bond in the hydrocarbon chain (Astrup et al., 2020; Calder, 2015). Examples are illustrated below.

Table 2.1: Examples of saturated fatty acids

Systematic name	Trivial name	Formula	Abbreviation
Butanoic	Butyric	$CH_3(CH_2)_2COOH$	4:0
Pentanoic	Valerie	$CH_3(CH_2)_3COOH$	5:0
Hexanoic	Caproic	$CH_3(CH_2)_4COOH$	6:0
Heptanoic	Enanthic	$CH_3(CH_2)_5COOH$	7:0
Octanoic	Caprylic	$CH_3(CH_2)_6COOH$	8:0
Nananoic	Pelargonic	$CH_3(CH_2)_7COOH$	9:0
Decanoic	Capric	$CH_3(CH_2)_8COOH$	10:0
Undecanoic	-	$CH_3(CH_2)_9COOH$	11:0
Dodecanoic	Lauric	$CH_3(CH_2)_{10}COOH$	12:0
Tridecanoic	-	$CH_3(CH_2)_{11}COOH$	13:0
Tetradecanoic	Myristic	$CH_3(CH_2)_{12}COOH$	14:0
Pentadecanoic	-	$CH_3(CH_2)_{13}COOH$	15:0
Hexadecanpic	Palmitic	$CH_3(CH_2)_{14}COOH$	16:0
Heptadecanoic	Margarin	$CH_3(CH_2)_{15}COOH$	17:0
Octadeconoic	Stearic	$CH_3(CH_2)_{16}COOH$	18:0
Nonadecanoic	-	$CH_3(CH_2)_{17}COOH$	19:0
Eicosanoic	Arachidic	$CH_3(CH_2)_{18}COOH$	20:0
Docosanoic	Behenic	$CH_3(CH_2)_{20}COOH$	22:0
Tetracosanoic	Lignoceric	$CH_3(CH_2)_{22}COOH$	24:0
Hexaeosanoic	Cerotic	$CH_3(CH_2)_{24}COOH$	26:0

2.2.1.2 Unsaturated fatty acids

Unsaturated fatty acids contain one or more carbon - carbon double bonds (Takashima et al., 2020; Moretti and Corino, 2008). The double bonds can either be cis or trans isomers.

2.2.1.3 Monoenoic (Monounsaturated) fatty acids

These fatty acids are classified as unsaturated with a single double (Gunstone, 2018; Moretti and Corino, 2008; Vinet and Zhedanov, 2011). The most prevalent monoenoic fatty acid in tissues is cis-9-octadecenoic acid, commonly referred to as oleic acid. Additional examples of monoenoic fatty acids are listed in the accompanying table (O'Connor et al., 2019).

Table 2.2: Examples of monounsaturated fatty acids

Common Name	Lipid Number	Structural Formula
Myristoleic Acid	C14:1	$CH_3(CH_2)_3CH = CH(CH_2)_7COOH$
Palmitoleic Acid	C16:1	$CH_3(CH_2)_5CH = CH(CH_2)_7COOH$
Sapienic Acid	C16:1	$CH_3(CH_2)_4CH = CH(CH_2)_6COOH$
Oleic Acid	C18:1	$CH_3(CH_2)_7CH = CH(CH_2)_7COOH$
Elaidic Acid	C18:1	$CH_3(CH_2)_7CH = CH(CH_2)_7COOH$
Vaccenic Acid	C18:1	$CH_3(CH_2)_7CH = CH(CH_2)_9COOH$
Gadoleic Acid	C20:1	$CH_3(CH_2)_9CH = CH(CH_2)_8COOH$
Eicosenoic Acid	C20:1	$CH_3(CH_2)_8CH = CH(CH_2)_9COOH$
Erucic Acid	C22:1	$CH_3(CH_2)_{11}CH = CH(CH_2)_8COOH$
Nervonic Acid	C24:1	$CH_3(CH_2)_{13}CH = CH(CH_2)_8COOH$

2.2.1.4 Polyunsaturated fatty acids

These contain two or more double bonds between carbon atoms (Michalak et al., 2016). Here are two main categories of polyunsaturated fatty acids (PUFAs) that are advantageous for human health: omega-3 and omega-6. These fatty acids are predominantly found in fish oils and are also present in smaller quantities in certain meats (Moretti and Corino, 2008; Calder, 2010; Scialabba, 2021).

Table 2.3: Examples of polyunsaturated fatty acids

Type	Common Name	Lipid Number	Structural Formula
Omega-3	Alpha-Linolenic Acid (ALA)	C18:3	$CH_3(CH_2CH=CH)_3(CH_2)_7COOH$
Omega-3	Eicosapentaenoic Acid (EPA)	C20:5	$CH_3(CH_2CH=CH)_5(CH_2)_3COOH$
Omega-3	Docosahexaenoic Acid (DHA)	C22:6	$CH_3(CH_2CH=CH)_6(CH_2)_2COOH$
Omega-6	Linoleic Acid (LA)	C18:2	$CH_3(CH_2)_4(CH_2CH=CH)_2(CH_2)_6COOH$
Omega-6	Gamma-Linolenic Acid (GLA)	C18:3	$CH_3(CH_2)_4(CH_2CH=CH)_2(CH_2)_6COOH$

2.2.1.5 Essential fatty acids

Essential fatty acids (EFAs) are polyunsaturated fatty acids (PUFAs) that must be obtained through the diet because the body cannot synthesize them, despite their crucial role in maintaining health (Kaur et al., 2014; Das, 2006). These fatty acids are termed "essential" due to their involvement in regulating various physiological processes, including blood pressure, blood viscosity, vasoconstriction, and immune and inflammatory responses.

EFAs are classified into two main categories: omega-3 and omega-6. Omega-6 fatty acids include linoleic acid, gamma-linolenic acid, dihomogamma-linolenic acid, and arachidonic acid (C20:4). Conversely, omega-3 fatty acids encompass alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid (Maroon et al., 2006; Kaur et al., 2014; Simopoulos, 2009; Das, 2006; Tallima and El Ridi, 2018).

2.3 Factors affecting fatty acid composition.

Various studies (Bednárová et al., 2013., Alabiso et al., 2020; Samková et al., 2012; Samková et al., 2012), explain the following factors which affect fatty acid composition in beef: muscle type, animal category (type of breed), sex, age of the animal, diet, slaughter season, genetic factors (genetic variability), live weight, degree of fattening.

2.3.1 Muscle type

Muscle fat content and fatty acid profiles can differ significantly across various types of tissues, including intramuscular, intermuscular, abdominal, and subcutaneous fat. The muscle fiber type is a key factor in lipid metabolism and meat quality, influencing attributes such as marbling, flavor, and nutritional value (Zhao et al., 2019; Smet et al., 2004; Mansbridge and Blake, 1997; Sexten et al., 2012).

2.3.2 Sex

Sex is a significant factor influencing variations in beef muscle and fat distribution within the carcass. It affects several meat quality attributes, including muscle chemical composition, fat deposition, and levels of protein and ash content. Zhang et al. (2010) reported that these differences arise because sex hormones, such as testosterone and estrogen, drive divergent growth patterns and metabolic processes.

2.3.3 Animal category (Breed type)

Breed type significantly influences fatty acid composition due to variations in fat deposition patterns (Demirel et al., 2006). Dairy breeds, for instance, often accumulate a greater proportion of their total fat as intramuscular fat, even though

they generally have leaner carcasses compared to traditional beef breeds (Alabiso et al., 2020b; Choi et al., 2000).

2.3.4 Diet

The final composition of beef is significantly influenced by the diet, particularly concerning lipid components that affect consumer health. Various feeding systems can modify the levels of functional lipids, including saturated and trans fatty acids, in beef (Ponnampalam et al., 2002; Nogoy et al., 2022; Ogura et al., 2010; Zhang et al., 2007). This variation results from how different feeds impact the animal's lipid metabolism and fat deposition. For example, grain-based diets often lead to higher levels of saturated and trans fats in the meat, whereas forage-based diets can increase the concentration of beneficial omega-3 fatty acids and other health-promoting lipids (Whetsell and Rayburn, 2022; Decsi and Kennedy, 2011)

2.3.5 Slaughter age

Kumar (2018) reported that slaughter age is a critical factor in determining meat quality, noting that meat from younger animals is generally more valued than that from older ones. Research shows that slaughter age influences various meat quality traits, including the fatty acid profile. This impact is primarily due to the correlation between slaughter age and muscle fiber size, as well as overall muscle volume (Al-Suwaiegh and Al-Shathri, 2014; Li et al., 2020).

2.3.6 Degree of fattening

De Smet et al.(2004b) observed that the level of fatness has a significant effect on the fatty acid composition of meat. As fat content increases, the concentrations of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) tend to

rise more rapidly than those of polyunsaturated fatty acids (PUFA). Consequently, the proportion of PUFA relative to SFA decreases, which in turn reduces the polyunsaturated-to-saturated fatty acid (P/S) ratio (Carrilho et al., 2009; Ayuso et al., 2020; Smet et al., 2004).

2.3.7 Genetic variability

Genetic variability includes differences observed between species, breeds, or lines, as well as variations arising from crossbreeding and among individuals within a breed (Smet et al., 2004).The deposition of fat is significantly influenced by genetic factors, owing to the phenotypic and genetic correlations between overall carcass fatness and intramuscular fat content (Saxena et al., 2017; Feitosa et al., 2017).

2.4 Effect of fatty acids on human health

The connection between dietary fat intake and health outcomes, including cardiovascular disease and cancer mortality, has been extensively studied. Wang and Hu (2017) reported that saturated fatty acids (SFA), which are predominantly found in animal fats, are consistently linked to adverse health effects, particularly concerning cardiovascular disease.

High consumption of SFA has been found to elevate levels of low-density lipoprotein cholesterol, a known risk factor for coronary heart disease (Perna and Hewlings, 2023; Siri-Tarino et al., 2010; Sanders, 2013) . Conversely, Coniglio et al. (2023) indicated that polyunsaturated fatty acids (PUFA), commonly present in vegetable oils, offer beneficial effects by serving as biological mediators in various physiological processes. However, not all fats impact health in the same way; while SFA is associated with negative health outcomes, certain

PUFAs have been observed to provide protective benefits. Consequently, dietary guidelines recommend reducing saturated fat intake, particularly from red and processed meats, to lower the risk of chronic diseases.

The increase in dietary fat consumption over the past century, especially from animal sources, parallels the rise in cardiovascular diseases such as coronary heart disease and arteriosclerosis, as observed by Lee et al. (2022). This trend underscores the critical role of dietary fat composition in health outcomes. To mitigate the risk of cardiovascular diseases, which are significant causes of mortality in Western societies, it is essential to reduce intake of saturated fats, especially from animal sources (Tarino et al., 2015). The U.S. dietary guidelines strongly recommend adopting dietary patterns low in red and processed meats to alleviate the burden of chronic diseases linked to excessive saturated fat consumption (Van Horn et al., 2016; Lichtenstein et al., 2021b; Tapsell et al., 2016).

2.5 Studies on Fatty Acid Profiles in Beef

Significant progress has been made in understanding the fatty acid profile of beef, influenced by factors such as age at slaughter, dietary composition, live weight, breed type, degree of fattening, sex, and genetic predispositions (Muchenje et al., 2009a; Nfor et al., 2014; Daley et al., 2010b; Fiorentini et al., 2015; Gill et al., 2008; Hwang and Joo, 2017).

Alfaia et al. (2006) conducted a study to investigate the impact of breed on fatty acid composition, focusing on crossbred and purebred bullocks reared in a semi-extensive system in Alentejana, Portugal. Their findings indicated significant differences in fat characteristics between the two breeds. In a review, De Smet et

al. (2004b) examined the effects of fatness and genetic factors on fatty acid composition, reporting that both factors significantly influence fatty acid profiles. Additionally, Vera et al. (2009a) explored fat, cholesterol, and fatty acid contents in eight beef cuts from un-supplemented, suckling, 7-8 month-old male and female Hereford, Aberdeen Angus, and crossbred calves reared on permanent pastures in Chile. Their study revealed that all meat cuts had lower values than typically reported, with significant differences among them.

Noviandi et al. (2012) found that fatty acid composition in the adipose tissue of beef steers varied significantly between those finished on pasture and those finished in feedlots, with pasture-finished steers showing higher concentrations of stearic acid, trans-fatty acids, and polyunsaturated fatty acids (PUFAs).

Despite these advances, there remains a gap in research on the fatty acid composition of various beef cuts, including liver, heart, kidney, brisket, round, lungs, and chuck. Therefore, this research aims to address this gap by investigating the fatty acid profiles across different tissues in cattle meat.

2.6 Cholesterol

Cholesterol (**Figure 2.1**) is a type of sterol, which is classified as a steroid lipid, and is present in the tissues and blood plasma of vertebrates (Kumar et al., 2018; Ramachandra Rao and Fliesler, 2021). Chemically isolated cholesterol appears as a yellowish crystalline solid. Its structure is distinguished by a fused four-ring hydrocarbon framework known as the steroid nucleus, and it includes a hydrocarbon tail consisting of an eight-carbon (Alamgir, 2018; Craig et al., 2018). The molecular formula of cholesterol is $C_{17}H_{46}O$, and its molecular structure is illustrated below:

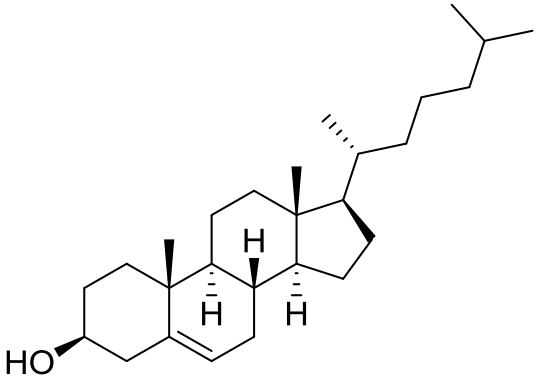


Figure 2.1: The structure of cholesterol (Lin et al., 2015)

Cholesterol is a vital component of cell membranes, contributing to their stability. It has been reported that cholesterol serves as a precursor for the synthesis of vitamin D and various hormones, including cortisol, cortisone, and aldosterone from the adrenal glands, as well as sex hormones like progesterone, estrogen, and (Rezen et al., 2011; Craig and Malik, 2019; Orth and Bellosta, 2012). Additionally, it plays a crucial role in brain synapses and supports the immune system.

Blood cholesterol primarily comes from two sources: dietary cholesterol and endogenous cholesterol. Alphonse and Jones (2016) indicated that dietary cholesterol, found in animal tissues, is abundant in foods such as egg yolks, shrimp, beef, pork, poultry, butter, and cheese. Endogenous cholesterol is synthesized within the body, mainly in the liver, intestines, and reproductive organs (Cortes et al., 2014; Lecerf and De Lorgeril, 2011)

Kapourchali et al. (2016) confirmed that while plants contain only small amounts of cholesterol, they produce larger quantities of phytosterols. These phytosterols are chemically similar to cholesterol and can compete with it for absorption in the digestive tract (Li et al., 2022).

2.6.1 Effect of cholesterol on human health

An isolated cholesterol molecule is insoluble in water, and as a result, it dissolves in blood only at very low concentrations (Huff and Jialal, 2018; Vourakis et al., 2021). Over time, high levels of cholesterol can lead to plaque buildup inside blood vessels, a condition known as atherosclerosis. This buildup significantly increases the risk of various cardiovascular conditions, such as coronary artery disease (CAD), which can lead to heart attacks, angina, and high blood pressure (Poznyak et al., 2022; Shao et al., 2020; Poznyak et al., 2022).

It has been reported that foods high in cholesterol are often also high in saturated fatty acids, which can further increase the risk of cardiovascular disease due to their impact on lipid levels (Shao et al., 2020b; Soliman, 2018). High levels of saturated fatty acids are undesirable because they can elevate low-density lipoprotein (LDL) cholesterol, affecting the LDL to high-density lipoprotein (HDL) ratio—a marker for cardiovascular disease (Perna and Hewlings, 2023)

Elevated LDL levels transport cholesterol to peripheral tissues, where it can deposit in the arterial lumen, leading to plaque formation and narrowing of blood vessels, which are characteristic of atherosclerosis. Conversely, HDL is responsible for reverse cholesterol transport, carrying cholesterol from peripheral tissues back to the liver for bile synthesis and cholesterol disposal (Wang et al., 2017b; Ikonen, 2006).

2.6.2 Studies on cholesterol in various body parts of cattle

Numerous researchers have conducted various studies on cholesterol in beef, focusing on specific muscles and other factors affecting lipid composition in meat (Hwang and Joo, 2017; Violeta et al., 2020; Cifuni et al., 2004). Bragagnolo and

Rodriguez-Amaya (2003b) performed simultaneous analyses of total lipids, cholesterol, and fatty acids in raw and grilled beef longissimus dorsi, trimmed of external fat, using three cattle breeds from Brazil: Nelore, Canchim, and Beefalo. Their study confirmed that cholesterol concentrations in the longissimus dorsi were higher in cooked meat (67-70 mg/100 g) compared to raw samples (40-43 mg/100 g).

In another study, Abonyi et al. (2020) observed that cholesterol levels varied significantly across different body parts of cattle, goats, and pigs from the Nsukka Municipal Abattoir in Nigeria. They found out that while cholesterol levels in the liver, kidney, and intestine of goats were significantly higher ($p < 0.05$) compared to pigs and cattle, cholesterol concentrations were notably higher ($p < 0.05$) in the muscle and skin of pigs compared to goats and cattle. Overall, lipid profiles were similar between goats and cattle but differed for pigs.

Additionally, Ejike and Emmanuel (2009) investigated cholesterol concentrations in various parts of bovine meat sold in Nsukka, Nigeria. They observed that among ten meat cuts (rib muscle, lungs, large intestine, small intestine, collar, liver, kidney, and heart), the liver had the highest cholesterol concentration (6.5 ± 0.15 mg/g), while the large intestine had the lowest (1.0 ± 0.01 mg/g).

Despite these efforts, the research scope has been limited, primarily focusing on specific muscles and factors. Further studies are needed to explore cholesterol levels across a broader range of body parts, especially those commonly consumed. Such comprehensive research could provide deeper insights into the

nutritional composition of beef and its implications for human health, facilitating more informed dietary recommendations.

2.7 Nutritional quality implications of the fatty acid profiles and cholesterol based on established dietary guidelines

Dietary fats, primarily consisting of fatty acids and cholesterol, have been extensively studied for their dual roles in disease prevention and treatment (Chen and Liu, 2020a). Fatty acids occur naturally as mixtures of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Orsavova et al., 2015; Chen and Liu, 2020b). Cholesterol, categorized into high-density lipoprotein (HDL) and low-density lipoprotein (LDL), plays different roles in health, with HDL generally being protective and LDL being associated with increased disease risk (Patel and Kashfi, 2022; Yadav and Prasad, 2021; Eren, 2012).

Recent studies have reported various indices used to assess the nutritional and health implications associated with dietary fats beyond conventional measures such as total SFA, MUFA, PUFA, and their ratios (Murariu et al., 2023; Samara et al., 2024; Dal Bosco et al., 2022a; Bušová et al., 2023; Ferrara et al., 2024; Łuczyńska et al., 2024). These indices provide a detailed evaluation of fatty acid profiles and include several key metrics: the PUFA/SFA ratio, index of atherogenicity (IA), index of thrombogenicity (IT), hypocholesterolemic / hypercholesterolemic (HH) ratio, health-promoting index (HPI), and the unsaturation index (UI).

The PUFA/SFA Ratio, which compares polyunsaturated fatty acids to saturated fatty acids, is crucial for assessing dietary quality. Recommendations indicate that

a higher PUFA/SFA ratio is beneficial for health, with an optimal ratio of at least 0.4 suggested to lower LDL cholesterol levels and improve cardiovascular outcomes (Dassanayake et al., 2024; Wu et al., 2020). The index of atherogenicity (IA) evaluates the risk of atherosclerosis by comparing SFAs to unsaturated fatty acids, with lower IA values indicating a reduced risk of plaque buildup in the arteries. Dietary guidelines recommend an IA value below 1.0 to support cardiovascular health (Fernandes and Gandin, 2015).

Similarly, the index of thrombogenicity (IT) assesses the risk of blood clot formation by comparing pro-thrombogenic SFAs with anti-thrombogenic unsaturated fats. It is recommended to maintain an IT value below 0.5 to minimize the risk of thrombosis and related cardiovascular conditions. The hypocholesterolemic/hypercholesterolemic (HH) Ratio compares fats that lower cholesterol with those that raise it, with a higher HH ratio being favorable. Guidelines suggest a ratio of at least 2.0 for effective cholesterol management and cardiovascular health.

The health-promoting index (HPI) measures the proportion of unsaturated fatty acids relative to atherogenic SFAs, with higher HPI values being preferred. Recommendations advocate for an HPI value above 1.0 to indicate a diet supportive of cardiovascular (Chen and Liu, 2020b) (Ratusz et al., 2018). The unsaturation Index (UI), which reflects the degree of unsaturation in fatty acids, also provides valuable insights. Higher UI values, ideally above 1.5, are recommended for optimal health due to their indication of a favorable balance of unsaturated fatty acids (Weijers, 2015; Dal Bosco et al., 2022b)

2.8 Lipid extraction methods

To extract lipids for analysis, tissues are processed using either chemical or mechanical methods (Mubarak et al., 2015a). Chemical methods include the Folch and Bligh and Dyer techniques, Soxhlet extraction, supercritical fluid extraction, and accelerated solvent extraction, each using different solvents or conditions to isolate lipids. Mechanical methods involve oil expellers, microwave-assisted extraction, and ultrasonic-assisted extraction, which physically or thermally disrupt the tissue to release lipids. The choice of method depends on the sample type and desired purity of the lipids (Gorgich et al., 2020 ; Mubarak et al., 2015).

Other methods of lipid extraction include: Matyash method of lipid extraction (Mubarak et al., 2015b). Enzyme assisted extraction, osmotic pressure method (Mubarak et al., 2015b). Blood serum lipids can be extracted by the following methods; detergent extraction, silica extraction, extraction with hexane and hexane – isopropanol (Shahidi and Wanasundara, 2002; Ferraz et al., 2004).

2.9 Analytical method for fatty acid analysis

Commonly employed analytical techniques for studying fatty acids and lipidomes include gas chromatography (GC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) (Jurowski et al., 2017; Jurowski et al., 2017; Correia et al., 2020). Each of these methods offers distinct advantages and limitations, and they are often used in combination to enhance analytical outcomes. For instance, GC, while effective, requires additional sample preparation to enhance the volatility of the compounds being analyzed. High-performance liquid chromatography (HPLC) is less frequently used for fatty acid quantification due

to the lack of chromophores or fluorescent groups in these compounds. Though HPLC can achieve satisfactory separation and precision compared to standard GC, it generally exhibits lower sensitivity (Amores and Virto, 2019; Zhang et al., 2015).

2.10 Method of preparing fatty and methyl esters, fame (derivatization)

Derivation involves converting fatty acids into fatty acid methyl esters (FAMEs) to facilitate their analysis. Fatty acids in their free form present analytical challenges due to their high polarity, which can lead to issues such as hydrogen bonding and poor absorption. Researchers have reported that analyzing underivatized fatty acids with polar columns often results in suboptimal peak shapes and extended retention times (Trivedi et al., 2022; Ng, 2002). To enhance peak shape and separation, derivatization is commonly employed, which contributes to more consistent and reproducible results.

Acidic catalysts, including sulphuric acid, acetyl chloride, and hydrochloric acid, are frequently used for this process (Fisk et al., 2014) Some studies have also reported the use of boron trifluoride for derivatization (David and Sandra, 2002 ; Hewavitharana et al., 2020). Additionally, base catalysts such as metal hydroxides and alkoxides like sodium hydroxide, potassium hydroxide, or sodium methoxide in methanol are employed to form methyl esters (Pradhan et al., 2014)

2.11 Cholesterol extraction methods

For many years, dietary cholesterol has been associated with elevated blood cholesterol levels, which can increase the risk of cardiovascular disease (CVD) (Soliman, 2018). This association has particularly raised concerns about meat

products, especially red meat. The focus on dietary cholesterol's impact on heart disease, coupled with mandatory nutritional labeling requirements in the United States, has underscored the need for precise and efficient methods to determine cholesterol levels (Warmate and Onarinde, 2023; Dinh et al., 2011; Viegas et al., 2012).

Cholesterol extraction is typically achieved through a saponification process, as described in various studies. During this process, the meat sample is treated with potassium hydroxide dissolved in methanol or ethanol. The mixture is then incubated for a specified period to ensure complete saponification of the lipids. Following saponification, cholesterol is extracted using n-hexane or another suitable organic solvent. This step is critical for isolating cholesterol from the saponified material, allowing for accurate analysis , (AOAC official method 976.26, AOAC official method 994.10; Dinh et al., 2008).

2.12 Analytical methods for cholesterol analysis

In a review article by (Li et al., 2010), analytical methods of cholesterol quantification include; Modified Abell- Kendall method, Fluorometric enzymatic essay, Electrospray ionization tandem mass spectrometry, Matrix assisted laser description ionization time of flight, Matrix assisted laser desorption/ionization-ion-mobility mass spectrometry, Description electrospray ionization mass spectrometry, Direct analysis real time mass spectrometry, Matrix assisted laser desorption/ionization mass spectrometry imaging, Description electron spray ionization mass spectrometry imaging.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Meat samples from long-horned Ankole cattle were purposively collected from Ntungamo Sub-County (Lat: -0.87, Long: 30.28) and Rubaare Sub-County (Lat: -0.82, Long: 30.22) in Ntungamo District, and Bukomero Sub-County (Lat: 0.51, Long: 31.25) and Lwamata Sub-County (Lat: 0.50, Long: 31.31) in Kiboga District, located in western and central Uganda respectively.

The Ankole cattle breed, common in Uganda, was studied in Ntungamo District and Kiboga District due to their significant cattle farming activities and high beef consumption rates. Ntungamo is noted for its extensive cattle farming and high beef consumption, while Kiboga is recognized for its growing beef industry and agricultural development. Within these districts, Ntungamo sub-county, Rubaare sub-county, Bukomero sub-county, and Lwamata sub-county were specifically chosen for their high levels of development and substantial beef consumption, providing a comprehensive overview of meat quality and farming practices in these representative areas.

Location map, District Information for sub – counties

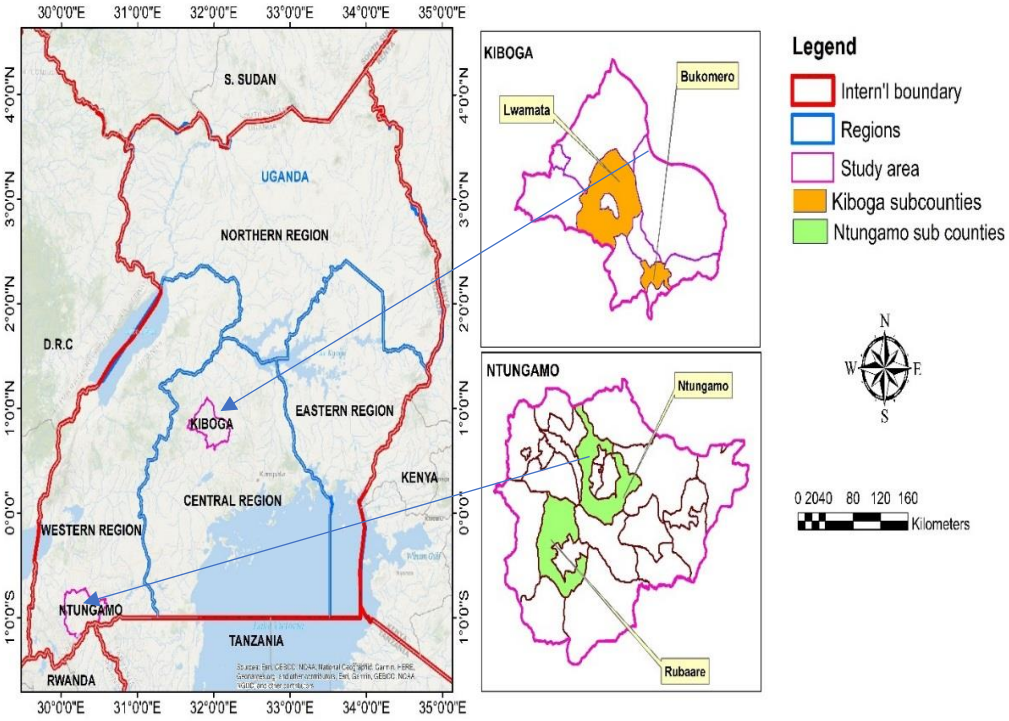


Figure 3.1: Location map, District Information for sub – counties

3.2 Materials

3.2.1 Sampling

A total of 32 meat samples were collected purposively from local abattoirs in Ntungamo, Rubaare, Bukomero, and Lwamata, with 8 samples from each sub-county. These samples included liver, kidney, heart, large intestines, round, rib, brisket, and chuck from mature long-horned Ankole cattle, selected based on their muscle type and breed.

3.2.2 Reagents and chemicals

Distilled water, NaOH pellets (95% assay), and KOH (85% assay) were used all of analytical grade. Organic solvents included acetone (AR, 99.5% assay), absolute ethanol (AR, 99.9% assay), methanol (AR, 99.8% assay), chloroform

(AR, 99.5% assay), and hexane (AR, 95% assay). All reagents were purchased from Labx Chemical Suppliers Uganda limited.

External Standards: Cholesterol Standard, ≥ 99% (HPLC) (Sigma-Aldrich, USA), was used for cholesterol analysis while an external standard FAME mixture solution, (Nu-Chek-Prep, Elysian, Minnesota, USA) was used for fatty acid analysis.

3.3 Methods

3.3.1 Sample collection and treatment

Muscle meat cuts from 4 different muscles (brisket, chuck, rib and round), four different organ meat cuts (liver, kidney, heart, and large intestines) of long-horned Ankole cattle were collected, washed in water to remove blood and chilled for about 24 hours at 0°C. They were then be frozen using liquid nitrogen, stored a freezer at -200C, transported to the chemistry laboratory at Kyambogo University, and stored at -20°C until analysis.

3.3.2 Sample preparation and analysis

3.3.2.1 Extraction of lipids

The lipid extraction was carried out by a chemical method described by Gerhardtova et al. (2024), which is briefly explained here below:

The tissue was homogenized with chloroform/methanol (2/1) to a final volume 20 times the volume of tissue sample (1g in 20 mL of solvent mixture). After dispersion, the whole mixture was agitated for 15-20 min in an orbital shaker at room temperature. The homogenate was filtered (funnel with folded filter paper) to recover the liquid phase. The solvent was washed with 0.2 volume (4 mL) of 0.9% sodium chloride solution. After vortexing for some seconds, the mixture

was centrifuged at low speed (2000 rpm) to separate the two phases. After centrifugation and siphoning of the upper phase, the lower chloroform phase containing lipids was evaporated under a nitrogen stream to obtain a residue of constant mass. Total lipids were measured gravimetrically, in triplicate, by weighing the fatty residue obtained after solvent evaporation.

3.3.2.2 Preparation of fatty acid methyl esters (FAMEs) standards

For fatty acid analysis, a comprehensive standard mixture of fatty acid methyl esters (FAMEs) was prepared, including a diverse array of saturated, monounsaturated, and polyunsaturated fatty acids. This mixture, consisting of 18 different fatty acids, was purchased from Sigma-Aldrich, Germany, and used to prepare a stock solution with a concentration of 50 mg/mL. A series of standards were prepared and used to generate a calibration curve for quantification of fatty acid compositions according to a method described by Igbakin et al.(2010) with a few modifications.

The inclusion of this broad range of fatty acids ensured a thorough representation of the typical fatty acid profiles found in Ankole cattle. The identification and quantification of fatty acids in the samples were achieved by comparing the retention times of the sample FAME peaks with those of the standard mixture and through mass spectrometry analysis, providing accurate and detailed results.

3.3.2.3 Preparation of fatty acid methyl esters (FAMEs)

Fatty acid methyl esters (FAME) were prepared by base-catalyzed methanolysis as described by Ostermann et al. (2014) with slight modifications. In this method 2 mL of methanolic potassium hydroxide were added to the dried lipid extract.

The reaction vial was placed in a water bath maintained at 50 °C for 30 min with shaking every 10 minutes.

The reaction vial was then removed and cooled down to room temperature. Afterwards, 2 mL ultra-pure water and 4mL heptane were added to the reaction vial, mixed, and the resulting mixture was transferred to a centrifuge tube. The mixture was vortexed for 1 min and centrifuged at 2000 rpm for 15 min at room temperature. After this, the mixture was transferred to a new vial containing sodium sulphate and centrifuged again. Finally, the supernatant was collected and analyzed by GC. The concentration of the FAMEs was adjusted by addition of hexane to obtain a suitable chromatographic response.

3.3.2.4 Gas chromatography FAME analysis

The fatty acid methyl esters (FAMEs) analysis was conducted using a method adapted from (Masa et al., 2011) . The analysis utilized gas chromatography/mass spectrometry (GC/MS) with an Agilent Technologies system. Specifically, an Agilent GC-5975T gas chromatograph, equipped with an Agilent 7683-B auto sampler and an MS-5975 mass selective detector, was employed. Prior to GC/MS analysis, the pooled extract was concentrated to 1 mL.

A 1 μL aliquot of this concentrated extract was then injected into an Agilent J&W HP-88 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness), which contains 88% cyanopropyl arylpolysiloxane as the stationary phase. The GC conditions were as follows: the injector and detector temperatures were set to 240°C and 260°C, respectively. The initial column temperature was held at 140°C for 2 minutes, then increased to 230°C at a rate of 4°C/min, and maintained at

230°C for 5 minutes. The split ratio was 1:50, and nitrogen was used as the carrier gas with a flow rate of 0.8 mL/min.

For the mass spectrometry analysis, the instrument was operated in electron impact (EI) mode at 70 eV. The ion source temperature was set to 230°C, the quadrupole temperature to 150°C, and the translating line temperature to 260°C. The mass scan range was 50 to 550 m/z, with an emission voltage of 1035 V. FAME peak identification was achieved by comparing their mass spectra and retention times (Rt) with those of known standards. For quantification, an external standard mixture of FAMEs was used. Calibration curves for each FAME were generated by preparing a series of standard solutions with known concentrations. These standards were analyzed under the same GC/MS conditions, and their peak areas were plotted against their concentrations to create calibration curves.

Quantification of FAMEs in the sample was achieved by comparing the peak areas of the sample FAMEs to the corresponding calibration curves. This allows for the determination of the concentration of each FAME in the sample based on its peak area relative to the standard mixture.

3.3.2.5 Preparation of cholesterol

Each sample (2g) was saponified according to a modified version of the method described by Stewart et al. (1992), with 4 mL of 50% potassium hydroxide and 6 mL of 95% ethanol absolute heated for complete solubilization at 40 ºC, and then heated for 10 min at 60ºC. After this, 5 mL of water was added and the sample cooled. The non-saponifiable fraction was extracted three times using 10 mL of hexane. Aliquots of hexane extracts (3 mL) were dried under a nitrogen flow.

After saponification, samples were analyzed by high-performance liquid chromatography (HPLC).

3.3.2.6 Preparation of cholesterol standard

The cholesterol standard (Sigma and Polyscience, U.S.A. ® C8667), was used for calibration. Stock solutions were prepared by dissolving the standard in methanol. Calibration standards were created at the following cholesterol concentrations: 2000 mg/L, 1000 mg/L, 500 mg/L, and 100 mg/L as modified method of (Mazalli et al., 2006). These standards were injected into an HPLC system with a mobile phase of acetonitrile and isopropanol (70:30, v/v) at a flow rate of 1 mL/min. Chromatograms were recorded using a UV detector at 210 nm. A calibration curve was established by plotting concentration against peak area. Sample cholesterol concentrations were determined by comparing their peak areas to the calibration curve, with periodic quality control and recalibration performed as needed.

3.3.2.6 HPLC Cholesterol analysis

The extract was dissolved in 3 mL of an acetonitrile-isopropanol solution (70:30, v/v), and 1 mL of this solution was injected into the HPLC system (Bragagnolo and Rodriguez-Amaya, 2001). The mobile phase consisted of acetonitrile and isopropanol in a 70:30 (v/v) ratio, with a flow rate of 1 mL/min. The chromatograms were processed at a wavelength of 210 nm.

Cholesterol was identified by comparing the retention times of the sample with those of a cholesterol standard. Quantification was performed using external standardization with a calibration curve. Cholesterol standards at various concentrations were prepared and analyzed to generate this calibration curve. The

response factors from the calibration curve were used to quantify cholesterol in the samples. This approach ensured precise and accurate measurement of cholesterol concentrations.

3.3.3 Determining the nutritional quality implications of the fatty acid profiles and cholesterol based on established dietary guidelines.

Evaluating health risks associated with dietary fats involved analyzing fatty acid profiles and cholesterol levels through various nutritional indices (Products et al., 2016; Aranceta and Pérez-Rodrigo, 2012). These indices helped align dietary intake with health guidelines and provided insights into cardiovascular and other health risks. The calculations were based on formulars reported by Chen and Liu, (2020b) and Al-Amiri et al., (2020).

Polyunsaturated Fatty Acid/Saturated Fatty Acid (PUFA/SFA) ratio: This ratio compared the amount of polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFAs). A higher ratio, typically suggested to be at least 0.4, was associated with a favorable impact on cholesterol levels, particularly in lowering LDL cholesterol (Dassanayake et al., 2024; Wu et al., 2020). The formula used was:

$$\mathrm{PUFA/SFA} = \frac{\sum \mathrm{PUFA}}{\sum \mathrm{SFA}}$$

Index of atherogenicity (IA): This index evaluated the risk of atherosclerosis by comparing SFAs to unsaturated fatty acids. A lower IA value, ideally below 1.0, indicated a reduced risk of plaque buildup in the arteries (Fernandes and Gandin, 2015). The formula was:

$$\mathrm{IA} = \frac{[\mathrm{C12{:}0} + (4 \times \mathrm{C14{:}0}) + \mathrm{C16{:}0}}{\sum \mathrm{UFA}}$$

Index of Thrombogenicity (IT): This index assessed the risk of blood clot formation by comparing pro-thrombogenic SFAs with anti-thrombogenic unsaturated fats. Lower IT values, generally recommended to be below 0.5, were linked to a decreased risk of thrombosis. The formula used was:

$$\mathrm{IT} = \frac{(\mathrm{C14{:}\,0} + \mathrm{C16{:}\,0} + \mathrm{C18{:}\,0})}{\left[(0.5 \times \sum \mathrm{MUFA}) + (0.5 \times \sum \mathrm{n-6\ PUFA}) + 3 \times \sum \mathrm{n-3\ PUFA}) + \left(\frac{\mathrm{n-3}}{\mathrm{n-6}}\right)\right]}$$

Hypocholesterolemic / hypercholesterolemic (HH) Ratio: This ratio compared fats that lower cholesterol with those that raise it. A higher HH ratio, recommended to be at least 2.0, was indicative of better cholesterol management. The formula was:

$$\mathrm{HH} = \frac{\mathrm{Cis-C18{:}\,1} + \sum \mathrm{PUFA}}{(\mathrm{C12{:}\,0} + \mathrm{C14{:}\,0} + \mathrm{C16{:}\,0})}$$

Health-promoting Index (HPI): This index measured the proportion of unsaturated fatty acids relative to atherogenic SFAs. Higher HPI values, ideally above 1.0, suggested a diet more supportive of cardiovascular health (Chen and Liu, 2020b; Ratusz et al., 2018). The formula used was:

$$\mathrm{HPI} = \frac{\sum \mathrm{UFA}}{\mathrm{C12{:}\,0} + 4 \times \mathrm{C14{:}\,0} + \mathrm{C16{:}\,0}}$$

Unsaturation Index (UI): This index assessed the degree of unsaturation in fatty acids. Higher UI values, recommended to be at least 1.5, reflected a greater proportion of beneficial unsaturated fats (Weijers, 2015; Dal Bosco et al., 2022b). The formula was:

$$\mathrm{UI} = 1 \times (\%\ \text{monoenoics}) + 2 \times (\%\ \text{dienoics}) + 3 \times (\%\ \text{trienoics}) + 4 \times (\%\ \text{tetraenoics}) + 5 \times (\%\ \text{pentaenoics}) + 6 \times (\%\ \text{hexaenoics})$$

These nutritional indices and their formulas provided a comprehensive framework for assessing the impact of fatty acids and cholesterol profiles on health.

3.4 Statistical analysis

Students' t-test and one-way ANOVA was performed using SPSS. All analyses were performed in triplicate (Singer et al., 2007; Beam et al., 2000) and reported as means ± standard error of the mean (s.e). One-way ANOVA was used to compare the levels of fatty acid and cholesterol yield of muscle meat (chuck, rib, round, brisket) and organ meat (liver, kidney, heart, large intestines). Students' t-test was employed to assess the statistical significance of the difference between the means of cholesterol content and fatty acid profiles in the districts of Kiboga and Ntungamo. Differences between means were considered significant at $p < 0.05$ otherwise they were insignificant (Smucker et al., 2007; White et al., 2022).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Lipid content

The data presented in Table 4.1 shows the mean lipid content of various meat cuts sourced from Kiboga District, specifically from Bukomero and Lwamata sub-counties.

4.1.1 Lipid content for Kiboga meat cuts.

In both Bukomero and Lwamata sub-counties of Kiboga District, the liver had the highest lipid content, with means of 2.38 ± 0.36% in Bukomero and 3.08 ± 0.91% in Lwamata, while the chuck cut had the lowest lipid content, showing 0.42 ± 0.20% in Bukomero and 0.51 ± 0.09% in Lwamata (**Table 4.1**). Intermediate lipid levels were observed in various cuts, with rib meat and heart presenting higher levels compared to brisket and round cuts, though still significantly lower than organ meats. The statistical analysis indicated significant differences in the mean lipid content among various meat cuts ($p < 0.05$).

Table 4.2 further indicates that there were no significant differences in the mean lipid content of meat cuts between Bukomero and Lwamata sub-counties in Kiboga District (Student's t-test; $P > 0.05$). Specifically, the proportion of lipids in meat cuts of Bukomero was similar to that of Lwamata. These results demonstrate that the lipid content across various meat cuts did not vary significantly between the two sub-counties. A comparison of the mean lipid content analyzed in eight meat cuts from both sub-counties is shown in **Figure 4.1.** All organ meat had high proportion of lipids.

Table 4.1: Mean lipid content (%) for meat cuts from Bukomero and Lwamata sub - counties in Kiboga district

Lipid content (%) for meat cuts in Kiboga sub - counties		
Tissue	Bukomero	Lwamata
Brisket	0.61 ± 0.05^{ab}	0.81 ± 0.38^{a}
Chuck	0.42 ± 0.20^{a}	0.51 ± 0.092^{a}
Rib	1.22 ± 0.03^{abc}	1.01 ± 0.140^{b}
Round	0.67 ± 0.02^{ab}	0.78 ± 0.100^{a}
Liver	2.38 ± 0.36^{e}	3.08 ± 0.905^{c}
Kidney	2.23 ± 0.31^{de}	2.09 ± 0.340^{c}
Heart	1.39 ± 0.04^{bcd}	1.29 ± 0.158^{b}
Large intestines	1.92 ± 0.13^{cde}	1.73 ± 0.04^{b}
P – value (ANOVA)	0.00	0.00

Data are derived from the analysis of lipid content of different meat cuts. Values within columns with different superscript letters are significantly different ($p < 0.05$). The values presented are means of three replicates $\pm$ standard errors.

Table 4.2: Comparison of mean lipid content (%) of Bukomero with Lwamata sub-counties meat cuts

Lipid content (%) for meat cuts in Kiboga sub - counties					
Meat cut	Bukomero	Lwamata	t	P – value	df
Brisket	0.61 ± 0.05	0.81 ± 0.38	-0.9	0.42	2
Chuck	0.42 ± 0.20	0.51 ± 0.09	-0.93	0.40	2
Rib	1.22 ± 0.03	1.01 ± 0.14	1.43	0.23	2
Round	0.67 ± 0.02	0.78 ± 0.10	-1.09	0.34	2
Liver	2.38 ± 0.36	3.08 ± 0.91	-0.32	0.77	2
Kidney	2.23 ± 0.31	2.09 ± 0.34	0.38	0.72	2
Heart	1.39 ± 0.04	1.29 ± 0.16	0.61	0.57	2
Large intestines	1.92 ± 0.13	1.73 ± 0.04	1.54	0.20	2

Data are derived from the comparison of different meat cuts in Bukomero sub – county with Lwamata sub - county. The values presented are means from three replicates ± standard errors. Degrees of freedom (df), t-values (t), and p-values (p) are shown for the student's t-test.

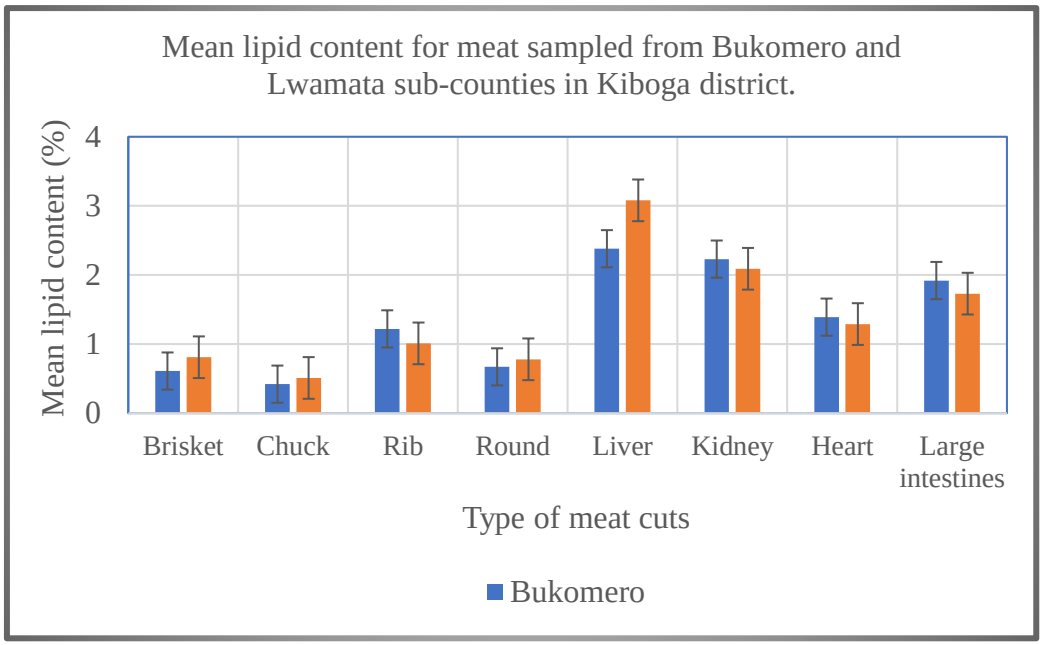


Figure 4.1: Mean lipid content (%) for various meat cuts sampled from Bukomero and Lwamata sub - counties.

4.1.2 Lipid content for Ntungamo meat cuts

In both Ntungamo and Rubaare sub-counties, the analysis (**Table 4.3**) indicated significant differences in lipid content across various meat cuts ($p < 0.05$,). Organ meats consistently had higher fat content compared to muscle meats. Specifically, liver had the highest lipid content, with values of 3.55 ± 0.46% in Ntungamo and 3.00 ± 0.22% in Rubaare. In contrast, the chuck cut had the lowest fat content, particularly in Ntungamo at 0.69 ± 0.04%. Intermediate levels of lipid content were observed in cuts such as the rib and round, with Rubaare generally showing higher levels than Ntungamo.

Table 4.4 further indicates that there were no significant differences in the mean lipid content of meat cuts between Ntungamo and Rubaare sub-counties in Ntungamo District (Student's t-test; $P > 0.05$). Specifically, the lipid content of meat cuts from Ntungamo was similar to that of meat cuts from Rubaare. These

results demonstrate that the lipid content across various meat cuts did not vary significantly between the two sub-counties. A comparison of the mean lipid content analyzed in eight meat cuts from both sub-counties is shown in **Figure 4.2.**

Table 4.3: Mean Lipid content (%) of Ntungamo and Rubaare sub – county meat cuts

Lipid content (%) for meat cuts in Ntungamo sub – counties		
Type of meat cut	Ntungamo sub - county	Rubaare sub - county
Brisket	1.07 ± 0.07^{a}	1.22 ± 0.21^{a}
Chuck	0.69 ± 0.04^{a}	1.28 ± 0.12^{a}
Rib	0.87 ± 0.17^{a}	1.65 ± 0.18^{a}
Round	1.04 ± 0.04^{a}	1.63 ± 0.31^{a}
Liver	3.55 ± 0.46^{b}	3.00 ± 0.22^{c}
Kidney	1.21 ± 1.21^{c}	2.23 ± 0.35^{a}
Heart	1.57 ± 0.25^{d}	1.96 ± 0.41^{b}
Large intestines	1.70 ± 0.86^{e}	2.18 ± 0.30^{b}
P – value (ANOVA)	0.02	0.01

Data are derived from the analysis of different meat cuts in Ntungamo and Rubaare sub – counties of Ntungamo district. Values within columns with different superscript letters are significantly different ($p < 0.05$). The values presented are means of three replicates $\pm$ standard errors.

Table 4.4: Comparison of mean lipid content of Ntungamo with Rubaare sub – county in Ntungamo district.

Lipid content (%) for meat cuts in Ntungamo sub - counties					
Meat cut	Ntungamo	Rubaare	t	p	df
Brisket	1.07±0.07	1.22±0.21	0.67	0.54	2
Chuck	0.69±0.04	1.28±0.12	4.70	0.00	2
Rib	0.87±0.17	2.50±0.18	3.15	0.03	2
Round	1.04±0.04	1.65±0.31	0.53	0.03	2
Liver	3.55±0.46	3.00±0.22	-1.06	0.35	2
Kidney	1.21±1.21	2.23±0.35	3.38	0.62	2
Heart	1.57±0.25	1.96±0.41	0.82	0.46	2
Large intestines	1.70±0.86	2.18±0.30	0.34	0.75	2

Data are derived from the comparison of lipid content in different meat cuts. The values presented are means from three replicates ± standard errors. Degrees of freedom (df), t-values (t), and p-values (p) are shown for the student's t-test.

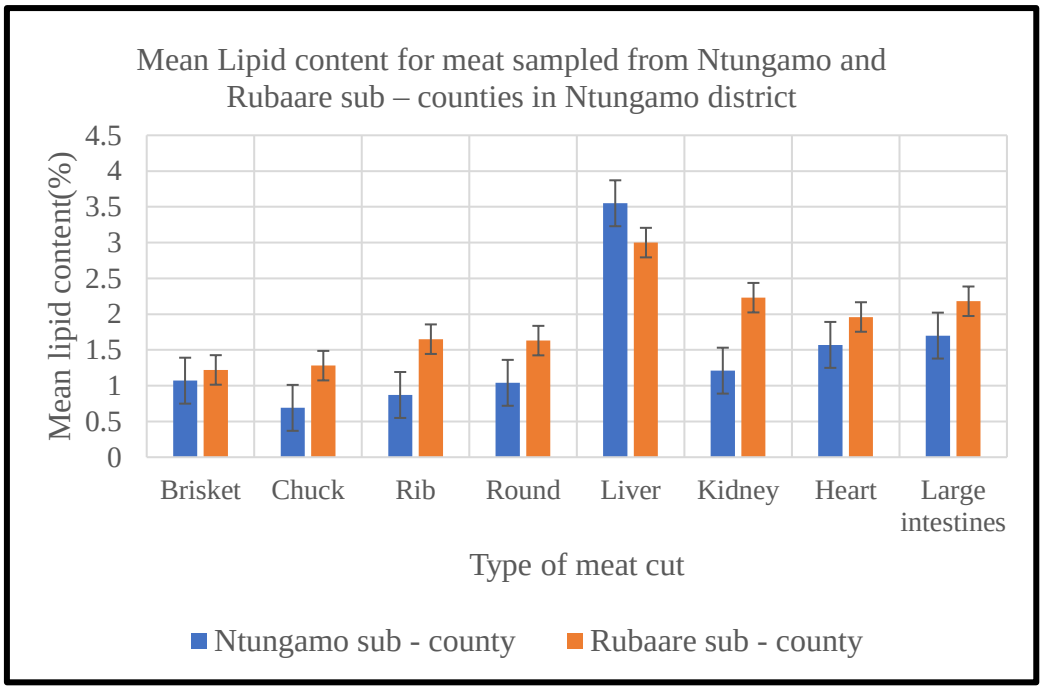


Figure 4.2: Mean Lipid content (%) of Ntungamo and Rubaare sub – county meat cuts

4.1.3 Comparison of total lipid content between Kiboga district and Ntungamo district

The findings presented in **Table 4.5** indicate that, overall, there were no statistically significant differences in the mean lipid content of meat cuts between Ntungamo and Kiboga districts (Student's t-test; $P > 0.05$). This suggests that, in general, the lipid composition of meat from both districts is comparable.

However, a closer examination of individual meat cuts shows that Ntungamo district had slightly higher lipid levels in several cuts, including brisket, chuck, and round, although these differences were not statistically significant. Organ meats such as liver consistently exhibited higher lipid content than muscle meats across both districts, reflecting the known metabolic role of these organs in lipid storage and metabolism. For instance, liver tissue had mean lipid contents of

3.27% in Ntungamo and 2.73% in Kiboga, which were higher than values observed in muscle cuts such as rib (Ntungamo 1.26%, Kiboga 1.12%).

The comparison of mean lipid content across the eight meat cuts from both districts is illustrated in **Figure 4.3.** These results indicate that while district-level differences in lipid content are minimal, tissue type is a major determinant of lipid concentration, with organ meats storing more lipids than muscle cuts.

Table 4.5: Comparison of mean lipid content (%) of Kiboga district and Ntungamo district meat cuts

Lipid content (%) for meat cuts in each district					
Meat cut	Ntungamo	Kiboga	t	p	df
Brisket	1.38	0.78	2.60	0.03	5
Chuck	1.14	0.47	3.87	0.03	5
Rib	1.26	1.12	0.37	0.71	5
Round	1.42	0.72	1.84	0.10	5
Liver	3.27	2.73	1.02	0.33	5
Kidney	1.63	2.26	1.73	0.11	5
Heart	1.57	1.34	1.15	0.29	5
Large intestines	2.09	1.83	0.42	0.68	5

Data are derived from the comparison of lipid content in different meat cuts. The values presented are averages for each district. Degrees of freedom (df), t-values (t), and p-values (p) are shown for the student's t-test.

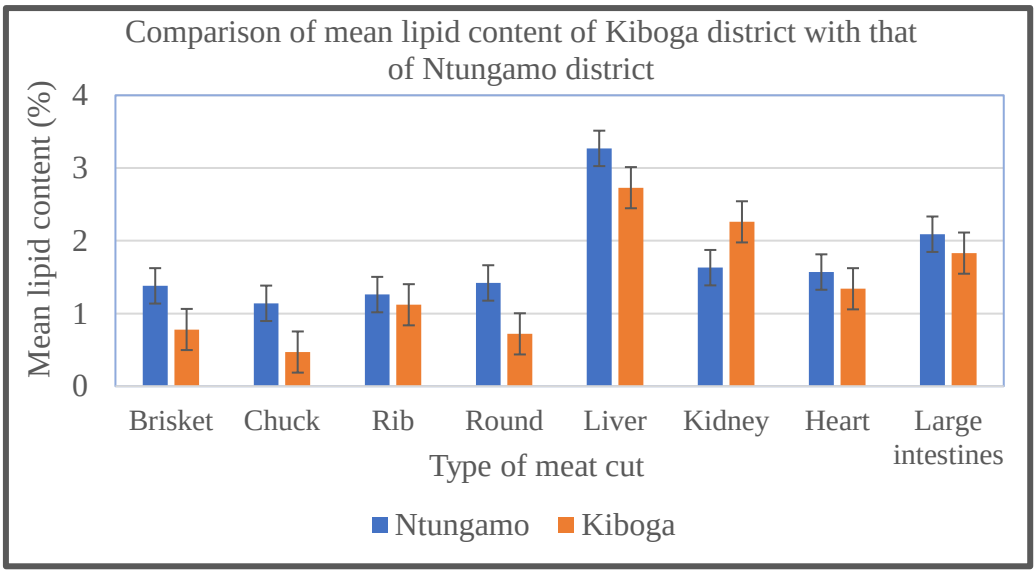


Figure 4.3: Comparison of mean lipid content (%) of Kiboga district and Ntungamo district meat cuts

The study (**Tables 4.1, 4.3 and 4.5**), indicated that organ meats, including heart, liver, and kidney, had the highest proportion of total lipids in all the sub – counties in each district. The highest lipid content is attributed to their physiological roles in lipid metabolism and storage, particularly in the liver (Zaefarian et al., 2019; Descalzo and Sancho., 2008). Both the heart and liver are rich in adipose tissue, the primary site for de novo lipid synthesis (Jayathilakan et al., 2012; Dodson et al., 2010).

Conversely, the low lipid content in chuck tissue and other muscle meats can be linked to its muscular nature, as muscles generally contain less fat compared to other body tissues (Aubrey et al., 2014). This is due to lipids being primarily stored in adipose tissue for energy and insulation, rather than in muscle tissues. Additionally, muscle function requires lipids, which may lead to lower lipid content in muscle tissues (Shimizugawa et al., 2002). The lipid content in muscle tissues is also influenced by the animal's diet; a diet low in fats but high in

carbohydrates or proteins can result in lower lipid levels in the muscles (Hausman et al., 2008; Mourot and Hermier, 2001).

High lipid content in organ meats, such as the heart, liver, and kidneys, compared to muscle meats, contributes to elevated cholesterol levels, which poses a potential risk by increasing the likelihood of developing cardiovascular diseases (Beriain et al., 2018; Smet et al., 2018).

In the sub-counties of both districts, the differences in lipid content among the eight meat cuts were statistically significant ($P < 0.05$), aligning with the findings of Restrepo-Molina et al. (2022), who observed significant differences among seven meat cuts of beef from Antioquia, Colombia. Their study, however, reported higher fat content compared to the current study. Gálvez et al. (2019) also noted varying fat levels in different meat cuts of female calves, which supports the trends observed in Kiboga and Ntungamo districts. The variations in lipid content among the meat parts could be attributed to factors such as muscle type, and anatomical location (Bianchi et al., 2006).

The current study indicated lower lipid content in meat cuts, with Kiboga district showing an average of approximately 1.53 ± 0.32% (**Table 4.1** and **Figure 4.1**) and Ntungamo district showing approximately 1.78 ± 0.31% (**Table 4.3** and **Figure 4.2**), compared to higher values reported in previous studies. Vera et al. (2009) reported lipid content ranging from 0.68% to 4.23% in various meat cuts of calves reared on permanent pastures, which is slightly higher than the 0.73% to 3.55% range observed in the current study.

Similarly, Bragagnolo and Rodriguez-Amaya (2003) reported higher lipid content, ranging from 2.1% to 2.6%, in longissimus dorsi muscles from different

cattle breeds. The lower lipid content in the current study's meat cuts may be due to grass-based feeding practices, which generally lead to leaner carcasses with less intramuscular fat, and breed types, which influence intramuscular fat content (Liu et al., 2022; Nogoy et al., 2022; Mordenti et al., 2019)

The lack of significant differences (student's t – test, $p > 0.05$) in lipid content between sub – counties and districts may be because of similar dietary habits and environmental factors influencing cattle. Additionally, genetic and breed similarities among the livestock commonly raised in both regions could also contribute to this result (Všetičková et al., 2020; Warner et al., 2010).

4.2 Cholesterol content

The data presented below shows the mean cholesterol content across various meat cuts sourced from Bukomero sub – county and Lwamata sub – county in Kiboga district, Ntungamo sub – county and Rubaare sub- county in Ntungamo district.

4.2.1 Cholesterol content for Kiboga district

The study results, presented in **Table 4.6**, indicated significant differences in cholesterol content among various meat cuts within each sub-county ($P < 0.05$). Organ meats, including liver, kidney, and large intestines, generally had higher cholesterol levels compared to muscle meats.

Specifically, the liver had the highest cholesterol content, with values of 206.57 ± 2.88 mg/100g in Bukomero and 177.18 ± 3.28 mg/100g in Lwamata. The kidney also showed higher cholesterol levels, especially in Lwamata (176.14±12.06 mg/100g). Intermediate levels were found in the heart and large intestines, with the heart showing 49.79 ± 3.57 mg/100g in Bukomero and 83.46

± 1.70 mg/100g in Lwamata, and large intestines having 59.91 ± 4.15 mg/100g in Bukomero and 70.35 ± 3.14 mg/100g in Lwamata.

Muscle meats, including brisket, chuck, rib, and round, had lower cholesterol levels. Notably, rib and round cuts consistently showed the lowest cholesterol content. In Bukomero, the rib had a cholesterol level of 24.75 ± 1.17 mg/100g, and in Lwamata, it was slightly lower at 24.17 ± 0.00 mg/100g. Similarly, the round cut had low cholesterol levels with 27.62 ± 0.25 mg/100g in Bukomero and 33.13 ± 0.13 mg/100g in Lwamata.

Table 4.7 further reveals that there were no significant differences in the mean cholesterol content of rib, liver, and large intestines between the two sub-counties (Student's t-test, $p > 0.05$). However, significant differences were observed in brisket, round, heart, chuck, and kidney (Student's t-test, $p < 0.05$).

Conclusively, organ meats had the highest cholesterol levels across both sub-counties, while muscle meats had lower levels. Detailed comparisons of mean cholesterol contents for each meat cut are illustrated in **Figure 4.4.**

Table 4.6: Mean cholesterol content (mg/100g) for meat cuts sampled from Bukomero and Lwamata sub-counties in Kiboga district

Cholesterol content (mg/100g) for meat cuts Kiboga sub – counties		
Meat cut	Bukomero	Lwamata
Brisket	44.13 ± 1.73^{b}	37.16 ± 0.71^{a}
Chuck	29.95 ± 0.50^{a}	42.19 ± 0.89^{a}
Rib	24.75 ± 1.17^{a}	24.17 ± 0.00^{a}
Round	27.62 ± 0.25^{a}	33.13 ± 0.13^{a}
Liver	206.57 ± 2.88^{e}	177.18 ± 3.28^{b}
Kidney	156.16 ± 0.70^{a}	176.14 ± 12.06^{b}
Heart	49.79 ± 3.57^{bc}	83.46 ± 1.70^{c}
Large intestines	59.91 ± 4.15^{c}	70.35 ± 3.14^{c}
P -value (ANOVA)	0.00	0.00

Data are derived from the analysis of cholesterol content in different meat cuts in Bukomero and Lwamata sub – counties in Kiboga district. Values within columns with different superscript letters are significantly different ($p < 0.05$). The values presented are means of three replicates $\pm$ standard errors.

Table 4.7: Comparison of the cholesterol content (mg/100g) of Bukomero sub – county with Lwamata sub – county meat cut

Cholesterol content (mg/100g) for meat cuts Kiboga sub – counties					
Meat cut	Bukomero	Lwamata	t	p	df
Brisket	44.13 ± 1.73	37.16 ± 0.71	3.73	0.02	2
Chuck	29.95 ± 0.50	42.19 ± 0.89	-11.95	0.00	2
Rib	24.75 ± 1.17	24.17 ± 0.00	0.49	0.65	2
Round	27.62 ± 0.25	33.13 ± 0.13	-19.59	0.00	2
Liver	206.57 ± 2.88	177.18 ± 3.28	-6.27	0.07	2
Kidney	156.16 ± 0.70	176.14 ± 12.06	-6.27	0.00	2
Heart	49.79 ± 3.57b	83.46 ± 1.70	-8.51	0.00	2
Large intestines	59.91 ± 4.15	70.35 ± 3.14	-2	0.11	2

Data are derived from the comparison of cholesterol content in different meat cuts of Kiboga sub - counties. The values presented are means from three

replicates ± standard errors. Degrees of freedom (df), t-values (t), and p-values (p) are shown for the student's t-test.

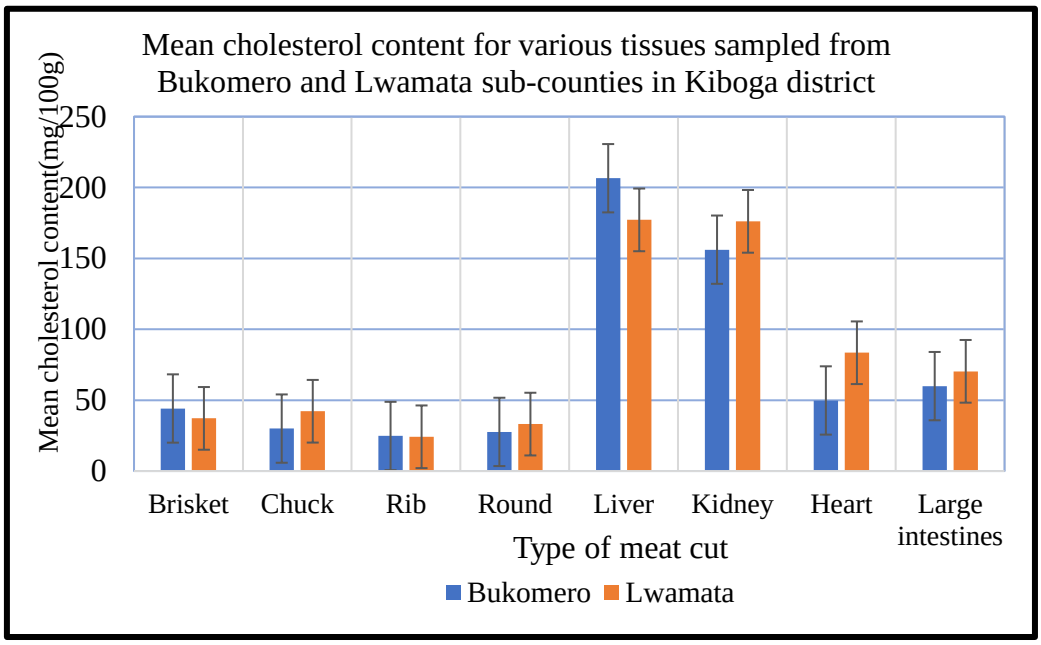


Figure 4.4: Mean cholesterol content (mg/100g) for various tissues sampled from Bukomero and Lwamata sub-counties in Kiboga district

4.2.2 Cholesterol content for Ntungamo district

The findings of this study revealed that the difference in the cholesterol content among eight meat cuts was statistically significant ($P < 0.05$) for both Ntungamo sub – county and Rubaare sub – county **(Table 4.8).** Organ meat which included liver, kidney, heart and large intestines had significantly higher cholesterol content. The liver tissue had the highest cholesterol content of 187.21 ± 2.92 mg/100g for Ntungamo sub - county and 155.95 ± 1.37 mg/100g for Rubaare sub – county. Muscle meat which included chuck, brisket, round and ribs had lower cholesterol content for both sub – counties **(Figure 4.5).** Furthermore, the findings indicated that the brisket and round emerge as the options with the lowest cholesterol content. In Ntungamo sub – county, the round tissue had the lowest

cholesterol content of 27.59 ± 1.16 mg/100g, while in Rubaare sub-county, it increased to 50.66 ± 0.81 mg/100g. Similarly, the brisket in Rubaare had the lowest cholesterol levels, with values of 24.24 ± 1.94 mg/100g and it increased slightly to 31.84 ± 1.18 mg/100g in Ntungamo sub-county, respectively.

Furthermore, the study's findings (**Table 4.9**) revealed that most meat cuts from the two sub-counties in Ntungamo District exhibited no significant differences in cholesterol content (Student's t-test, $p > 0.05$). However, significant differences were observed in the cholesterol content of brisket, rib, and heart between the two sub-counties (Student's t-test, $p < 0.05$). Detailed comparison of mean cholesterol contents for each meat cut are illustrated in **Figure 4.5.**

Table 4.8: Cholesterol content (mg/100g) of meat cuts from Rubaare and Ntungamo sub – county in Ntungamo district

Cholesterol content (mg/100g) for meat cuts Ntungamo sub – counties		
Meat cut	Ntungamo	Rubaare
Brisket	31.84 ± 1.184^{a}	24.58 ± 1.94^{a}
Chuck	36.55 ± 4.448^{a}	26.41 ± 1.12^{a}
Rib	28.5 ± 1.89^{a}	24.76 ± 0.55^{a}
Round	27.59 ± 1.16^{b}	50.66 ± 0.81^{b}
Liver	187.21 ± 2.92^{d}	155.95 ± 1.37^{d}
Kidney	129.75 ± 1.16^{c}	113.4 ± 6.62^{b}
Heart	56.14 ± 2.92^{b}	53.44 ± 2.67^{b}
Large intestines	73.54 ± 2.45^{b}	59.07 ± 1.31^{b}
P – Value (ANOVA)	0.00	0.00

Data are derived from the analysis of cholesterol content in different meat cuts in Ntungamo and Rubaare sub – counties in Ntungamo district. Values within columns with different superscript letters are significantly different ($p < 0.05$). The values presented are means of three replicates ± standard errors.

Table 4.9: Comparison in the cholesterol content (mg/100g) of Ntungamo with that of Rubaare sub – county meat cuts

Tissue	Ntungamo	Rubaare	t – value	p – value	df
Brisket	31.84 ± 1.18	24.24 ± 1.94	-3.35	0.03	2
Chuck	36.55 ± 4.44	26.41 ± 1.12	-2.21	0.09	2
Round	28.50 ± 1.89	24.76 ± 0.55	-1.56	0.19	2
Rib	27.59 ± 1.16	50.66 ± 0.89	16.23	0.00	2
Liver	187.21 ± 2.92	113.4 ± 6.62	-1.7	0.17	2
Liver	129.75 ± 1.16	155.95 ± 1.37	-9.69	0.00	2
Heart	56.14 ± 2.29	53.44 ± 2.67	-0.96	0.39	2
Large intestines	73.54 ± 2.45	59.07 ± 1.31	-5.2	0.01	2

Data are derived from the comparison of cholesterol content in different meat cuts of Ntungamo sub - counties. The values presented are means from three replicates ± standard errors. Degrees of freedom (df), t-values (t), and p-values (p) are shown for the student's t-test.

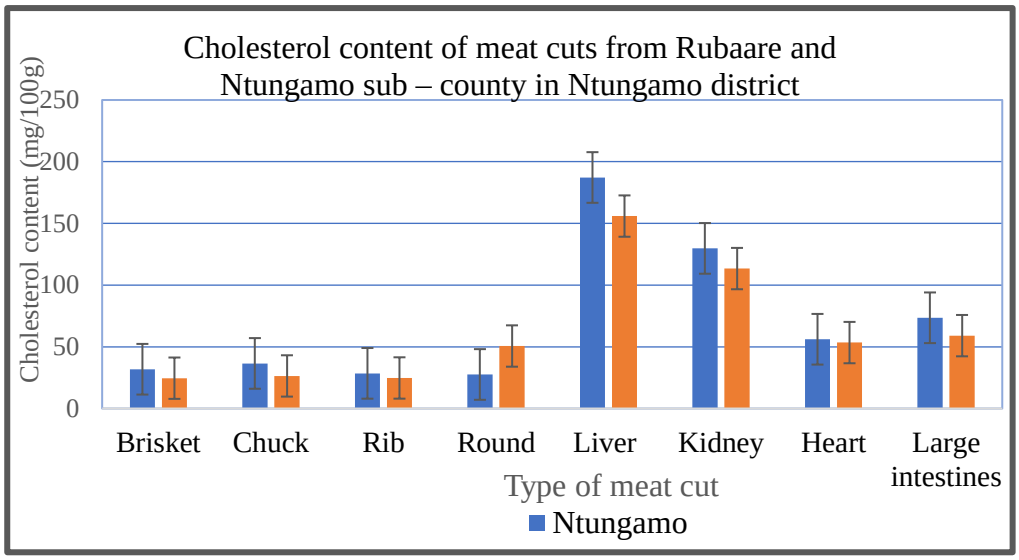


Figure 4.5: Cholesterol content of meat cuts from Rubaare and Ntungamo sub – county in Ntungamo district

4.2.3 Comparison in the mean cholesterol content of Kiboga and Ntungamo district meat cuts.

The results of the study **(Table 4.10)** indicated that most meat cuts between Ntungamo and Kiboga districts had no significant differences in mean cholesterol content (Student's t-test, $p > 0.05$). However, significant differences were observed in the brisket and kidney tissues ($p < 0.05$). For brisket, cholesterol levels in Ntungamo district were significantly lower at 28.04 mg/100g compared to 40.64 mg/100g in Kiboga district ($t = -4.75$, $p < 0.05$). Conversely, kidney tissue exhibited significantly higher cholesterol levels in Kiboga district (166.67 mg/100g) than in Ntungamo district (121.57 mg/100g) **(Figure 4.1).**

For other meat cuts, including chuck, rib, round, liver, heart, and large intestines, there were no significant differences between the districts. Specifically, the rib tissue had the lowest mean cholesterol content, with 26.15 mg/100g in Ntungamo and 24.46 mg/100g in Kiboga. The liver tissue had the highest cholesterol content

in both districts, averaging 191.36 mg/100g in Kiboga and 172.06 mg/100g in Ntungamo. Kidney tissue also had high mean cholesterol content, with Kiboga showing 166.67 mg/100g and Ntungamo 121.57 mg/100g

Overall, while the mean cholesterol content in most meat cuts was similar between the two districts, significant differences were found in specific cuts such as brisket and kidney. Muscle tissues consistently had the lowest cholesterol levels in both districts. The cholesterol levels of meat cuts in Ntungamo were slightly lower than in Kiboga district (**Figure 4.2**).

Table 4.10: Comparison in the mean cholesterol content (mg/100g) of various tissues between Kiboga district and Ntungamo district.

Meat cut	Ntungamo	Kiboga	t	p	df
Brisket	28.04	40.64	-4.75	0.00	5
Chuck	31.48	36.07	-1.11	0.29	5
Rib	26.15	24.46	1.35	0.20	5
Round	39.51	30.37	1.76	0.11	5
Liver	172.06	191.36	-1.69	0.12	5
Kidney	121.57	166.67	-6.01	0.00	5
Heart	55.09	66.63	-1.47	0.17	5
Large intestines	66.31	65.13	0.25	0.81	5

Data are derived from the comparison of cholesterol content in different meat cuts of Ntungamo with Kiboga district. The values presented are averages for each district. Degrees of freedom (df), t-values (t), and p-values (p) are shown for the student’s t-test.

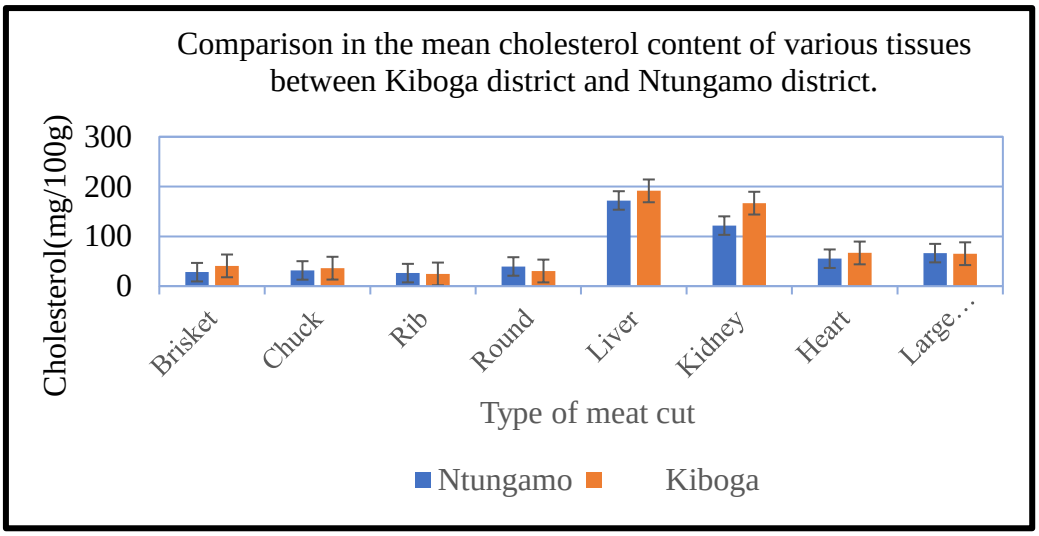


Figure 4.6: Comparison in the mean cholesterol content (mg/100g) of various tissues between Kiboga district and Ntungamo district

Organ meats, including the liver, kidneys, and large intestines, exhibited the highest cholesterol content levels across all sub-counties in each district. The higher cholesterol levels in these organs may be attributed to their crucial roles in metabolism and synthesis processes. The liver, for instance, is central to synthesizing and storing cholesterol, producing bile, and regulating lipid levels in the body (Chiang and Li, 2009; Alamri, 2018; Luo et al., 2020). The kidneys require cholesterol for maintaining cell membranes and for their role in filtering blood and managing waste products (Florens et al., 2016). The large intestines also contain high levels of cholesterol due to their involvement in nutrient absorption and the presence of various cells that need cholesterol for structural integrity and function (Kruit et al., 2006; Silva et al., 2018). These findings align with previous research by Abonyi et al. (2020b), which reported significantly higher cholesterol levels in organ meats like the kidneys, large intestines, and liver compared to muscle meat cuts.

In contrast, muscle meat cuts generally have lower cholesterol levels because they are leaner and contain less fat. Muscle meat primarily consists of protein and water, with relatively little fat (Listrat et al., 2016a; Listrat et al., 2016b; Williams, 2007). Consequently, high cholesterol levels in organ meats can pose health risks by increasing blood cholesterol and contributing to cardiovascular diseases. To reduce these risks, it may be advisable to limit intake of organ meats and focus on consuming lean muscle cuts (Das and Ingole, 2023; Upadhyay, 2023; Smet and Vossen, 2016).

The observed differences in cholesterol content among various meat cuts ($p < 0.05$) might be due to the anatomical location of different body parts (Burgos et al., 2010; Macedo et al., 2008). Factors such as the development rate and structural characteristics of these parts could influence cholesterol synthesis rates (Werdi Pratiwi et al., 2006).

Therefore consuming excessive liver and kidney, which are high in lipids, can raise cholesterol levels and increase the risk of cardiovascular diseases, obesity other health issues thus need to make regulate their consumption and rather choose leaner cuts (Vargas and Larraín, 2017).

Previous studies have also noted significant differences in cholesterol content among different meat cuts. Oliveira et al. (2015) reported significant variations in cholesterol levels between loin (L. thoracis) and rump (B. femoris) tissues in Nellore young bulls in Brazil. Similarly, Abonyi et al. (2020) found higher cholesterol levels in the kidneys and liver compared to other cuts in pigs, cattle, and goats slaughtered in Nigeria. The cholesterol content reported in the current study for various meat cuts in Ntungamo and Kiboga districts was lower than in

many earlier studies. Silva et al. (2022) reported higher cholesterol values in muscle tissues of Nelore, Canchim, and Beefalo breeds in Brazil, ranging from 43.3 mg/100g to 53.3 mg/100g, compared to the current study's range of 22.02 mg/100g to 36.55 mg/100g. The present study found cholesterol content in the chuck and round cuts to be approximately 33.03 mg/100g and 25.02 mg/100g, respectively, with liver cholesterol averaging around 168.21 mg/100g in Mbarara district and 171.58 mg/100g in Ntungamo district. Ejike and Emmanuel (2009) reported even higher cholesterol levels, ranging from 190 mg/100g to 650 mg/100g, in various bovine meat parts, including rib muscle, lungs, large intestine, small intestine, color, liver, kidney, and heart, which is higher than the range of 22.02 mg/100g to 187.89 mg/100g observed in the current study. However, the cholesterol levels were within the upper side of values reported by Igbakin et al. (2010). They reported 141.94 ± 2.12 mg/100g, 95.37 ± 1.59 for the liver and kidney respectively.

4.3 Fatty acids

The data presents the fatty acid content across various meat cuts sourced from Bukomero sub – county and Lwamata sub – county in Ntungamo district and Ntungamo and Rubaare sub- county in Ntungamo district.

4.3.1 Fatty acid composition of meat cuts from Bukomero and Lwamata sub – counties in Kiboga district

The proportions of fatty acids predominantly consisted of high levels of monounsaturated fatty acids (MUFA), followed by saturated fatty acids (SFA), and then polyunsaturated fatty acids (PUFA) (**Table 4.11 and 4.12**). In Bukomero, SFA ranged from 32.25% to 47.94%, while in Lwamata, it ranged

from 33.25% to 44.58%. MUFA ranged from 47% to 60.67% in Bukomero and from 30.64% to 64.78% in Lwamata. PUFA ranged from 3.47% to 26.37% in Bukomero and from 3.25% to 13.90% in Lwamata. The high levels of MUFA were attributed to substantial amounts of oleic acid and elaidic acid present in all meat samples from both Bukomero and Lwamata. SFA were predominantly composed of stearic acid and palmitic acid, while PUFA were primarily represented by Linolelaidic.

The fatty acid compositions differed significantly between various meat cuts within each district ($p < 0.05$). However, there were no statistically significant differences between the sub-counties (student's t – test, $p > 0.05$). This indicates that the fatty acid compositions of meats in Bukomero were similar to those in Lwamata. For a detailed comparative analysis, **Figure 4.7** was used, it showed only slight differences that were not statistically significant.

Table 4.11: Fatty acid composition (% of total fatty acids) in fat from meat cuts in Bukomero sub-county

	Fatty acid composition (%) of different meat cuts								
Fatty acids	Brisket	Chuck	Rib	Round	Liver	Kidney	Heart	Large Intestines	p – value
SFA									< 0.001
Myristic (C14:0)	2.02 ± 0.16^{a}	1.43 ± 0.10^{c}	2.22 ± 0.10^{de}	2.67 ± 0.21^{e}	0.76 ± 0.00^{ab}	0.36 ± 0.02^{a}	0.35 ± 0.03^{a}	1.32 ± 0.09^{bc}	< 0.001
Palmitic (C16:0)	11.99 ± 0.17^{b}	17.48 ± 1.07^{c}	22.77 ± 0.25^{d}	22.88 ± 0.27^{d}	16.94 ± 0.07^{b}	20.02 ± 0.17^{cd}	13.39 ± 0.33^{a}	19.21 ± 1.06^{bc}	< 0.001
Margaric (17:0)	1.22 ± 0.05^{b}	1.00 ± 0.01^{a}	0.89 ± 0.04^{a}	0.93 ± 0.03^{a}	2.24 ± 0.01^{d}	1.28 ± 0.04^{bc}	1.26 ± 0.01^{bc}	1.43 ± 0.08^{c}	< 0.001
Stearic (C18:0)	21.32 ± 0.16^{c}	14.62 ± 1.24^{ab}	12.53 ± 0.34^{a}	10.88 ± 0.18^{a}	28.00 ± 0.10^{d}	19.09 ± 0.19^{c}	18.59 ± 0.44^{bc}	19.39 ± 1.81^{c}	< 0.001
Total SFA (%)	36.55 ± 0.37	34.53 ± 1.34	38.41 ± 0.43	37.37 ± 0.29	47.94 ± 0.18	41.75 ± 0.24	33.59 ± 0.45	41.35 ± 2.04	< 0.001
MUFA									
Myristoleic (14:1n 5)	0.23 ± 0.02^{bc}	0.54 ± 0.07^{e}	0.36 ± 0.01^{cd}	0.36 ± 0.03^{b}	0.16 ± 0.02^{a}	0.00 ± 0.00^{dc}	0.38 ± 0.02^{a}	0.00 ± 0.00^{d}c	< 0.001
Palmitoleic (16:1n7)	2.69 ± 0.06^{c}	2.71 ± 0.16^{c}	2.76 ± 0.06^{c}	4.39 ± 0.19^{d}	1.24 ± 0.01^{a}	1.30 ± 0.06^{a}	1.91 ± 0.02^{b}	2.69 ± 0.19^{c}	< 0.001
Heptadecenoic (15:1n5)	0.22 ± 0.08^{a}	0.91 ± 0.03^{c}	0.41 ± 0.02^{bc}	0.49 ± 0.04^{c}	0.44 ± 0.02^{e}	0.61 ± 0.07^{c}	0.56 ± 0.08^{c}	0.00 ± 0.00^{a}	< 0.001
Elaidic (22:1n9)	29.94 ± 0.14^{c}	25.43 ± 3.46^{abc}	26.98 ± 0.72^{bc}	24.44 ± 0.01^{abc}	22.21 ± 0.07^{ab}	20.72 ± 0.34^{ab}	19.90 ± 0.27^{ab}	19.23 ± 0.11^{a}	< 0.001
Oleic (18:1n9)	17.56 ± 0.14^{a}	31.17 ± 0.58^{b}	27.61 ± 0.26^{b}	29.82 ± 0.11^{b}	16.65 ± 0.14^{a}	17.25 ± 0.11^{a}	17.29 ± 0.26^{a}	27.45 ± 2.10^{b}	< 0.001
Erucic (22:1n9)	2.32 ± 0.88^{b}	0.00 ± 0.00^{a}	0.60 ± 0.03^{ab}	0.57 ± 0.00^{ab}	7.10 ± 0.07^{cd}	16.28 ± 0.34^{e}	8.95 ± 0.27^{d}	6.44 ± 0.11^{c}	< 0.001
Total MUFA (%)	52.96 ± 0.23	61.76 ± 3.52	58.72 ± 0.92	60.67 ± 0.34	47.90 ± 0.18	56.16 ± 0.62	48.99 ± 0.66	56.81 ± 2.18	< 0.001
PUFA									
Linolelaidic (18:2n6)	10.87 ± 0.01^{c}	4.13 ± 0.81^{a}	2.99 ± 0.25^{a}	2.28 ± 0.10^{a}	8.40 ± 0.07^{b}	16.42 ± 0.07^{d}	23.33 ± 0.51^{e}	8.29 ± 0.75^{b}	< 0.001
Alpha-Linolenic (18:3n3)	0.26 ± 0.01^{b}	0.24 ± 0.01^{b}	0.00 ± 0.00^{a}	0.49 ± 0.02^{d}	0.62 ± 0.00^{e}	0.38 ± 0.04^{d}	0.24 ± 0.03^{ad}	0.00 ± 0.00^{a}	< 0.001
Gamma-Linolenic (18:3n6)	1.67 ± 0.01^{c}	0.35 ± 0.02^{a}	0.48 ± 0.10^{a}	0.38 ± 0.03^{a}	2.34 ± 0.01^{a}	2.58 ± 0.02^{de}	2.80 ± 0.07^{e}	0.99 ± 0.01^{b}	< 0.001
Total PUFA (%)	12.80 ± 0.04	4.72 ± 0.84	3.47 ± 0.28	3.15 ± 0.11	11.36 ± 0.08	19.38 ± 0.13	26.37 ± 0.60	9.28 ± 0.77	< 0.001

Data are derived from the analysis of fatty acids in different meat cuts in Bukomero sub - county. Values within rows with different superscript letters are significantly different ($p < 0.05$). The values presented are means of three replicates ± standard errors. S.F.A – saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – Polyunsaturated fatty acid

Table 4.12: Fatty acid composition (% of total fatty acids) in fat from meat cuts in Lwamata sub-county.

	Fatty acid composition (%) of different meat cuts								
Fatty Acid	Brisket	Chuck	Rib	Round	Liver	Kidney	Heart	Large Intestines	p – value
Myristic (14:0)	2.12 ± 0.71^{d}	0.71 ± 0.03^{a}	1.79 ± 0.10^{cd}	1.54 ± 0.19^{bc}	0.55 ± 0.00^{a}	0.29 ± 0.03^{a}	1.33 ± 0.10^{bc}	1.30 ± 0.07^{b}	< 0.001
Palmitic (16:0)	18.77 ± 0.16^{bc}	15.63 ± 0.31^{a}	21.02 ± 0.05^{cd}	19.06 ± 0.61^{cd}	15.91 ± 0.44^{a}	20.43 ± 0.11^{cd}	16.53 ± 0.94^{ab}	21.19 ± 0.24^{d}	< 0.001
Margaric (17:0)	1.05 ± 0.19^{a}	2.01 ± 0.00^{c}	1.79 ± 0.03^{bc}	1.04 ± 0.06^{a}	3.41 ± 0.05^{d}	1.30 ± 0.06^{ab}	3.15 ± 0.17^{d}	1.43 ± 0.06^{ab}	< 0.001
Stearic (18:0)	10.31 ± 0.33^{a}	17.38 ± 0.93^{b}	12.30 ± 0.16^{a}	11.20 ± 0.89^{a}	24.71 ± 0.13^{c}	18.51 ± 0.14^{b}	20.49 ± 1.10^{b}	20.36 ± 0.23^{b}	< 0.001
Total SFA (%)	32.25 ± 0.86	35.73 ± 1.27	37.90 ± 0.30	33.84 ± 0.90	44.58 ± 0.58	40.53 ± 0.40	41.50 ± 1.41	44.28 ± 0.40	< 0.001
MUFA									
Myristoleic (14:1n5)	0.22 ± 0.02^{b}	0.51 ± 0.05^{c}	0.35 ± 0.01^{b}	0.31 ± 0.03^{b}	0.25 ± 0.03^{b}	0.00 ± 0.03^{a}	0.28 ± 0.02^{b}	0.00 ± 0.00^{a}	< 0.001
Palmitoleic (16:1n7)	3.14 ± 0.29^{a}	1.71 ± 0.02^{b}	3.016 ± 0.36^{a}	3.29 ± 0.59^{a}	1.48 ± 0.21^{c}	1.50 ± 0.31^{c}	1.76 ± 0.57^{b}	1.57 ± 0.01^{b}	< 0.001
Heptadecenoic (15:1n5)	0.25 ± 0.11^{ab}	0.65 ± 0.03^{cd}	0.52 ± 0.00b^{cd}	0.38 ± 0.06^{bc}	0.32 ± 0.04^{b}	0.49 ± 0.01b^{cd}	0.70 ± 0.08^{d}	0.00 ± 0.00^{a}	< 0.001
Elaidic (22:1t9)	33.94 ± 0.14^{c}	24.77 ± 3.09^{ab}	27.15 ± 0.25^{bc}	25.42 ± 0.45^{ab}	21.38 ± 1.97^{ab}	18.44 ± 1.47^{a}	18.16 ± 1.29^{a}	20.80 ± 0.64^{ab}	< 0.001
Oleic (18:1n9)	26.84 ± 0.08^{c}	31.85 ± 0.43^{d}	27.73 ± 0.34^{c}	29.33 ± 0.73^{cd}	19.49 ± 0.94^{a}	18.46 ± 0.53^{a}	23.69 ± 0.20^{a}	27.19 ± 0.23^{ab}	< 0.001
Erucic (22:1n9)	0.39 ± 0.03^{a}	0.00 ± 0.00^{a}	1.25 ± 0.03^{a}	0.57 ± 0.45^{a}	4.22 ± 0.09^{b}	9.45 ± 0.65^{c}	1.76 ± 0.71^{a}	3.85 ± 0.24^{b}	< 0.001
Total MUFA (%)	64.78 ± 0.30	59.57 ± 3.59	60.02 ± 0.44	59.70 ± 1.45	47.84 ± 1.09	30.64 ± 1.01	46.39 ± 1.88	33.41 ± 0.88	< 0.001
PUFA									
Linolelaidic (18:2n6)	2.70 ± 0.42^{a}	4.22 ± 2.38^{a}	3.23 ± 0.57^{a}	7.08 ± 1.74^{ab}	8.47 ± 0.24^{ab}	16.88 ± 0.48^{c}	11.59 ± 2.16^{bc}	5.16 ± 0.07^{ab}	< 0.001
Alpha-Linolenic (18:3n3)	0.27 ± 0.01^{c}	0.22 ± 0.01^{bc}	0.00 ± 0.00^{a}	0.48 ± 0.02^{d}	0.72 ± 0.01^{e}	0.13 ± 0.04^{b}	0.19 ± 0.02^{bc}	0.00 ± 0.00^{a}	< 0.001
Gamma-Linolenic (18:3n6)	0.41 ± 0.00^{a}	0.34 ± 0.02^{a}	1.11 ± 0.02^{a}	1.01 ± 0.16^{a}	3.32 ± 0.02^{c}	3.33 ± 0.15^{c}	2.12 ± 0.41^{a}	1.00 ± 0.01^{a}	< 0.001
Total PUFA (%)	3.38 ± 0.43	4.78 ± 2.40	4.34 ± 0.59	8.57 ± 1.81	12.51 ± 0.27	20.34 ± 0.67	13.90 ± 2.57	6.16 ± 0.07	< 0.001

Data are derived from the analysis of fatty acids in different meat cuts in Lwamata sub – county. Values within rows with different superscript letters are significantly different ($p < 0.05$). The values presented are means of three replicates $\pm$ standard errors. S.F.A – saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – Polyunsaturated fatty acid.

Table 4.13: Comparison of SFA, MUFA and PUFA of various tissues from Lwamata sub – county with those from Bukomero sub – county

Total fatty acid composition (%) for Kiboga sub – counties						
Meat cut	Fatty acid	Bukomero	Lwamata	t	p	Df
Brisket	SFA	36.55	32.25	0.17	0.87	2
	MUFA	52.96	64.78	-0.25	0.81	2
	PUFA	12.8	3.38	0.92	0.41	2
Chuck	SFA	34.53	35.73	-0.05	0.96	2
	MUFA	61.76	59.57	0.03	0.98	2
	PUFA	4.72	4.78	-0.45	0.67	2
Rib	SFA	38.41	37.9	0.05	0.96	2
	MUFA	58.72	60.02	-0.03	0.98	2
	PUFA	3.47	4.34	-0.22	0.84	2
Round	SFA	37.37	33.84	0.17	0.87	2
	MUFA	60.67	59.7	0.02	0.99	2
	PUFA	3.15	8.57	-0.82	0.46	2
Liver	SFA	47.94	44.58	0.1	0.93	2
	MUFA	47.9	47.84	0.02	0.98	2
	PUFA	11.36	12.51	-0.12	0.91	2
Kidney	SFA	41.75	40.53	0.01	0.99	2
	MUFA	56.16	30.64	0.24	0.81	2
	PUFA	19.38	20.34	-0.04	0.97	2
Heart	SFA	33.59	41.5	-0.3	0.97	2
	MUFA	48.99	46.39	0.08	0.94	2
	PUFA	26.37	13.9	0.64	0.51	2
Large Intestines	SFA	41.35	44.28	-0.1	0.93	2
	MUFA	56.81	33.41	0.06	0.95	2
	PUFA	9.28	6.16	0.34	0.75	2

Data are derived from the comparison of fatty acid composition in different meat cuts in Kiboga sub - counties. The values presented are averages of total lipids in each sub – county. Degrees of freedom (df), t-values (t), and p-values (p) are

shown for the student's t-test. S.F.A – saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – Polyunsaturated fatty acid

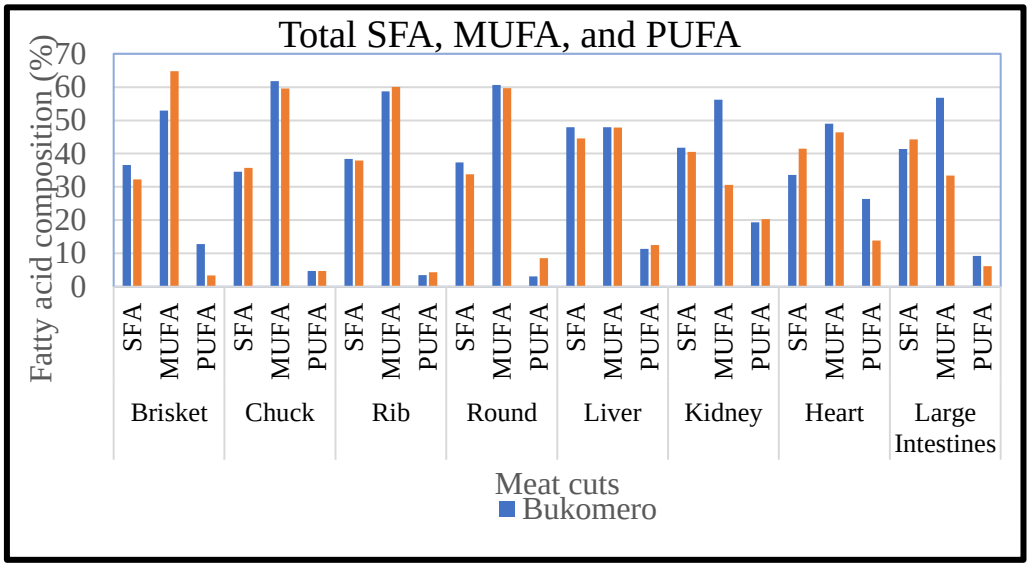


Figure 4.7: Comparison of SFA, MUFA and PUFA of various tissues from Lwamata sub – county with those from Bukomero sub – county

4.3.2 Fatty acid composition (%) of meat cuts from Ntungamo and Rubaare sub – counties in Ntungamo district

The proportions of various fatty acids varied significantly for most meat cuts in the two Ntungamo and Rubaare sub – county of Ntungamo district (**Table 4.14 and 4.15**), ($p < 0.05$),

The fatty acid profiles predominantly consisted of high levels of monounsaturated fatty acids (MUFA), followed by saturated fatty acids (SFA), and then polyunsaturated fatty acids (PUFA). In Ntungamo, the mean SFA ranged from 32.79% to 48.78%, while in Rubaare, it ranged from 34.83% to 55.00%. MUFA ranged from 40.47% to 63.15% in Ntungamo and from 29.54% to 64.07% in Rubaare. PUFA ranged from 3.00% to 11.91% in Ntungamo and from 2.09% to 17.71% in Rubaare. The high levels of MUFA were attributed to substantial

amounts of oleic acid and elaidic acid present in all meat samples from both Ntungamo and Rubaare. SFA were predominantly composed of stearic acid and palmitic acid, while PUFA were primarily represented by Linolelaidic.

The fatty acid compositions differed significantly between various meat cuts within each district ($p < 0.05$). However, there were no statistically significant differences between the sub-counties (student's t – test, $p > 0.05$). This indicates that the fatty acid compositions of meats in Ntungamo were similar to those in Rubaare. For a detailed comparative analysis, **Figure 4.8** was used, it showed only slight differences that were not statistically significant.

Table 4.14: Fatty acid composition (% total fatty acid) of meat cuts from Rubaare sub – county

Fatty Acid	Fatty acid composition (%) of different meat cuts								
	Brisket	Chuck	Rib	Round	Liver	Kidney	Heart	Large Intestines	P – value
SFA									
Myristic (14:0)	3.28 ± 0.11^{bc}	1.63 ± 0.70^{ab}	4.51 ± 0.43^{c}	3.84 ± 0.47^{c}	0.60 ± 0.12^{a}	0.36 ± 0.01^{a}	0.37 ± 0.09^{a}	2.46 ± 0.58^{a}	0.00
Palmitic (16:0)	21.02 ± 0.44^{a}	17.64 ± 0.57^{a}	25.77 ± 0.93^{a}	19.14 ± 8.07^{a}	10.30 ± 5.22^{a}	14.87 ± 0.13^{a}	17.24 ± 0.34^{a}	21.60 ± 1.04^{a}	0.00
Margaric (17:0)	2.33 ± 0.02^{a}	1.06 ± 0.34^{a}	0.97 ± 0.35^{a}	1.79 ± 0.93^{a}	1.83 ± 1.20^{a}	1.15 ± 0.06a^{a}	1.08 ± 0.31^{a}	1.46 ± 0.66a	0.15
Stearic (18:0)	14.78 ± 0.24^{ab}	12.46 ± 1.02^{ab}	11.16 ± 0.76^{a}	18.07 ± 4.24^{ab}	29.89 ± 4.04^{c}	16.69 ± 0.24^{ab}	23.35 ± 0.51^{bc}	23.26 ± 0.44^{bc}	0.84
Total SFA (%)	41.41 ± 1.71	32.79 ± 2.10	42.41 ± 1.82	42.84 ± 3.74	42.62 ± 5.02	33.07 ± 0.43	42.04 ± 0.64	48.78 ± 1.90	0.72
MUFA									
Myristoleic (14:1n5)	0.26 ± 0.04^{ab}	0.58 ± 0.10^{b}	0.32 ± 0.02^{ab}	0.47 ± 0.16^{b}	0.33 ± 0.05^{ab}	0.00 ± 0.00^{a}	0.41 ± 0.05^{b}	0.28 ± 0.03^{ab}	0.00
Palmitoleic (16:1n7)	1.48 ± 1.30	0.22 ± 0.06	2.23 ± 2.05	1.21 ± 1.08	0.41 ± 0.04	0.92 ± 0.35	0.69 ± 0.06	0.56 ± 0.04	0.00
Heptadecenoic (15:1n5)	0.17 ± 0.00^{bc}	0.19 ± 0.01^{bc}	0.12 ± 0.01^{b}	0.23 ± 0.04^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.15 ± 0.01^{bc}	0.00
Elaidic (18:1t9)	35.87 ± 1.14^{b}	36.18 ± 0.51^{b}	33.02 ± 0.76^{b}	35.01 ± 2.47^{b}	24.63 ± 3.30^{a}	16.72 ± 0.37^{b}	23.71 ± 0.34^{b}	22.20 ± 0.79^{b}	0.00
Erucic (22:1n9)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.58 ± 0.03^{a}	0.00 ± 0.00^{a}	15.10 ± 1.55^{d}	11.49 ± 0.60^{c}	0.00 ± 0.00^{a}	4.47 ± 0.55^{b}	0.00
Oleic (18:1n9)	16.73 ± 0.62^{b}	25.98 ± 1.45^{b}	18.81 ± 2.27^{b}	17.22 ± 3.96^{b}	0.00 ± 0.00a	25.90 ± 0.20^{b}	22.91 ± 0.75^{b}	19.21 ± 2.64^{b}	0.00
Total MUFA (%)	54.51 ± 1.71	63.15 ± 2.10	55.08 ± 1.82	54.14 ± 3.74	40.47 ± 5.02	77.94 ± 0.43	47.72 ± 0.64	47.87 ± 1.90	
PUFA									
Linolelaidic (18:2n6)	2.84 ± 0.21^{a}	3.23 ± 0.32^{a}	1.91 ± 0.19^{a}	2.12 ± 0.50^{a}	6.95 ± 0.95^{bc}	10.23 ± 0.21^{c}	9.07 ± 0.76^{c}	3.69 ± 1.35^{ab}	0.00
Alpha-Linolenic (18:3n3)	0.68 ± 0.09^{ab}	0.57 ± 0.07^{ab}	0.26 ± 0.03^{ab}	0.17 ± 0.04^{a}	2.07 ± 0.25^{c}	1.18 ± 0.36^{bc}	1.18 ± 0.11^{bc}	0.41 ± 0.08^{ab}	0.00
Gamma-Linolenic (18:3n6)	0.55 ± 0.00b^{c}	0.31 ± 0.03^{abc}	0.54 ± 0.03^{bcd}	0.71 ± 0.19^{cd}	0.86 ± 0.12^{d}	0.50 ± 0.02^{bcd}	0.00 ± 0.00^{a}	0.24 ± 0.07^{ab}	0.00
Total PUFA (%)	4.07 ± 0.21	4.11 ± 0.32	2.71 ± 0.19	3.00 ± 0.50	9.88 ± 0.95	11.91 ± 0.36	10.25 ± 0.76	4.34 ± 1.35	

Data are derived from the analysis of fatty acids in different meat cuts in Ntungamo sub – county. Values within rows with different superscript letters are significantly different ($p < 0.05$). The values presented are means of three replicates ± standard errors. S.F.A – saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – Polyunsaturated fatty acid.

Table 4.15: Fatty acid composition (% total fatty acid) of meat cuts from Rubaare sub – county

	Muscles and organ tissues								
Fatty Acid	Brisket	Chuck	Rib	Round	Liver	Kidney	Heart	Large Intestines	P – value
SFA									
Myristic (14:0)	3.45 ± 0.28^{e}	1.78 ± 0.25^{bc}	2.35 ± 0.49^{cd}	2.38 ± 0.10^{cd}	1.25 ± 0.07^{abc}	0.33 ± 0.05^{a}	0.56 ± 0.06^{ab}	3.32 ± 0.33^{de}	< 0.001
Palmitic (16:0)	23.36 ± 0.20^{b}	20.00 ± 1.45^{ab}	22.79 ± 1.07^{dc}	21.87 ± 0.88^{ab}	22.34 ± 0.27^{ab}	22.36 ± 0.82^{ab}	18.53 ± 0.29^{a}	22.48 ± 0.70^{ab}	0.323
Margaric (17:0)	2.26 ± 0.12^{bcd}	1.57 ± 0.13^{ab}	2.09 ± 0.05^{abcd}	1.83 ± 0.08^{abc}	2.71 ± 0.00^{d}	1.39 ± 0.00^{a}	2.60 ± 0.00^{d}	2.42 ± 0.01cd	0.017
Stearic (18:0)	14.40 ± 0.35^{ab}	10.48 ± 5.26^{a}	17.95 ± 0.51^{abc}	$17.51 \pm 1.06a^{bc}$	28.27 ± 0.81^{bc}	25.44 ± 3.79^{bc}	29.40 ± 0.51^{c}	27.78 ± 1.77^{bc}	< 0.001
Total SFA (%)	43.47 ± 1.26	34.83 ± 6.22	44.18 ± 1.12	43.59 ± 1.22	54.07 ± 1.27	49.52 ± 4.66	51.09 ± 0.56	55.00 ± 2.29	
MUFA									
Myristoleic (14:1n5)	0.31 ± 0.02^{a}	0.73 ± 0.13^{a}	0.41 ± 0.14^{a}	0.88 ± 0.60^{a}	0.17 ± 0.00^{a}	0.00 ± 0.00^{a}	0.76 ± 0.44^{a}	0.29 ± 0.02^{a}	< 0.001
Palmitoleic (16:1n7)	0.16 ± 0.00^{a}	3.10 ± 0.00^{e}	2.79 ± 0.15^{de}	2.57 ± 0.07^{d}	1.78 ± 0.03^{b}	1.61 ± 0.05^{b}	2.15 ± 0.06^{c}	0.44 ± 0.03^{a}	< 0.001
Heptadecenoic (15:1n5)	0.15 ± 0.01^{b}	0.19 ± 0.01^{bc}	0.15 ± 0.01^{b}	0.22 ± 0.02^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.14 ± 0.01^{b}	< 0.001
Elaidic (18:1t9)	37.76 ± 0.52^{cd}	35.81 ± 4.25^{d}	29.49 ± 0.23^{bcd}	30.79 ± 1.25^{bcd}	25.84 ± 0.31^{abc}	19.84 ± 2.95^{a}	22.04 ± 0.78^{ab}	21.74 ± 1.56^{ab}	< 0.001
Oleic (22:1n9)	16.16 ± 1.12^{b}	24.24 ± 0.63^{b}	19.52 ± 1.84^{b}	15.75 ± 3.44^{b}	0.00 ± 0.00^{a}	2.75 ± 0.11^{a}	2.52 ± 0.04^{a}	18.59 ± 2.97^{b}	< 0.001
Erucic (18:1n9)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.58 ± 0.02^{a}	0.00 ± 0.00^{a}	6.74 ± 0.31^{b}	14.63 ± 2.95^{c}	4.07 ± 0.78^{ab}	0.53 ± 0.03^{a}	< 0.001
Total MUFA (%)	54.58 ± 1.35	64.07 ± 5.18	52.94 ± 1.58	50.21 ± 6.00	34.33 ± 0.34	38.83 ± 3.21	29.54 ± 0.91	41.73 ± 3.26	
PUFA									
Linolelaidic (18:2n6)	3.13 ± 0.16^{ab}	1.26 ± 1.26^{a}	3.42 ± 0.28^{ab}	4.15 ± 0.85^{ab}	7.80 ± 0.78^{ab}	8.83 ± 3.36^{bc}	15.78 ± 0.66^{c}	1.61 ± 0.11^{ab}	< 0.001
Alpha-Linolenic (18:3n3)	$0.84 \pm 0.02b^{c}$	0.53 ± 0.00^{ab}	0.18 ± 0.01^{a}	1.42 ± 0.13^{cd}	2.29 ± 0.07^{c}	2.29 ± 0.32^{e}	1.61 ± 0.07^{ab}	0.37 ± 0.04^{a}	< 0.001
Gamma-Linolenic (18:3n6)	0.51 ± 0.00^{bcd}	0.30 ± 0.03^{abc}	0.22 ± 0.01^{ab}	0.68 ± 0.15^{d}	0.81 ± 0.02^{d}	0.54 ± 0.02^{ed}	0.32 ± 0.00^{abc}	0.30 ± 0.03^{abc}	< 0.001
Total PUFA (%)	4.48 ± 0.18	2.09 ± 1.29	3.82 ± 0.30	6.25 ± 1.01	10.90 ± 0.85	11.66 ± 3.55	17.71 ± 0.73	2.28 ± 0.16	

Data are derived from the analysis of fatty acids in different meat cuts in Rubaare sub – county. Values within rows with different superscript letters are significantly different ($p < 0.05$). The values presented are means of three replicates $\pm$ standard errors. S.F.A – saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – Polyunsaturated fatty acid.

Table 4.16: Comparison of SFA, MUFA and PUFA of various tissues from of Ntungamo with those from Rubaare sub – county

Total fatty acid composition (%) for Kiboga sub – counties					
Meat Cut	Fatty Acid	Bukomero	Lwamata	t-value	p-value
Brisket	SFA	41.41	43.47	-0.17	0.87
	MUFA	54.51	54.58	-0.25	0.81
	PUFA	4.07	4.48	0.92	0.41
Chuck	SFA	32.79	34.83	-0.05	0.96
	MUFA	63.15	64.07	0.03	0.98
	PUFA	4.11	2.09	-0.45	0.67
Rib	SFA	42.41	44.18	0.05	0.96
	MUFA	55.08	52.94	-0.03	0.98
	PUFA	2.71	3.82	-0.22	0.84
Round	SFA	42.84	43.59	0.17	0.87
	MUFA	54.14	50.21	0.02	0.99
	PUFA	3	6.25	-0.82	0.46
Liver	SFA	42.62	54.07	0.1	0.93
	MUFA	40.47	34.33	0.02	0.98
	PUFA	9.88	10.9	-0.12	0.91
Kidney	SFA	33.07	49.52	0.01	0.99
	MUFA	77.94	38.83	0.24	0.81
	PUFA	11.91	11.66	-0.04	0.97
Heart	SFA	42.04	51.09	-0.3	0.97
	MUFA	47.72	29.54	0.08	0.94
	PUFA	10.25	17.71	0.64	0.51
Large Intestines	SFA	48.78	55	-0.1	0.93
	MUFA	47.87	41.73	0.06	0.95
	PUFA	4.34	2.28	0.34	0.75

Data are derived from the comparison of fatty acid composition in different meat cuts of Ntungamo sub - counties. The values presented are averages of total lipids in each sub – county. Degrees of freedom (df), t-values (t), and p-values (p) are shown for the student's t-test. S.F.A – saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – Polyunsaturated fatty acid

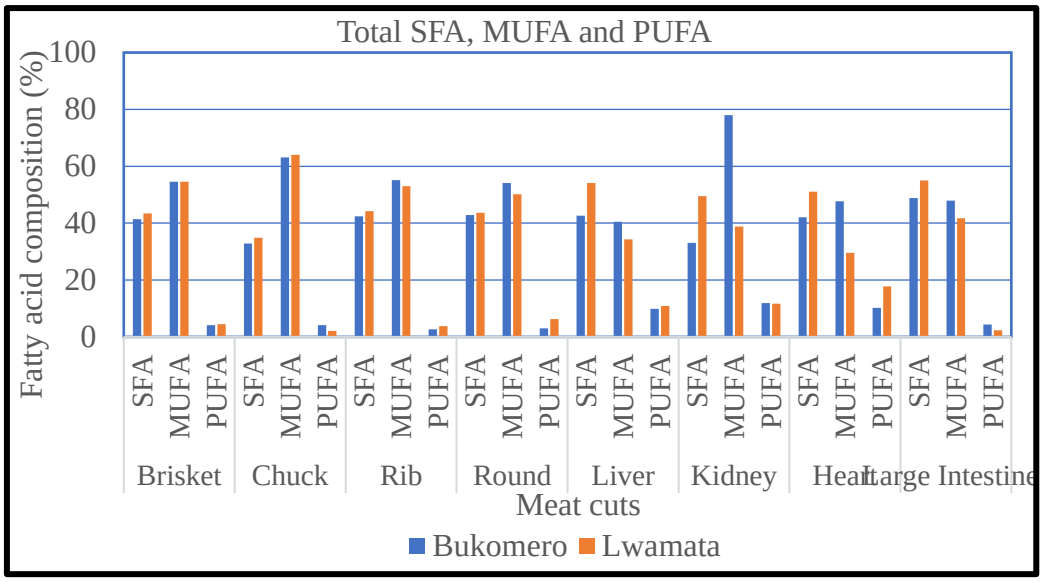


Figure 4.8: Comparison of SFA, MUFA and PUFA of various tissues from Lwamata sub – county with those from Bukomero sub – county

4.3.3 Comparison of the SFA, MUFA and PUFA of meat cuts from Kiboga district with those from Ntungamo district.

The fatty acid composition in each of the two districts predominantly consisted of monounsaturated fatty acids (MUFA), followed by saturated fatty acids (SFA) and then polyunsaturated fatty acids (PUFA) (**Table 4.17**). MUFA was largely represented by high proportions of oleic acid and elaidic acid. SFA was mainly composed of elevated levels of palmitic acid and stearic acid. While there was slight variation between the districts, these differences were not statistically significant (Student's t-test, $p > 0.05$) for the majority of fatty acids (**Figure 4.9**). Further analysis indicated that the fatty acid proportions were slightly lower in the Kiboga district compared to the Ntungamo district.

Table 4.17: Comparison in the SFA, MUFA and PUFA of various tissues from Kiboga district with those from Ntungamo district.

Total fatty acid composition (%) for Ntungamo and Kiboga district					
Meat cut	Fatty acid	Ntungamo	Kiboga	t	p
Brisket	SFA	42.44	34.40	0.05	0.96
	MUFA	54.545	58.87	0.00	1.00
	PUFA	4.275	8.09	-0.12	0.91
Chuck	SFA	33.81	35.13	-0.11	0.91
	MUFA	63.61	60.67	-0.02	0.99
	PUFA	3.1	4.75	-0.58	0.57
Rib	SFA	43.295	38.16	0.32	0.75
	MUFA	54.01	59.37	0.05	0.96
	PUFA	3.265	3.91	-0.27	0.80
Round	SFA	43.215	35.61	0.46	0.65
	MUFA	52.175	60.19	0.08	0.93
	PUFA	4.625	5.86	-0.34	0.74
Liver	SFA	48.345	46.26	0.1	0.92
	MUFA	37.4	47.87	0.16	0.87
	PUFA	10.39	11.94	-0.27	0.80
Kidney	SFA	41.295	41.14	0.03	0.98
	MUFA	58.385	43.40	0.48	0.64
	PUFA	11.785	19.86	-0.73	0.48
Heart	SFA	46.565	37.55	0.44	0.67
	MUFA	38.63	47.69	0.45	0.66
	PUFA	13.98	20.14	-0.45	0.66
Large intestines	SFA	51.89	42.82	0.44	0.66
	MUFA	44.8	45.11	0.15	0.89
	PUFA	3.31	7.72	-0.98	0.35

Data are derived from the comparison of fatty acid composition in different meat cuts of Kiboga and Ntungamo districts. The values presented are averages of total lipids in each sub – county. Degrees of freedom (df), t-values (t), and p-values (p) are shown for the student's t-test. S.F.A – saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – Polyunsaturated fatty acid

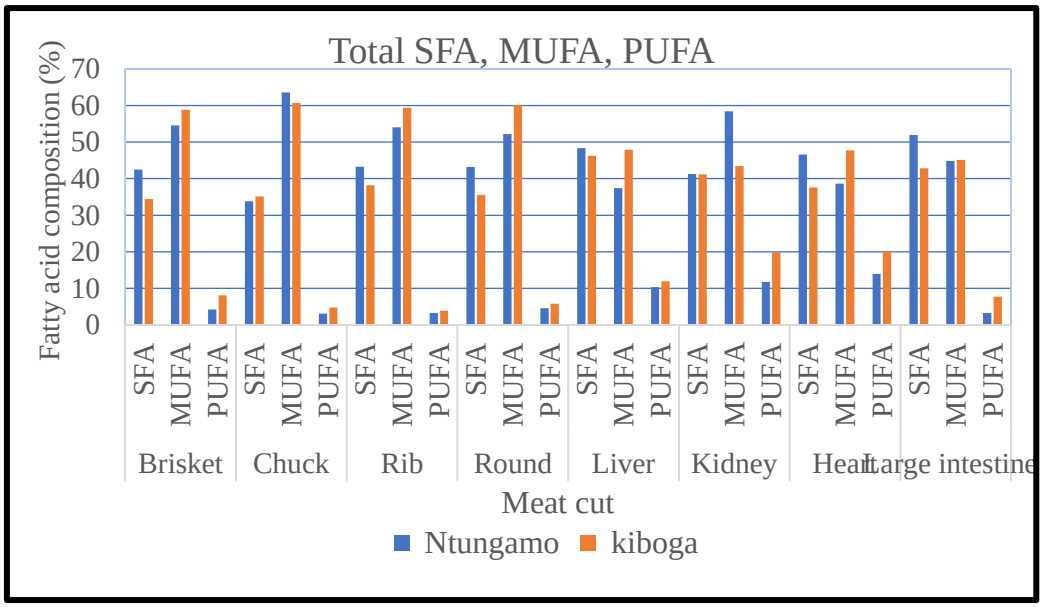


Figure 4.9: Comparison in the SFA, MUFA and PUFA of various tissues from Kiboga district with those from Ntungamo district.

Generally, proportions of all fatty acids varied significantly for meat cuts within the same geographical region (**Table 4.11, 4.12, 4.14 and 4.15**), there was a significant different in the proportions of fatty acids among meats ($p < 0.05$) in each of the sub – counties Bukomero, Lwamata, Ntungamo and Rubaare, primarily stem from their distinct biological functions and tissue characteristics (Webb and O'Neill, 2008). These findings concur with the findings of Turk and Smith. (2009) who observed a significant difference in fatty content in round, sirloin, loin, rib, chuck, brisket, plate and flank of beef. The findings indicated that the fatty acid composition across the meat cuts between the sub-counties and districts showed insignificant differences in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) content (Student's t-test, $p > 0.05$). This lack of significant difference can be attributed to the relatively uniform feeding practices, genetic homogeneity of the

Ankole cattle, and similar environmental conditions within the districts. (Budge et al., 2002; Koçyigit et al., 2023, Smet et al., 2004, Mwangi et al., 2019).

Proportions of fatty acids consisted of mostly MUFA followed by SFA and the PUFA. MUFA levels were due to high levels of oleic acid and elaidic that dominated most meat samples, probably because of conversion of some saturated fatty acids to MUFA by the enzyme by the enzyme stearoyl Co-A desaturase (Piccinin et al., 2019; Noto et al., 2013; Kamal et al., 2018; Wood et al., 2008). The high levels of oleic and elaidic acids in the some cuts could be due to differences in their anatomical location, dietary influences, and specific fat metabolism (Smith, 2024a).

In the analyzed tissues, stearic acid and palmitic acids were the most abundant SFA, with concentrations reaching up to 28.27 ± 0.81% in the liver and 29.40 ± 0.51% in the heart, reflecting its significant roles in energy storage and membrane stability (Javadi et al., 2004). Palmitic acid was notably higher in muscle tissues, such as the brisket and rib, with concentrations of up to 23.36 ± 0.20% and 22.79 ± 1.07%, respectively, highlighting its importance as an energy source and in fatty acid synthesis (Fatima et al., 2019). This finding is supported by the finding of Koçyigit et al. (2023); Jang et al. (2017); Boni et al. (2010) who found out that stearic acid and palmitic acid were present in high quantity among the meat cuts. This because palmitic acid and stearic are naturally synthesized by animals then become part of the fat composition in the animal's tissues, including the meat (Smith, 2024a; Martins et al., 2018).

In both regions, the large intestines consistently showed the highest SFA content, with stearic acid being the predominant fatty acid. This indicates that large

intestines are rich in saturated fats, which are mainly composed of stearic acid. This could be due to biohydrogenation in the rumen by microbes which converts unsaturated fatty acids to saturated fatty acids (Dewanckele et al., 2020; Baldin et al., 2022).

The Heart exhibited the highest polyunsaturated fat (PUFA) content (**26.37%**), particularly due to linolelaidic and gamma-linolenic acids, highlighting its beneficial fat profile.

4.4 Nutritional quality implications of fatty acid profiles and cholesterol based on established dietary guidelines

The data were used to assess the nutritional quality implications associated with fatty acids and cholesterol profiles using nutritional indices such as the PUFA/SFA ratio, the Index of atherogenicity (IA), the Index of thrombogenicity (IT), the Hypocholesterolemic / Hypercholesterolemic ratio (HH), the health-promoting index (HPI), and the Unsaturation Index (UI) of fats in different tissues.

4.4.1 Nutritional indices for meat cuts from Ntungamo sub – county

The findings (**Table 4.18**) showed that PUFA/SFA ratio was highest in kidney (0.36) and lowest in rib (0.06), suggesting kidney had a more favorable fat balance. The Index of Atherogenicity (IA**)** was highest in rib (0.82) and lowest in brisket (0.71), indicating higher cardiovascular risk for rib. The Index of thrombogenicity (IT) was highest in large intestines

(0.83), suggesting a greater risk of thrombosis. The HH Ratio was highest in kidney (0.70), indicating better cholesterol profile potential. The health-promoting index (HPI) was highest in liver (0.60), reflecting a better-unsaturated

fat profile. The Unsaturation index (UI) was highest in heart (49.90), showing it had the greatest degree of unsaturation. For a detailed analysis, the nutritional indices are compared using **figure 4.10.**

Table 4.18: Nutritional indices for meat cuts from Ntungamo sub – county

	Meat cuts and their nutritional indices							
Fatty acid index	Brisket	Chuck	Rib	Round	Liver	Kidney	Heart	Large Intestines
PUFA/SFA	0.10	0.13	0.06	0.07	0.23	0.36	0.24	0.09
IA	0.71	0.73	0.82	0.74	0.76	0.74	0.75	0.83
IT	0.65	0.80	0.80	0.82	0.79	0.78	0.78	0.83
HH	0.50	0.62	0.53	0.53	0.48	0.70	0.65	0.50
HPI	0.55	0.58	0.50	0.53	0.60	0.57	0.58	0.50
UI	26.20	28.63	21.97	21.97	20.11	2.10	49.90	27.82

PUFA/SFA: Polyunsaturated fatty acid/Saturated fatty acid; IA: Index of atherogenicity; IT: Index of thrombogenicity; HH: Hypocholesterolemic /hypercholesterolemic. HPI: Health-promoting Index;

UI: Unsaturation index.

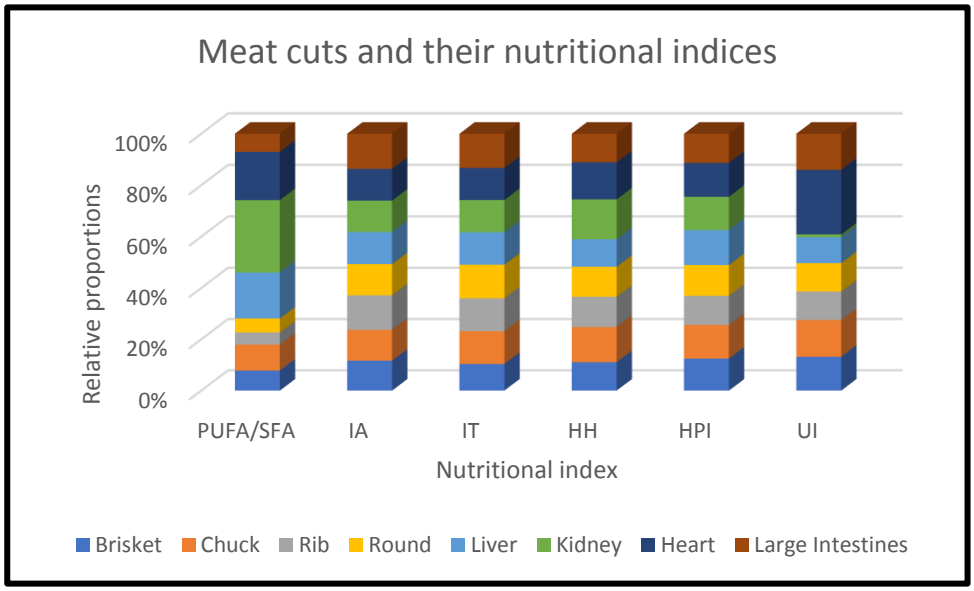


Figure 4.10: Nutritional indices for meat cuts from Ntungamo sub – county

4.4.2 Nutritional indices for meat cuts from Rubaare sub – county

The results (**Table 4.19**) showed that the PUFA/SFA ratio was highest in the heart (0.35) and lowest in intestines (0.04), indicating a better fat balance in the heart compared to other tissues. The index of atherogenicity (IA) was highest in the chuck (0.95), suggesting an increased cardiovascular risk, while the index of thrombogenicity (IT) was elevated in large intestines (2.17), reflecting a higher risk of clot formation. The hypercholesterolemic-hyperlipidemic (HH) index was lowest in the liver (0.21), implying a lower impact on cholesterol levels. The Unsaturation index (UI) was highest in the heart (42.46), highlighting a potentially greater overall health risk. For a detailed analysis, the nutritional indices are compared using **figure 4.11**

Table 4.19: Nutritional indices for meat cuts from Rubaare sub – county

	Meat cuts and their nutritional indices							
Fatty acid index	Brisket	Chuck	Rib	Round	Liver	Kidney	Heart	Large intestines
PUFA/SFA	0.10	0.06	0.09	0.14	0.20	0.24	0.35	0.04
IA	0.80	0.95	0.82	0.93	0.72	0.75	0.62	0.87
IT	1.09	1.23	1.12	1.20	0.47	0.71	1.74	2.17
HH	0.50	0.82	0.54	0.53	0.21	0.30	0.42	0.39
HPI	0.50	0.72	0.49	0.48	0.38	0.49	0.51	0.35
UI	26.43	32.78	30.54	33.12	24.04	29.97	42.46	24.25

PUFA/SFA: Polyunsaturated fatty acid/saturated Fatty Acid; IA: Index of atherogenicity; IT: Index of thrombogenicity; HH: Hypocholesterolemic/ hypercholesterolemic;

HPI: Health-promoting index; UI: Unsaturation Index.

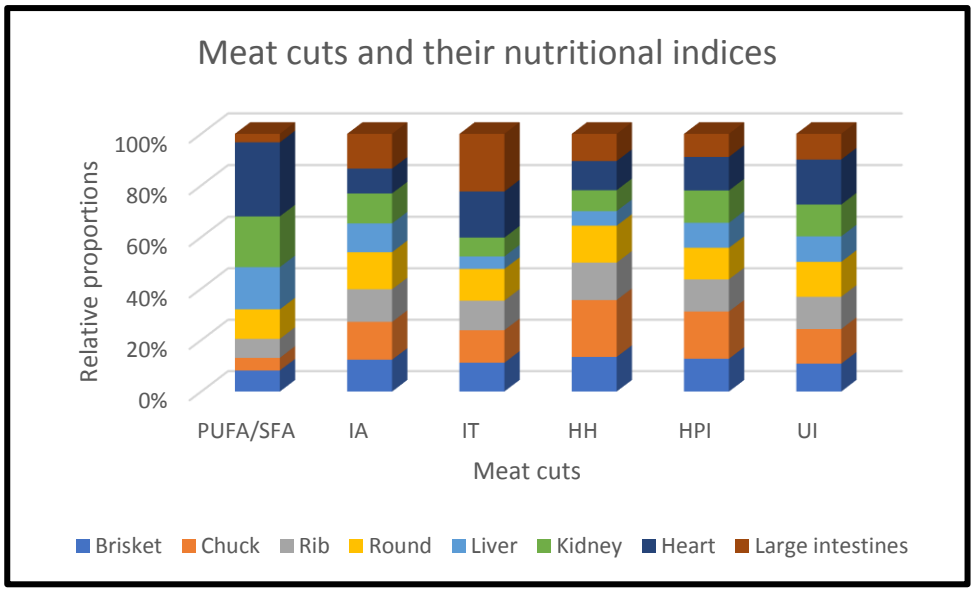


Figure 4.11: Nutritional indices for meat cuts from Rubaare sub – county

4.4.3 Nutritional indices for meat cuts from Bukomero sub – county

The findings (**Table 4.20**) showed that the PUFA/SFA ratio was highest in the heart (0.78) and lowest in the rib (0.09), indicating a less favorable balance of polyunsaturated to saturated fats compared to other tissues. The index of atherogenicity (IA) was highest in the rib (1.70), suggesting a greater risk of heart disease, whereas the heart had the lowest IA (0.96). The Index of Thrombogenicity (IT) was highest in the liver (1.50), signaling increased thrombosis risk. Both the hypercholesterolemic-hyperlipidemic index (HH) and hypercholesterolemic Potential Index (HPI) were very high in large intestines (3.01), reflecting the highest cholesterol risk. The Unsaturation index (UI) was also highest in the heart (101.73), indicating a high overall health risk. For a detailed analysis, the nutritional indices are compared using **figure 4.12.**

Table 4.20: Nutritional indices for meat cuts from Bukomero sub – county:

Meat cuts and their nutritional indices								
Fatty acid index	Brisket	Chuck	Rib	Round	Liver	Kidney	Heart	Large intestines
PUFA/SFA	0.35	0.14	0.09	0.08	0.24	0.46	0.78	0.21
IA	1.08	1.29	1.7	1.64	1.63	1.32	0.96	1.2
IT	1.02	0.95	1.14	1.06	1.5	1.05	0.87	0.63
HH	1.86	1.98	1.66	1.75	1.3	1.91	2.33	3.01
HPI	1.86	1.98	1.66	1.75	1.3	1.91	2.33	3.01
UI	78.56	71.2	65.66	66.97	70.62	94.92	101.73	75.37

PUFA/SFA: Polyunsaturated fatty acid/Saturated fatty acid; IA: Index of atherogenicity; IT: Index of thrombogenicity; HH: Hypocholesterolemic/ hypercholesterolemic.

HPI: Health-promoting index; UI: Unsaturation Index.

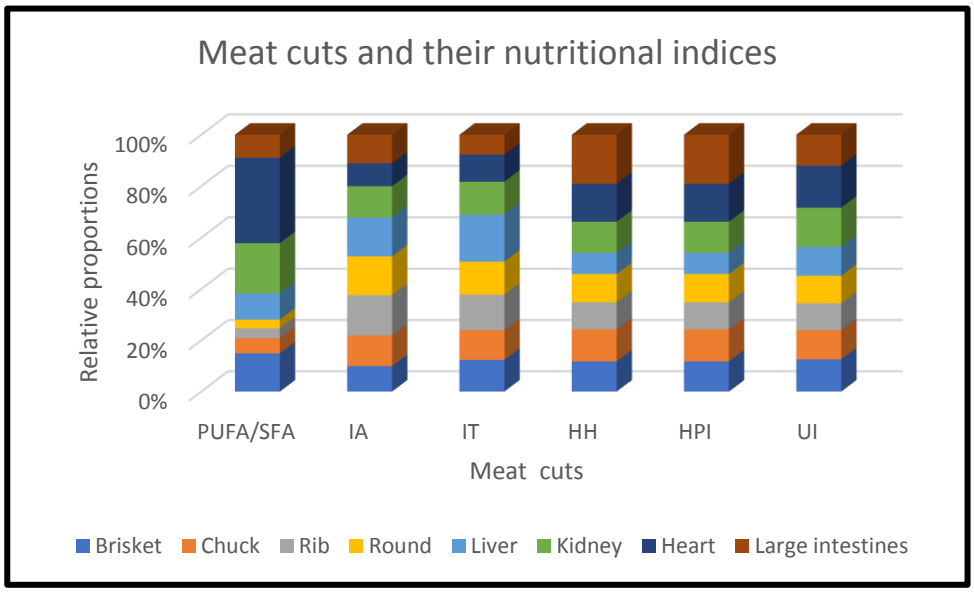


Figure 4.12: Nutritional indices for meat cuts from Bukomero sub – county

4.4.4 Nutritional indices for meat cuts from Lwamata sub – county

The results (**Table 4.21**) indicated that the PUFA/SFA ratio was highest in kidney (0.50) and lowest in brisket (0.10), indicating a more favorable balance of polyunsaturated to saturated fats in the kidney. The Index of atherogenicity (IA) peaked in large intestines (2.69), suggesting the highest cardiovascular risk, while the lowest IA was found in brisket (0.35). The Index of thrombogenicity (IT) was elevated in intestines (2.10), pointing to increased clot risk, with the lowest IT in round (0.80). Both the hypercholesterolemic-hyperlipidemic index (HH) and Hypercholesterolemic potential index (HPI) were highest in brisket and lowest in intestines, reflecting higher cholesterol risks in brisket. The Unsaturation index (UI) was highest in round (76.84), highlighting a potential overall health risk, while intestines had the lowest UI (45.73). For a detailed analysis, the nutritional indices are compared using **figure 4.13**

Table 4. 21: Nutritional indices for meat cuts from Lwamata sub – county

	Meat cuts and their nutritional indices							
Fatty acid	Brisket	Chuck	Rib	Round	Liver	Kidney	Heart	Large intestines
PUFA/SFA	0.10	0.13	0.11	0.25	0.28	0.50	0.33	0.14
IA	0.35	1.25	0.47	1.30	1.48	1.97	1.46	2.69
IT	0.85	0.93	1.03	0.80	1.26	1.50	1.21	2.10
HH	3.16	1.91	1.83	2.14	1.47	1.30	1.57	0.92
HPI	3.16	1.91	1.83	2.14	1.47	1.30	1.57	0.92
UI	71.54	69.13	68.70	76.84	72.86	71.32	74.19	45.73

PUFA/SFA: Polyunsaturated fatty acid/saturated fatty acid; IA: Index of atherogenicity; IT: Index of thrombogenicity; HH: Hypocholesterolemic/ hypercholesterolemic.

HPI: Health-promoting index; UI: Unsaturation Index.

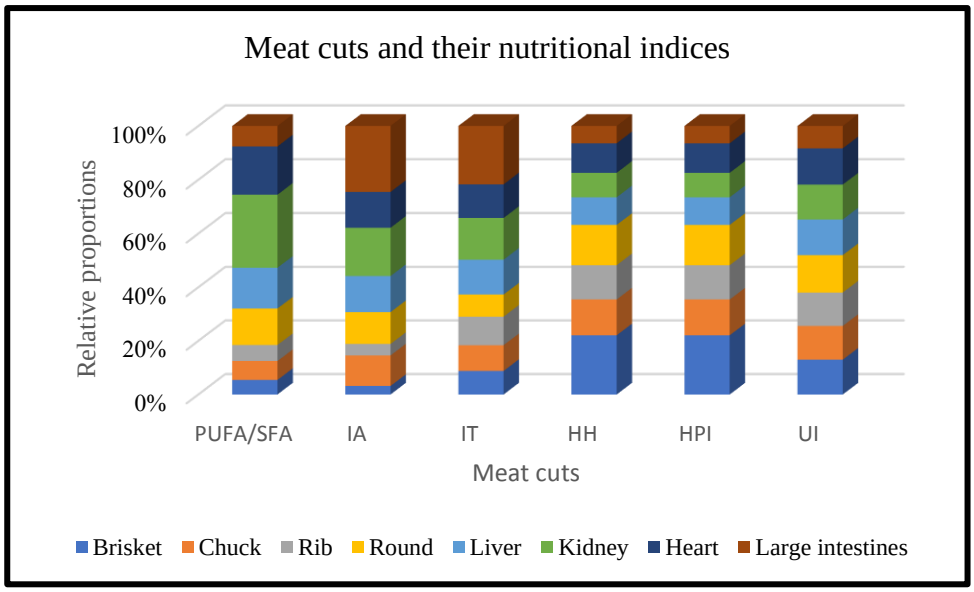


Figure 4.13: Nutritional indices for meat cuts from Lwamata sub – county

The nutritional indices of various organs in long-horned Ankole cattle showed notable differences that significantly impact cardiovascular health. Among these organs, the kidney, liver, and heart exhibited more favorable nutritional indices compared to the rib, large intestines, and chuck. Specifically, the kidney, liver, and heart demonstrated higher PUFA/SFA, HH, and HPI ratios, suggesting a beneficial balance of fats associated with lower LDL cholesterol levels and reduced cardiovascular risk (Goluch et al., 2023; Wood and Enser, 2017). In contrast, the rib, large intestines, and chuck showed higher health risks due to elevated Index of Atherogenicity (IA) and Index of thrombogenicity (IT), indicating an increased risk of blood clot formation and thrombosis. Barrea et al. (2023) noted that foods with high atherogenic and thrombogenic potential could stimulate platelet aggregation, raising concerns about cardiovascular health.

The nutritional ratios observed in this study varied notably from those reported in earlier research. For example, the PUFA/SFA ratio ranged from 0.04 to 0.78, generally exceeding the narrower ranges documented in previous studies (Chen

and Liu, 2020c; Barrea et al., 2023). The Index of atherogenicity (IA) ranged from 0.35 to 1.70, often surpassing earlier values, while the hypercholesterolemic-hyperlipidemic index (HH) ranged from 0.21 to 3.01, showing a broader range and lower average than previously reported. The Unsaturation index (UI) also displayed a wider range (24.04 to 101.73), indicating a higher degree of unsaturation in some samples. Despite this variability, the PUFA/SFA ratios were lower than the recommended threshold of 0.4 for a healthy cardiovascular status (Ospina et al., 2011; Wood et al., 2004)

Overall, while the kidney, liver, and heart demonstrated beneficial nutritional profiles, they still contained significant amounts of palmitic, myristic, and stearic acids, which can be detrimental. Attia et al. (2015) highlighted that myristic and palmitic acids are highly atherogenic, and stearic acid is known to be thrombogenic. Conversely, the rib, large intestines, and chuck presented higher health risks due to less favorable indices related to atherosclerosis and thrombosis. The 2005 US Dietary Guidelines recommend moderating red and processed meat intake due to concerns over saturated fat's effects on LDL cholesterol (Flock and Kris-Etherton, 2011). However, the relationship between meat consumption and chronic diseases like coronary heart disease, stroke, and type 2 diabetes remains complex and debated, with conflicting results in the literature (Shi et al., 2023). Further research is needed to clarify the impact of meat consumption on health and to develop more precise dietary recommendations (Zeraatkar et al., 2019; Bechthold et al., 2019).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The study indicated that organ meats including liver, kidney, heart, and large intestines—consistently contained the highest lipid and cholesterol levels across all sub-counties in Ntungamo (Ntungamo and Rubaare) and Kiboga (Bukomero and Lwamata) districts. In Kiboga, liver had the highest lipid content (2.38 ± 0.36% in Bukomero and 3.08 ± 0.91% in Lwamata) and the highest cholesterol (206.57 ± 2.88 mg/100 g in Bukomero and 177.18 ± 3.28 mg/100 g in Lwamata), while chuck, rib, and round cuts had the lowest values.

In Ntungamo, liver again contained the highest lipids (3.55 ± 0.46% in Ntungamo sub-county and 3.00 ± 0.22% in Rubaare) and cholesterol (187.21 ± 2.92 mg/100 g in Ntungamo sub-county and 155.95 ± 1.37 mg/100 g in Rubaare), with muscle cuts such as chuck, brisket, rib, and round showing consistently lower levels. Across districts, most meat cuts showed no significant differences in lipid content ($P > 0.05$), although Ntungamo generally had slightly higher lipids, while cholesterol differed significantly in specific cuts such as brisket and kidney. The high lipid and cholesterol levels in organ meats suggest potential health risks, including hypercholesterolemia, atherosclerosis, and cardiovascular diseases, if consumed frequently without balance.

Analysis of fatty acids revealed that monounsaturated fatty acids (MUFA) were dominant across all meat cuts, particularly in organ meats such as kidney (**77.94%**) and chuck (**63.15%**), providing potential benefits for cardiovascular health. Saturated fatty acids (SFA) were also significant (**32.25–55.00%**), with

palmitic and stearic acids being the most prevalent, while polyunsaturated fatty acids (PUFA) were lower, peaking in the heart (**17.71%**). Despite the presence of beneficial MUFAs, the substantial SFA content raises concerns regarding increased risks of obesity, coronary heart disease, and other lifestyle-related disorders. Overall, MUFA levels were moderate across districts, PUFA/SFA ratios were low (**0.04–0.05**), and there were no significant differences between Ntungamo and Kiboga, indicating that tissue type rather than district largely determines fatty acid profiles.

Nutritional indices highlighted that certain cuts offered more favourable health profiles. The kidney exhibited a beneficial PUFA/SFA ratio (**0.36**) and health-promoting index (HH = **0.70**), while the liver and heart also had relatively healthier fatty acid compositions. Conversely, rib, large intestines, and chuck had elevated atherogenicity (IA) and thrombogenicity (IT) indices, indicating higher risks of cardiovascular diseases.

These findings underscore the need for balanced consumption: while organ meats provide beneficial MUFAs, their high SFA and cholesterol content necessitates moderation, especially for individuals with heart disease or hyperlipidemia. Lean muscle cuts, such as rib and round, are better suited for cardiovascular health-conscious diets, providing lower lipid and cholesterol intake while still contributing essential fatty acids. Overall, meats from Ntungamo and Kiboga offer moderate health benefits, but careful selection and portion control are critical to minimize potential dietary-related health risks.

5.2 Recommendations

There is a need to compare the fatty acid and cholesterol profiles of long-horned Ankole cattle with those of other cattle breeds to understand their unique nutritional characteristics. This comparative analysis could reveal distinctive features or advantages of Ankole cattle, contributing to a better understanding of their potential role in promoting health through dietary choices.

Consumers should be encouraged to select meat cuts with lower levels of total lipids and cholesterol, such as brisket and round cuts, to support better heart health and manage cholesterol levels effectively.

Dietary interventions should aim to modify the fatty acid composition of Ankole cattle to enhance their nutritional profile. Controlled feeding trials with various supplements or forage types can help identify the best strategies for increasing beneficial fatty acids while reducing undesirable ones.

Breeding programs should be implemented to select for desirable fatty acid profiles in Ankole cattle. Utilizing genomic selection and targeted breeding strategies focused on traits such as desaturase activity and lipid deposition can improve the nutritional quality of Ankole beef, aligning it with consumer preferences for healthier meat options.

Furthermore, identifying lower lipid profiles in meat cuts suggests potential health benefits for cardiovascular and metabolic health. Additional research is needed to explore these effects and develop effective dietary strategies. Collaboration among researchers, policymakers, and healthcare professionals is

essential to apply these findings and promote healthier diets while reducing disease risk.

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APPENDICES

Appendix 1: Total Lipid content in Rubaare sub – county

Meat cut	Replicate	Sample Wt (g)	Wt. of empty vial (g)	Wt. of vial+lipid (g)	Wt of lipid(g)	Percentage
Brisket	A	1.0095	16.0144	16.0307	0.0163	1.6147
Brisket	B	1.0702	17.3286	17.3408	0.0122	1.1400
Brisket	C	1.0618	16.1304	16.1399	0.0095	0.8947
Chuck	A	1.0955	15.9927	16.0041	0.0114	1.0406
Chuck	B	1.1027	16.4522	16.4677	0.0155	1.4056
Chuck	C	1.0692	12.8884	12.8964	0.008	1.4056
Rib	A	1.0828	17.0121	17.0186	0.0065	0.5999
Rib	B	1.0738	12.4481	12.4796	0.0315	2.9310
Rib	C	1.0003	16.7969	16.8111	0.0142	1.4193
Round	A	1.0022	17.6494	17.6729	0.0235	2.3456
Round	B	1.0902	16.3929	16.4081	0.0152	1.3969
Round	C	1.058	16.4891	16.5012	0.0121	1.1475
Liver	A	1.0018	10.9834	11.0093	0.0259	2.5853
Liver	B	1.0983	13.225	13.2588	0.0338	3.0775
Liver	C	1.0608	10.8137	10.849	0.0353	3.3277
Kidney	A	1.0374	11.1294	11.1562	0.0268	2.5834
Kidney	B	1.0456	16.3113	16.3273	0.016	1.5302
Kidney	C	1.0454	16.1448	16.1716	0.0268	2.5636
Heart	A	1.0415	16.4219	16.4345	0.0126	1.2098
Heart	B	1.0837	15.8849	15.9131	0.0282	2.6022
Heart	C	1.032	16.1484	16.1696	0.0212	2.0543
Offals	A	1.0968	13.5795	13.5997	0.0202	1.8437
Offals	B	1.1107	12.7126	12.7437	0.0312	2.7968
Offals	C	1.055	16.2812	16.3012	0.0200	1.8993

Lipid content for Ntungamo sub – county

Tissue	Replicates	Sample Wt (g)	Wt. of empty vial (g)	Wt. of vial+lipid (g)	Wt of lipid(g)	Percentage
Brisket	A	1.0778	15.6038	15.618	0.0142	1.3175
Brisket	B	1.0105	16.3022	16.3194	0.0172	0.7021
Brisket	C	1.0646	17.5274	17.5443	0.0169	1.1875
Chuck	A	1.0573	16.8578	16.8753	0.0175	0.6952
Chuck	B	1.0102	16.5366	16.5428	0.0062	0.6287
Chuck	C	1.0438	16.377	16.3847	0.0077	0.7397
Rib	A	1.098	16.1766	16.1842	0.0076	0.6922
Rib	B	1.0477	16.2784	16.2857	0.0073	0.6968
Rib	C	1.061	17.4437	17.4565	0.0128	1.2064
Round	A	1.0513	15.9681	15.9689	0.0008	1.0761
Round	B	1.0522	16.429	16.4395	0.0105	0.9979
Round	C	1.0519	16.5545	16.5814	0.0269	1.0573
Liver	A	1.0834	15.7223	15.754	0.0317	2.926
Liver	B	1.0713	16.6031	16.6378	0.0347	3.2391
Liver	C	1.0302	15.0414	15.0876	0.0462	4.4846
Kidney	A	1.0781	16.454	16.4643	0.0103	0.9554
Kidney	B	1.0322	16.445	16.456	0.011	1.3657
Kidney	C	1.0726	16.1431	16.1549	0.0118	1.3001
Heart	A	1.0251	16.9677	16.9806	0.0129	1.2584
Heart	B	1.0105	15.7305	15.7446	0.0141	1.3953
Heart	C	1.0271	16.0067	16.0278	0.0211	2.0543
Large intestines	A	1.0761	16.1006	16.11	0.0094	0.8735
Large intestines	B	1.1013	16.6433	16.6947	0.0514	3.7672
Large intestines	C	1.1412	15.7733	15.7785	0.0052	0.4557

Lipid content in Bukomero sub – county

Tissue	Replicate	Sample wt (g)	Vial wt (g)	Vial+ Sample wt (g)	Wt of Lipid	Percentage
Brisket	A	1.0325	15.7919	15.7992	0.0073	0.7070
Brisket	B	1.0168	17.2829	17.2882	0.0053	0.5212
Brisket	C	1.0247	16.5374	16.5437	0.0063	0.6148
Chuck	A	1.0040	16.3987	16.4026	0.0039	0.3884
Chuck	B	1.0713	16.0665	16.0714	0.0049	0.4574
Chuck	C	1.0377	16.2326	16.2370	0.0044	0.4240
Rib	A	1.0433	16.3436	16.3558	0.0122	1.1694
Rib	B	1.0439	16.4055	16.4187	0.0132	1.2645
Rib	C	1.0436	16.3746	16.3873	0.0127	1.2169
Round	A	1.0940	15.6357	15.6426	0.0069	0.6307
Round	B	1.0586	16.4269	16.4344	0.0075	0.7085
Round	C	1.0763	16.0313	16.0385	0.0072	0.6690
Liver	A	1.0824	16.5566	16.5891	0.0325	3.0026
Liver	B	1.0843	15.6923	15.7114	0.0191	1.7615
Liver	C	1.0834	16.1245	16.1503	0.0258	2.3815
Kidney	A	1.0393	15.9475	15.9762	0.0287	2.7615
Kidney	B	1.0191	16.5863	16.6036	0.0173	1.6976
Kidney	C	1.0292	16.2669	16.2899	0.0230	2.2347
Heart	A	1.0989	16.3965	16.4111	0.0146	1.3286
Heart	B	1.0377	17.3522	17.3673	0.0151	1.4551
Heart	C	1.0683	16.8744	16.8892	0.0149	1.3901
Offals	A	1.0758	17.1397	17.1628	0.0231	2.1472
Offals	B	1.0670	15.8657	15.8838	0.0181	1.6963
Offals	C	1.0714	16.5027	16.5233	0.0206	1.9227

Appendix 2: Cholesterol concentrations determined by HPLC

Cholesterol content for Rubaare sub – county

Sample code	Area	Average Area	Wt in gm	Conc in mg/L	Final Vol. in litre	Final Conc in mg/Kg	Conc in g/100g	conc. mg /100g
Brisket	241512				0.003			
	240562	239979.3333	2.0405	155.6460624	0.003	228.8352	0.022884	22.8835
	237864				0.003			
					0.003			
Brisket	220822				0.003			
	251467	230057	2.0405	148.1205916	0.003	217.771	0.021777	21.7771
	217882				0.003			
					0.003			
Brisket	287057				0.003			
	286024	286474.3333	2.0405	190.9096195	0.003	280.6806	0.028068	28.0681
	286342				0.003			
					0.003			
Chuck	252295				0.003			
	259832	257445.3333	2.0366	168.8929339	0.003	248.7866	0.024879	24.8787
	260209				0.003			
					0.003			
Chuck	288718				0.003			
	293390	290689.6667	2.0366	194.1066869	0.003	285.9276	0.028593	28.5928
	289961				0.003			
					0.003			
Chuck	269052				0.003			
	264385	265241	2.0366	174.8054608	0.003	257.496	0.02575	25.7496
	262286				0.003			

					0.003			
Rib	258161				0.003			
	256538	256775	2.0229	168.3845279	0.003	249.7175	0.024972	24.9718
	255626				0.003			
					0.003			
Rib	244886				0.003			
	244672	245534	2.0229	159.8589306	0.003	237.0739	0.023707	23.7074
	247044				0.003			
					0.003			
Rib	261330				0.003			
	261164	262397	2.0229	172.6484642	0.003	256.041	0.025604	25.6041
	264697				0.003			
					0.003			
Round	479820				0.003			
	480616	483095	2.0726	340.0341297	0.003	492.1849	0.049218	49.2185
	488849				0.003			
					0.003			
Round	503612				0.003			
	511215	508799.3333	2.0726	359.5292631	0.003	520.4033	0.05204	52.0403
	511571				0.003			
					0.003			
Round	491799				0.003			
	505956	496865	2.0726	350.4778157	0.003	507.3017	0.05073	50.7302
	492840				0.003			
					0.003			
Liver	924290				0.003			
	921499	922823.6667	2.0088	673.5408924	0.003	1005.885	0.100589	100.5885

	922682				0.003			
					0.003			
Liver	1116767				0.003			
	1118594	1118280.333	2.0088	821.7825812	0.003	1227.274	0.122727	122.7274
	1119480				0.003			
					0.003			
Liver	1063602				0.003			
	1066963	1066594.667	2.0088	782.5822273	0.003	1168.731	0.116873	116.8731
	1069219				0.003			
					0.003			
Kidney	1458820				0.003			
	1461588	1460810	2.064	1081.569966	0.003	1572.049	0.157205	157.2049
	1462022				0.003			
					0.003			
Kidney	1426664				0.003			
	1422074	1424506.667	2.064	1054.036152	0.003	1532.029	0.153203	153.2029
	1424782				0.003			
					0.003			
Kidney	1462453				0.003			
	1461901	1462924.333	2.064	1083.173556	0.003	1574.38	0.157438	157.4380
	1464419				0.003			
					0.003			
Heart	523485				0.003			
	504531	515800.6667	2.0962	364.8393376	0.003	522.1439	0.052214	52.2144
	519386				0.003			
					0.003			
Heart	572385				0.003			

	575136	574252.3333	2.0962	409.1712805	0.003	585.59	0.058559	58.5590
	575236				0.003			
					0.003			
Heart	493891				0.003			
	491718	491218.3333	2.0962	346.1951713	0.003	495.4611	0.049546	49.5461
	488046				0.003			
					0.003			
Large intestines	580705				0.003			
	569719	573282.3333	2.0407	408.435596	0.003	600.4346	0.060043	60.0435
	569423				0.003			
					0.003			
Large intestines	541848				0.003			
	539524	541318	2.0407	384.1926432	0.003	564.7954	0.05648	56.4795
	542582				0.003			
					0.003			
Large intestines	578390	579131.5	2.0407	412.871824	0.003	606.9562	0.060696	60.6956
	579873				0.003			
	578910				0.003			

Cholesterol content for Ntungamo sub – county

Sample code	Area	Average Area	Wt in gm	Conc in mg/L	Final Vol. in litre	Final Conc in mg/Kg	Conc in g/100g	conc. mg /100g
	293495				0.003			
Brisket	294010	294323.6667	2.0012	196.8628492	0.003	295.1172	0.029512	29.5117
	295466				0.003			
					0.003			
	328606				0.003			
Brisket	326616	328229	2.0012	222.5779295	0.003	333.6667	0.033367	33.3667
	329465				0.003			
					0.003			
	323134				0.003			
Brisket	322750	321911	2.0012	217.7861206	0.003	326.4833	0.032648	32.6483
	319849				0.003			
					0.003			
	317260				0.003			
Chuck	315041	316161	2.0199	213.4251043	0.003	316.9837	0.031698	31.6984
	316182							
	324825							
Chuck	323146	323500	2.0199	218.991278	0.003	325.2507	0.032525	32.5251
	322529							
	448515							
Chuck	435965	438131	2.0199	305.9317406	0.003	454.3766	0.045438	45.4377
	429913							

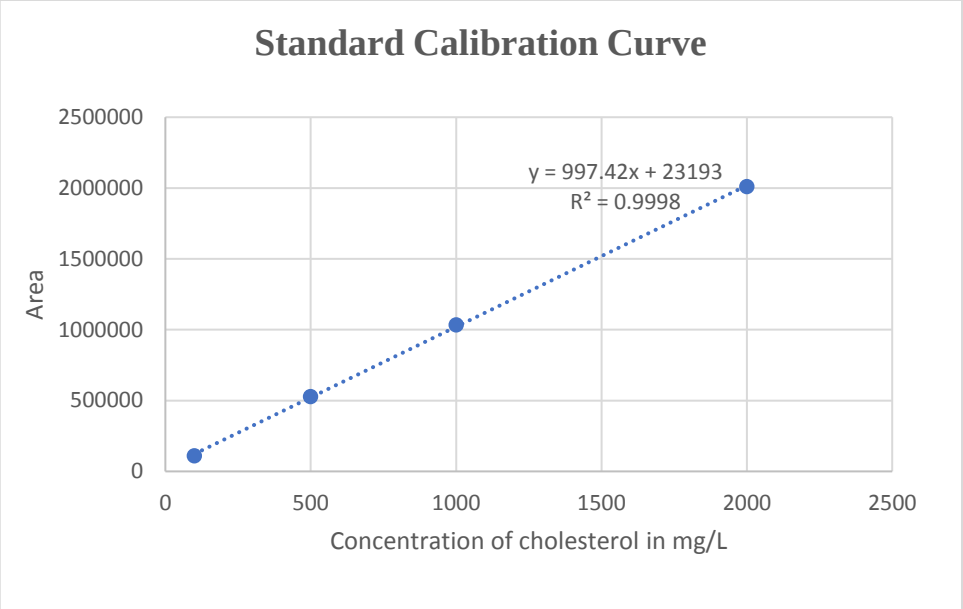
	320748							
Rib	318456	318158.6667	2.0441	214.9402098	0.003	315.4545	0.031545	31.5455
	315272				0.003			
					0.003			
	262460				0.003			
Rib	257568	259747.6667	2.0441	170.6391101	0.003	250.4365	0.025044	25.0437
	259215				0.003			
					0.003			
	273995				0.003			
Rib	266405	268738.3333	2.0438	177.4579699	0.003	260.4824	0.026048	26.0482
	265815				0.003			
					0.003			
	301664				0.003			
Round	303019	303095.6667	2.0438	203.515864	0.003	298.7316	0.029873	29.8732
	304604				0.003			
					0.003			
	275234				0.003			
Round	275962	275971	2.0438	182.9434964	0.003	268.5343	0.026853	26.8534
	276717				0.003			
					0.003			
	1319018				0.003			
Liver	1292874	1295375.333	2.0535	956.0980913	0.003	1396.783	0.139678	139.6783
	1274234				0.003			
					0.003			
	1087492				0.003			
Liver	1076252	1083806.667	2.0535	795.6364556	0.003	1162.362	0.116236	116.2362
	1087676				0.003			

					0.003			
	1233763				0.003			
Liver	1233710	1238121.333	2.0535	912.6745039	0.003	1333.345	0.133334	133.3345
	1246891				0.003			
					0.003			
	1735962				0.003			
Kidney	1759092	1743673.667	2.0983	1296.104412	0.003	1853.078	0.185308	185.3078
	1735967				0.003			
					0.003			
	1819852				0.003			
Kidney	1811249	1814108.333	2.0983	1349.524712	0.003	1929.454	0.192945	192.9454
	1811224				0.003			
					0.003			
	1727009				0.003			
Kidney	1725086	1725987	2.0983	1282.690178	0.003	1833.899	0.18339	183.3899
	1725866				0.003			
					0.003			
	547323				0.003			
Heart	535825	543296	2.0554	385.6928328	0.003	562.9457	0.056295	56.2946
	546740				0.003			
					0.003			
Heart	520626				0.003			
	532098	528396.3333	2.0554	374.3923651	0.003	546.4518	0.054645	54.6452
	532465				0.003			
					0.003			
	551719				0.003			
Heart	556810	553921.3333	2.0554	393.7514853	0.003	574.7078	0.057471	57.4708

	553235				0.003			
					0.003			
	727575				0.003			
Large instestines	728252	727109.3333	2.0149	525.1037795	0.003	781.831	0.078183	78.1831
	725501				0.003			
					0.003			
	676535				0.003			
Large intestines	677918	677573.3333	2.0149	487.5338137	0.003	725.8928	0.072589	72.5893
	678267				0.003			
					0.003			
	656191				0.003			
Large intestines	648167	653333	2.0149	469.149033	0.003	698.5196	0.069852	69.8520
	655641				0.003			

Appendix 3: Calibration curves for cholesterol and standard mixture for FA

Conc in mg/L	Area
2000	2009820
1000	1035389
500	527702
100	110582



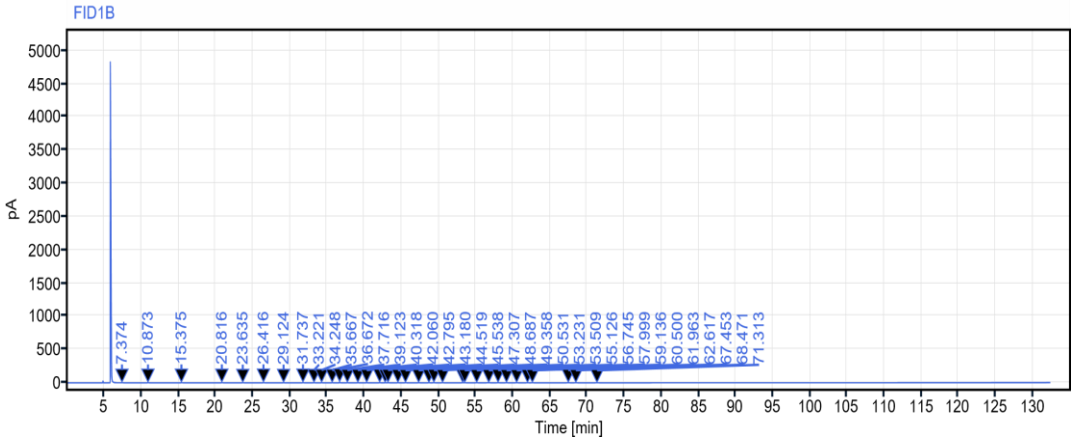
Appendix 4: Standard mixture for fatty acid analysis

Single Injection

Agilent

Data 2023-09-08 11-52-06+03-00-

Sequence Name:	051	Project Name:	UNBS 8890 GC
Sample name:	STD	Operator:	SYSTEM
Instrument:	UNBS 8890 GC	Injection date:	2023-09-08 11:56:04+03:00
Inj. volume:	1.000	Location:	102
Acq. method:	FAME2.amx	Type:	Sample
Processing method:	*GC_LC Area Percent_DefaultMethod.pmx	Sample amount:	0.00



Signal: FID1B

C4	7.37	0.54	30.97	5.56	2.49
C6	10.87	0.71	39.39	6.16	3.17
C8	15.37	0.76	44.88	6.39	3.61
C10	20.82	1.08	47.94	6.68	3.86
C11	23.64	0.83	24.43	3.39	1.97
	26.42	1.10	50.19	7.21	4.04
C13	29.12	0.93	25.12	3.69	2.02
C14	31.74	0.79	50.69	7.84	4.08
(C14:1[cis-9])	33.22	0.62	24.50	3.66	1.97
	34.25	0.71	25.91	4.07	2.09
(C15:1 [cis-10])	35.67	0.71	25.41	3.85	2.04
C16	36.67	0.77	80.62	13.09	6.49
(C16:1[cis-9]	37.72	0.84	25.65	3.78	2.06
C17:0	39.12	0.91	27.13	3.88	2.18
(C17:1 [cis-10])	40.32	0.87	26.11	3.26	2.10
C18:0	42.06	0.74	53.51	6.39	4.31

RT	[min]	Width	Area	Height	(%)
	42.79	0.41	25.53	2.90	2.05
(C18:1 [trans-9])	43.18	0.96	53.94	5.79	4.34
(C18:2 [trans-9,12])	44.52	0.91	25.07	2.63	2.02
(C18:2 [Cis-9,12])	45.54	0.70	25.16	2.52	2.03
C20:0	47.31	0.94	23.53	2.15	1.89
(C18:3 [cis-6,9,12])	48.69	0.64	22.15	2.04	1.78
(C18:3 [cis-9,12,15])	49.36	0.83	54.53	5.42	4.39
(C20:1 [cis-11])	50.53	0.98	27.23	2.52	2.19
	53.23	0.44	23.80	2.16	1.92
C21:0	53.51	0.81	28.84	2.52	2.32
C22:0	55.13	0.97	24.00	1.92	1.93
(C22:1 [cis-13])	56.74	1.45	46.36	2.22	3.73
(C20:4 [cis-5,8,11,14])	58.00	1.17	55.10	4.71	4.43
(C20:3 [cis-8,11,14])	59.14	1.20	27.31	2.28	2.20
C23:0	60.50	1.09	22.11	1.58	1.78
	61.96	0.65	25.06	2.00	2.02
(C22:2 [cis-13,16])	62.62	0.77	27.47	2.33	2.21
Methyl cis 5,8,11,14,17eicosapentaenoate	67.45	1.03	55.08	4.39	4.43
(C24:1 [cis-15])	68.47	0.95	27.19	2.16	2.19
Methyl docosahexaenoate (cis4,7,10,13,16,19)	71.31	1.11	20.70	1.42	1.67
		Sum	1242.57		

Appendix 5: HPLC Sample Chromatograms

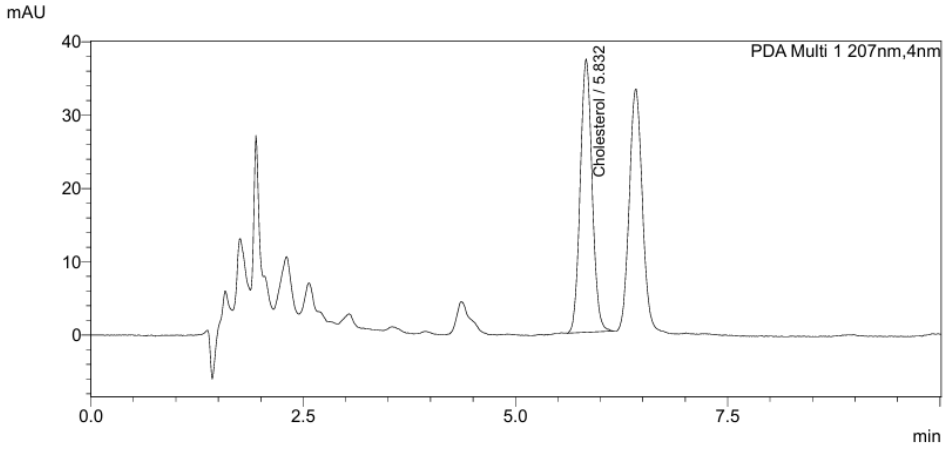
SHIMADZU
LabSolutions

DGAL
FOOD AND DRUGS LAB
ANALYSIS REPORT

<Sample Information>

Sample Name	: CholesterolNTM 11A-1		
Sample ID	:		
Data Filename	: CholesterolNTM 11A-1_29072023_017.lcd		
Method Filename	: Cholesterol Method.lcm		
Batch Filename	: Cholesterol-1.lcb		
Vial #	: 1-9	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 29/07/23 21:20:06	Acquired by	: System Administrator
Date Processed	: 29/07/23 21:30:08	Processed by	: System Administrator

<Chromatogram>



<Peak Table>

Peak Table

PDA Ch1 207nm

Peak#	Name	Ret. Time	Area	Conc.	Unit
1	Cholesterol	5.832	362571	0.000	mg/L
Total			362571		

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<Sample Information>

Sample Name	: CholesterolNTM 11B-2		
Sample ID	:		
Data Filename	: CholesterolNTM 11B-2_29072023_050.lcd		
Method Filename	: Cholesterol Method.lcm		
Batch Filename	: Cholesterol-1.lcb		
Vial #	: 1-17	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 30/07/23 03:10:18	Acquired by	: System Administrator
Date Processed	: 30/07/23 03:20:21	Processed by	: System Administrator

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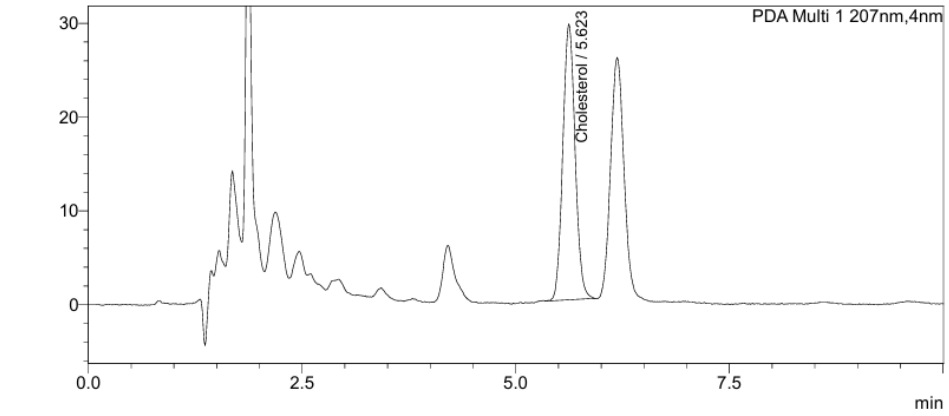

<Peak Table>

Peak Table

PDA Ch1 207nm

Peak#	Name	Ret. Time	Area	Conc.	Unit
1	Cholesterol	5.623	294589	0.000	mg/L
Total			294589		

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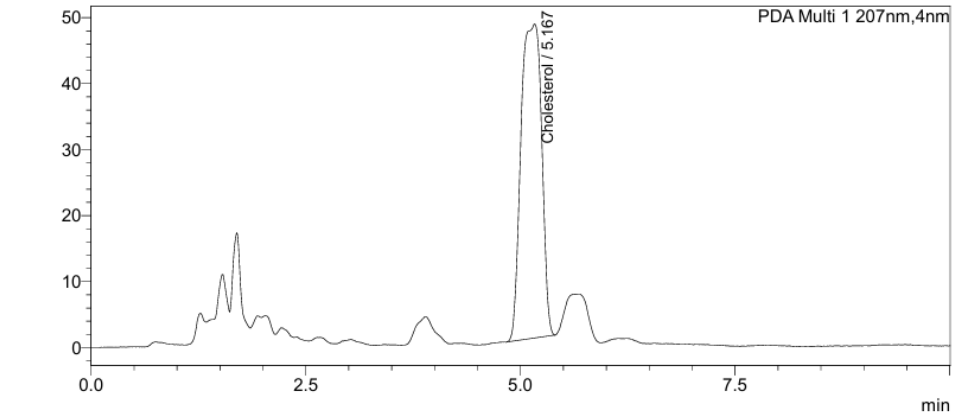
<Sample Information>

Sample Name	: Cholesterol KBO 38B-2		
Sample ID	:		
Data Filename	: Cholesterol KBO 38B-2_03082023_036.lcd		
Method Filename	: Cholesterol Method.lcm		
Batch Filename	: Cholesterol-2.lcb		
Vial #	: 1-105	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 04/08/23 02:19:58	Acquired by	: System Administrator
Date Processed	: 04/08/23 02:30:01	Processed by	: System Administrator

<Chromatogram>



<Peak Table>

Peak Table

PDA Ch1 207nm

Peak#	Name	Ret. Time	Area	Conc.	Unit
1	Cholesterol	5.167	795244	0.000	mg/L
Total			795244		

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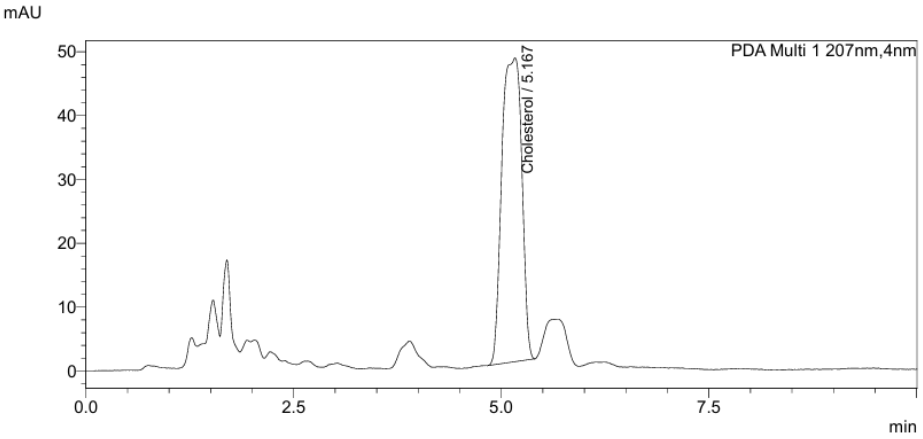
ANALYSIS REPORT

<Sample Information>

Sample Name	: Cholesterol KBO 38B-2		
Sample ID	:		
Data Filename	: Cholesterol KBO 38B-2_03082023_036.lcd		
Method Filename	: Cholesterol Method.lcm		
Batch Filename	: Cholesterol-2.lcb		
Vial #	: 1-105	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 04/08/23 02:19:58	Acquired by	: System Administrator
Date Processed	: 04/08/23 02:30:01	Processed by	: System Administrator

<Chromatogram>



<Peak Table>

Peak Table

PDA Ch1 207nm

Peak#	Name	Ret. Time	Area	Conc.	Unit
1	Cholesterol	5.167	795244	0.000	mg/L
Total			795244		