Protein and lipid gastro-small intestinal digestibility *in vitro* of pasture-fed beef, grain-finished beef, and meat alternative: A comparative study

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Technology

in

Food Technology

at Massey University, Palmerston North, New Zealand.

Amrutha Elamurugan

2022

Abstract

This study addresses the protein and lipid digestibility of meat and a plant-based meat alternative using a static model *in vitro* digestion system. Three commercially relevant beef cuts (tenderloin, striploin and topside) from five carcasses of two different production system namely, pasture-feeding and grain-finishing along with a plant-based meat alternative (Beyond Burger® from Beyond Meat™) were chosen for this study.

Breakdown of proteins and the release of peptides during digestion were analysed using tricine SDS-PAGE. The free amino nitrogen released during *in vitro* digestion was determined using ninhydrin assay. The results showed that there were no significant differences ($p<0.05$) between the pasture-fed and grain-finished meat digests in terms of *in vitro* protein digestibility. Both striploin and tenderloin gave good *in vitro* protein digestibility, but the topside did not perform well, mainly due to the longer cooking time and higher cook loss. The plant-based meat alternative performed relatively poorly in *in vitro* protein digestion experiments, possibly due to the formation of digestion-resistant protein aggregates formed during the manufacturing process.

In terms of *in vitro* lipid digestibility, this study concludes that pasture-fed beef showed higher amounts of total long chain (LCn-3) polyunsaturated fatty acids (PUFAs) and lower amounts of many free individual saturated fatty acids (SFAs) than those from grain-finished animals. However, grain-finished meat digests were high in total monounsaturated fatty acids (MUFAs) when compared to pasture-fed meat digests. The plant-based meat alternative digests had the highest amount of total SFAs, mostly contributed by lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0). The MUFAs were also significantly higher ($p< 0.05$) in the Beyond Burger® owing mainly to high amounts of individual free oleic acid (C18:1c9) and vaccenic acid (C18:1c11). In Beyond Burger® there was an abundance of n-6 PUFAs in the form of the individual free linoleic acid (C18:2). However, no LCn-3 PUFAs were detected in the plant-based meat alternative.

Acknowledgements

My Master's research and thesis writing journey has been extremely rewarding and challenging at the same time. I am extremely grateful to several people who have provided constant encouragement and unending support through the ups and downs of this unforgettable and amazing journey. I would like to start by expressing my deepest gratitude to Dr. Lovedeep Kaur, my supervisor for giving me the opportunity to take up this research work, encouraging me to put on my 'scientist hat' from day one, for being patient and providing invaluable guidance throughout my research journey. I would like to wholeheartedly thank Dr. Mike Boland my co-supervisor for the prompt, valuable and constructive feedback on my research work and thesis. I am extremely grateful to Dr. Feng Ming Chian for providing valuable insights, training, and for also carrying out *in vitro* digestion experiments and analysis of topside and tenderloin cuts. I would like to thank Dr. Scott Knowles and Dr. Emma Bermingham from AgResearch and Dr. Andrea Braakhuis from University of Auckland for their suggestions for choosing the meat cuts and providing valuable suggestions throughout the study. I would also like to thank the Meat Industry Association, Beef + Lamb New Zealand Ltd, the High-Value Nutrition National Science Challenge and the Ministry of Business, Innovation and Employment for funding this research programme.

I am thankful to all the laboratory managers Mrs. Michelle Tamehana, Mr. Steve Glasgow, Mr. Warwick Johnson, and Mr. Garry Radford from the School of Food and Advanced Technology, Ms Maggie Zou and Dr. Peter Zhu from the Riddet Institute and Mr. Michael Agnew from AgResearch for their assistance in lab and equipment training. I am thankful to Dr Nihal Jayamaha for helping me with statistical analysis of my data. I would like to show my appreciation for the support provided by the administrative staff from both the School of Food and Advanced technology and the Riddet Institute thanks to Ms Karen Pickering, Ms. Dilantha Punchihewa, Mrs. Miria Busby, Mr. John Henley-King and Ms. Terri Palmer.

I'm grateful to my wonderful colleagues and officemates, for supporting me and giving valuable inputs in my research journey and helping me embrace different cultures during my stay in New Zealand. To my amazing friends turned NZ family: Shawn, Osho, Phaveethra, and Arun thank you all for the countless pep talks, board game nights, and trips around New Zealand that has given me many memories to be cherished for a lifetime.

I am extremely lucky to be showered with lots of love from my extended family in Chennai. The never-ending support provided by my family back in India has made me who I am today.

Words cannot express how grateful I am to my parents for all their sacrifices. Their emotional and financial support along with their trust and confidence in me has given me the strength, positivity, and belief to pursue and complete my Master's in New Zealand.

Table of Contents

List of figures

List of tables

Abbreviations

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AUS-MEAT	Authority for the Uniform Specification of Meat and Livestock
BB	Beyond Burger
CIE	Commission Internationale de l‘Eclairage
CLA	Conjugated linoleic acids
CVD	Cardiovascular diseases
DHA	Docosahexaenoic acid
DI	Desaturase indices
EPA	Eicosapentaenoic acid
FAMEs	Fatty acid methyl esters
FFA	Free fatty acids
GC	Gas Chromatography
IMF	Intramuscular fat
LCn-3 PUFAs	Long chain n-3 polyunsaturated fatty acids
MSA	Meat Standards Australia
MUFAs	Monounsaturated fatty acids
PCA	Principle component analysis
PUFAs	Polyunsaturated fatty acids
SCD	Stearoyl CoA desaturases
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SFAs	Saturated fatty acids

SLG	Striploin grain-finished
SLP	Striploin pasture-fed
TLG	Tenderloin grain-finished
TLP	Tenderloin pasture-fed
TSG	Topside grain-finished
TSP	Topside pasture-fed

Chapter 1: Introduction

Meat has been a part of the human diet for at least 2.6 million years (Wyness, 2016). It is the edible portion especially from the flesh of mammals such as cattle, goat, sheep, swine, or even rabbits, (commonly known as red meat) and other non-mammals such as chicken, ducks, and turkey (white meat) and varieties of seafood (Vaclavik & Christian, 2014). Nowadays, meat is predominantly sold either fresh or frozen and frequently consumed throughout the world. The demand for meat is on the rise due to the increase in demographic growth, income, and shift in consumer preferences (Scozzafava et al., 2016; Vaclavik & Christian, 2014).

Red meat is an integral part of the human diet as it provides a good source of protein that contains essential amino acids; glutamine (about 16.5%) is the most abundantly found amino acid in meat followed by arginine, alanine, and aspartic acid (Roseland et al., 2018). Meat also contains fatty acids such as saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) and a wide range of micronutrients like zinc, iron, selenium, phosphorous, folic acid, and traces of vitamins A and B complex (especially vitamin B12 in higher amounts). Red meat especially contains a significant amount of highly bioavailable haem iron that is contributed by its myoglobin content (Wyness., 2016). All these nutrients help to maintain an overall good health and may vary in profiles between different muscle cuts and among animal species. Generally, red meat contains about 70 to 75% water, 5 to 20% fat (depending on the location of the cut), 16 to 23% protein and 3.5 to 5% non-protein substances and inorganic compounds. (Roseland et al., 2018; Wyness., 2016).

For many years, there have also been health claims that relate to the risk of chronic diseases like cardiovascular disease (CVD) and colon cancer with prolonged consumption of red meat (De la Fuente et al., 2009; Jung et al., 2016). The presence of saturated fatty acids and the amount of fat content in different cuts have been related to health complications in several epidemiological studies conducted in western countries (Jung et al., 2016; Wyness, 2016). On the other hand, contradicting popular perceptions the fat content in many retail beef cuts has been declining due to more fat trimming, production of leaner cattle breeds, and enhanced animal husbandry practices. Hence it has become crucial to communicate these changes to consumers (Roseland et al., 2018). In New Zealand, premium meat products are sourced from animals that are mostly raised on pasture and hence any differences from grain-finished beef that prove to be nutritionally beneficial will be an advantage. The incorporation and moderate

consumption of lean red meat in the diet has been shown to positively influence nutrient uptake and overall long-term health (Mora et al., 2017).

In recent times, there has been a slight transition to plant-based diets by consumers for being more sustainable and healthier when compared to meat-related products, although the data on sustainability and nutritional quality of plant-based meat alternative products is lacking. Indeed, meat still occupies an integral position in Western food culture and everyday meals. It is still seen as an irreplaceable source of vitality. Hence the main challenge for the meat substitute product developers is to make their product appeal to meat-eating consumers and significantly improve the sensory attributes so it resembles meat as much as possible (Graça et al., 2015).

Studies have shown that meat protein is highly digestible, but residual undigested protein has been known to cause changes in the metabolism and population structure of the colon microbiome in the human body, which can lead to adverse health conditions (Bax et al., 2012). In recent studies it has been argued that higher rates of release of amino acids (particularly branched chain amino acids) during the digestion of meat lead to anabolic effects in muscle structure, which leads to maintenance and gain in muscle mass. This is an essential process for elderly people to manage sarcopenia (muscle wasting), fitness seekers, athletes and body builders who look to increase the muscle mass (Berrazaga et al., 2019).

The nutritional quality of the meat and its related products is determined by the digestibility of the proteins and lipids in the gastro-intestinal transit. The digestion of meat is a complex phenomenon. There are studies in the literature that compare the composition of grass-fed and grain-finished beef (Daley et al., 2010; van Elswyk & McNeill, 2014), but as yet there is little knowledge in the literature about how red meat is digested in the human digestive tract and how grass-fed may differ from grain-finished meat, particularly with respect to both kinetics of protein digestion and the digestion and release of lipids (including those lipids hypothesised to be important for health).There are also not many studies conducted on how different feeding regimes during cattle rearing can impact the protein and lipid digestion after human consumption (Mora et al., 2017). In the case of New Zealand pasture-fed and grain-finished meat, any differences that prove to be beneficial to overall health will serve as an advantage to the New Zealand meat production industry and the consumers.

We have no information yet about the digestion of the meat lipids, but it is clear that grass-fed meat contains significantly enhanced amounts of some of the nutritionally important

polyunsaturated fatty acids (Bermingham et al., 2018; Lukic et al., 2021). Because the structure of meat is based around protein, it can be deduced that a higher rate of digestion of proteins can result in the release of fat molecules from within the meat structure and impact fat digestion and bioavailability. It is important for essential fatty acids and bioactive peptides to resist degradation by gastro-intestinal proteases and lipases to be absorbed in the epithelial cells of the intestines and distributed by the bloodstream to be utilized and bio-accessed by the body. There is also very little information available about the digestibility and digestion kinetics of highly processed plant-based meat alternatives (Zhou et al, 2021), and it is important to know how these compare with natural meat.

This research study is a part of the programme called "The New Zealand Pasture-Raised Advantage" funded by the Meat Industry Association, Beef + Lamb New Zealand Ltd, the High-Value Nutrition National Science Challenge and the Ministry of Business, Innovation and Employment. A panel of meat scientists, a nutritionist and research dietitian reviewed available options to decide on the meat cuts and the meat alternative. This research study was designed to understand protein and fat digestion (*in vitro*) characteristics of New Zealand beef from pasture-fed and grain-finished animals. The results were also compared with a commercially available plant-based meat alternative (Beyond Burger®). The criteria for the meat alternative included the following 1) nutrition matched to beef (macronutrients), 2) readily available in the retail space, and 3) matched the appearance of beef.

Chapter 2: Literature Review

2.1 Meat Structure and Composition

Red meat is composed of three primary tissues: skeletal muscle tissues, connective tissue, and adipose tissue. The meat quality traits vary from cut to cut throughout the animal due to muscle fibre composition, protein content, deposition of fat and moisture (Jung et al., 2016).

The primary portion of the meat is contributed by the skeletal muscles which provide up to 90% of the muscle volume. Skeletal muscles are the largest edible part of the animal in terms of meat quantity and economic value. The skeletal muscles support the mass of the carcass and effect the locomotion of the animal. The skeletal muscles are made up of multinucleated muscle fibres that consist of elongated myofibrils in a parallel configuration (Strasburg & Xiong, 2017). Figure 2.1 elaborates on the structure of the skeletal muscle in meat.

Several myofilaments form the muscle structure called the myofibril. Myofibrils are spindle-shaped cylindrical filaments with tapered ends. The number of myofibrils in the muscle structure is determined in the genes of the animal prior to development. Around 20 to 40 myofibrils are surrounded by the endomysium (a thin sheath that is made up of connective tissues) to form the primary muscle fiber or fascicle. This structure represents the grain of the meat. Several primary muscle fibers are further encased in perimysium connective tissues to form the secondary muscle bundle. These bundles are determined by environmental factors and nutrition intake of the animal. In between these muscle bundles there are small pockets of fat cells and blood vessels. Several secondary muscle bundles are then covered by inextensible connective tissue called the epimysium, this last connective tissue layer serves to differentiate one skeletal muscle from another and to also help in the attachment of bones and muscles (Guo & Greaser, 2017; Lonergan et al., 2018; Vaclavik & Christian, 2014).

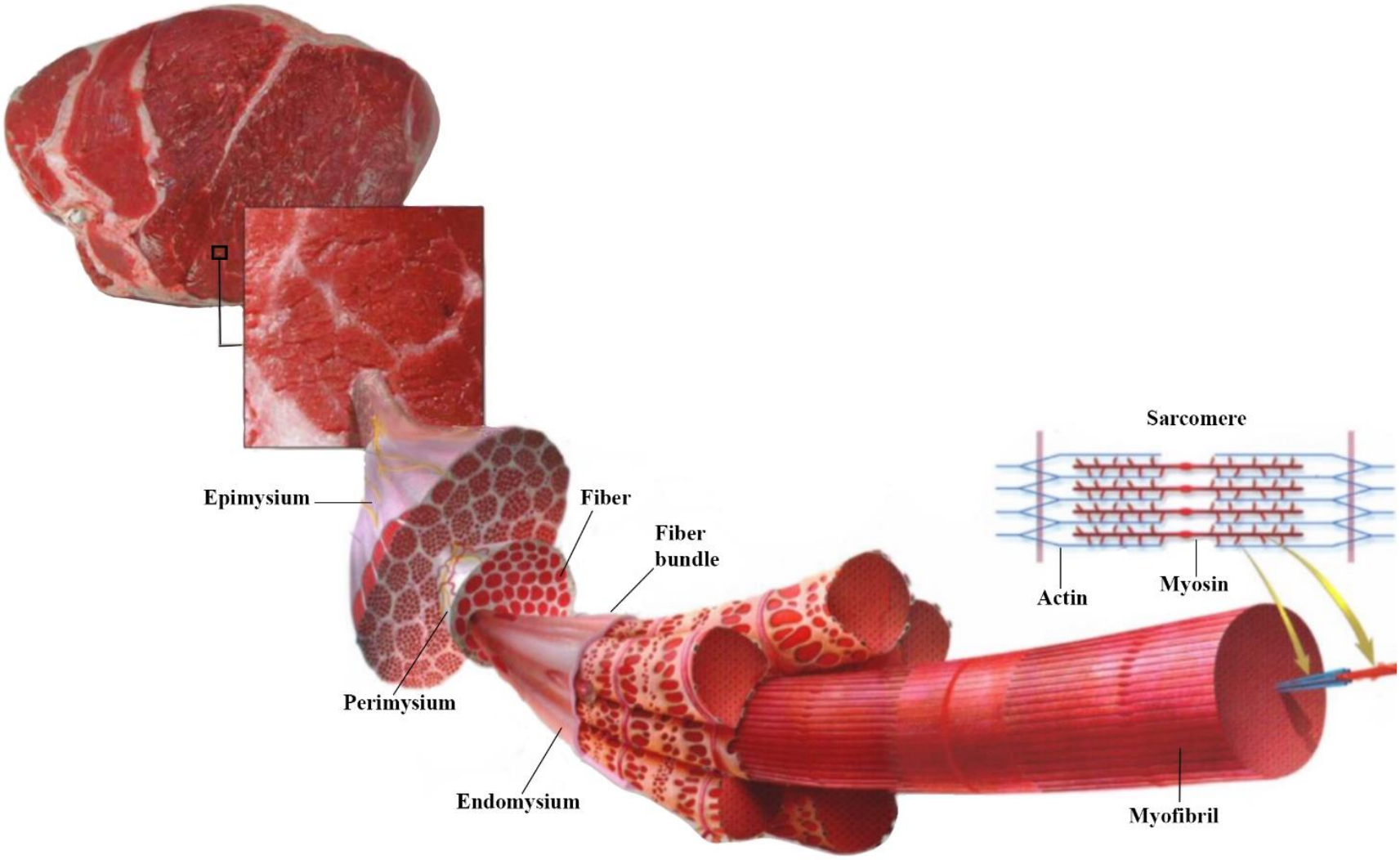


Figure 2.1: Skeletal muscle structure an example with meat. (Modified from Listrat et al., 2016).

2.1.1 Proteins in Meat

The muscle tissue contains different proteins that have different functional properties that contribute to the overall meat structure (Vaclavik & Christian, 2014). There are 30 different types of proteins that make up about 500-800 different types of muscles in animals and these muscles are distinguished based on the muscle fibre length (a few mm to 30 cm), diameter (10 μm to 100 μm), muscle size, orientation (parallel or at a specific angle), anatomical location, animal species and regulatory functions (Astruc, 2014; Guo & Greaser, 2017; Swartz et al., 2009; Vaclavik & Christian, 2014).

The skeletal muscle proteins in muscle tissue are segregated based on their biological functions (muscle structure and metabolism) and differential solubility in varying salt concentrations which yields the three primary classes of proteins found in skeletal muscle - myofibrillar (50% to 60%), sarcoplasmic (30%) and stromal proteins (10% to 20%) (Strasburg & Xiong, 2017).

2.1.1.1 Myofibrillar proteins

Myofibrillar proteins are soluble at high salt concentration (> 0.3 M NaCl) and are further divided into 3 types – contractile, regulatory, and structural proteins. Major contractile myofibrillar proteins include myosin and actin. Myosin is the most abundant myofibrillar protein that contributes to the structural component of the thick filament. Myosin has a molecular weight of 520 kDa. On the other hand, actin forms the structure of the thin filament and has a molecular weight of 42-47 kDa. These contractile proteins along with regulatory proteins such as troponin and tropomyosin are the muscle proteins that are directly involved in the relaxation and contraction of skeletal muscles. When viewed under the electron microscope the filaments appear as alternating light and dark bands in a pattern of cross striations. The overlapping of these filaments leads to the formation of actomyosin complex which has myosin filaments in the center of the complex that overlaps actin filaments at the ends and are further held together by the Z-line. The myofilaments are held in position by cytoskeletal or structural proteins which include titin, nebulin and desmin among other proteins. These structural proteins are present mostly in the Z-line region and control the integrity of myofibrils. The filaments are arranged in a repeating yet specific longitudinal pattern to form a sarcomere, which is the basic unit of the muscular cells. Each sarcomere unit is separated by a Z-line.

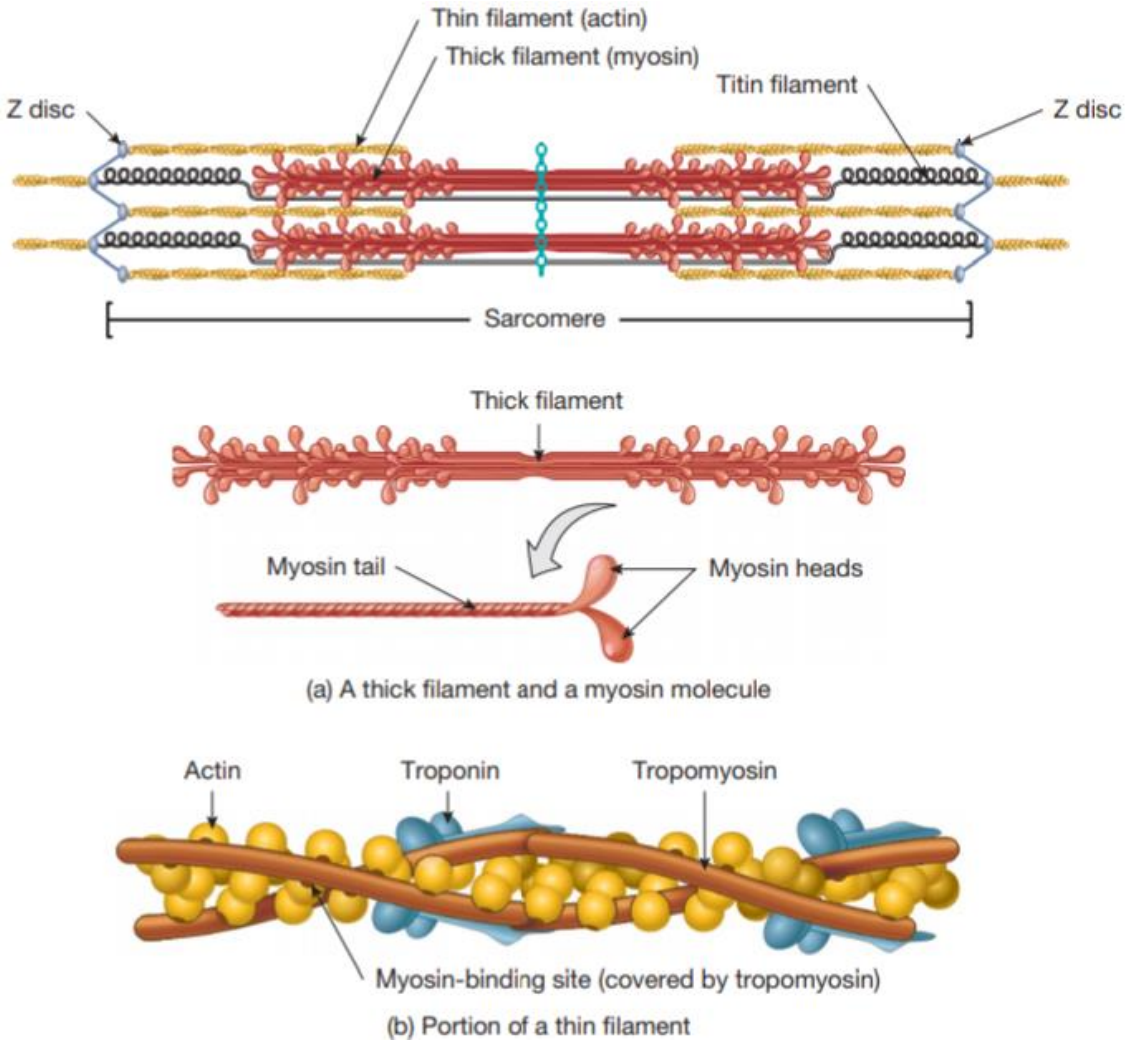


Figure 2.2: Components of a sarcomere (Reproduced with permission from Tortora & Derrickson, 2018, John Wiley and Sons).

In a muscle that is relaxed the sarcomeres are extended and the overlapping of filaments is minimal while for a muscle that is contracted the filaments have many overlaps and the sarcomeres tend to shorten in the process (Vaclavik & Christian, 2014). During pre-slaughter and post-mortem handling, the control of muscle contraction is very important as it determines the tenderness of the meat. If muscles are contracted with more overlapping filaments the meat produced is very tough in texture (Belk, 2015).

2.1.1.2 Sarcoplasmic proteins

The soluble muscle proteins (soluble in low salt concentrations (< 0.3 mM) that include pigments such as myoglobin and enzymes such as cathepsins, are called sarcoplasmic proteins. The red colour of the muscle is determined by the presence of two major pigments - myoglobin and haemoglobin. Around 80-90% of the total meat pigment is myoglobin (contains 1 haem

group in its structure) and the remaining 10-20% is haemoglobin (contains 4 haem groups in its structure). Myoglobin is present in a fluid state primarily in the cardiac and skeletal muscle cells and assists in the storage and replenishment of oxygen in the muscle (Vaclavik & Christian, 2014).

2.1.1.3 Stromal proteins

Stromal proteins like collagen, elastin and reticulin are relatively insoluble in salt solutions and constitute the connective tissue. Connective tissues are extracellular matrix components that provide structural support to the animal's body and help in the attachment of bones and muscles. Collagen is thin or watery, white, or transparent, inextensible, and appears to be crosslinked when observed under the microscope (Belk, 2015). As animals grow older, the collagen content and the number of crosslinks increases, which makes it less susceptible to heat-induced solubilization. Hence, the meat of older animals is tough in texture and difficult to digest. In general, the less connective tissue found in a cut of meat the more tender it will be (Calkins & Sullivan, 2007; Guo & Greaser, 2017; Toldrá, 2017; Vaclavik & Christian, 2014). Elastin is a minor constituent of connective tissue. It is hydrophobic, insoluble, heat stable, highly crossed linked with enhanced elasticity in the presence of water (Toldrá, 2017).

2.1.2 Fats in Meat

Besides skeletal muscle tissue and connective tissue, adipose or fat tissue is the third tissue component found in meat. Fats and fatty acids are stored as triglycerides in the adipocytes or fat cells and serve as reservoirs of energy and help to cushion and protect other tissues. Fat is deposited in several parts of the animal's body in the form of visceral fat (around organs), subcutaneous fat (under the skin), intermuscular fat (between the muscles) and intramuscular fat (within the muscles); IMF. The percentage of fat deposits tends to increase as the animal ages, and it is mainly controlled by feed intake. The fat content varies between different cuts of meat (Calkins & Sullivan, 2007; Lonergan et al., 2018; Toldrá, 2017; Vaclavik & Christian, 2014).

2.1.2.1 Marbling

In retail beef cuts, the intramuscular fat content (marbling) plays an important role when evaluating the quality of the carcass. The marbling scores are linearly related to the percentage fat content, and hence when the amount of fat increases the marbling score increases, which accounts for good quality meat (Calkins & Sullivan, 2007). The presence of intramuscular fat

in marbled beef denotes a superior meat quality and hence boosts its economic value to a certain level. The marbling in meat is graded on subjective visual basis and it assessed on the exposed rib eye from the 5^{th} to 13^{th} rib on the carcass. The two most common grading methods used in New Zealand are Authority for the Uniform Specification of Meat and Livestock (AUS-MEAT) and Meat Standards Australia (MSA). The AUS-MEAT marbling score ranges from 0 (none) to 9 (abundant) and is assessed using a standard marbling reference. The MSA uses a finer scale and is assessed based on the amount and distribution of the marbling on the rib eye. The grading range is from 100 to 1190 in increments of 10.

The fatty acid profile is known to be affected by the feeding regime, which in turn affects the beef flavour. However, studies have shown that the breed of the cattle can also affect the adipose tissue distribution. In the case of Angus, which is a breed found in many countries, the deposition of subcutaneous fat is higher, and the intramuscular fat deposition is relatively less compared to many other breeds. Extensive research has been undertaken to understand the genetics behind the intramuscular fat deposition and marbling in breeds such as Angus (Frank et al., 2016; Frank et al., 2017).

Consumer interest in fatty acids and fat content has grown in recent years. The deposition of fat in cattle has been reported to be altered by manipulating the by-products of rumen fermentation or by feeding a high energy diet. Bacteria that utilize fibre/forage (mainly pasture based) as the main energy source produce more acetate as their by-product while, bacteria that utilize starch (grain-based) as their energy source produce more propionate as the by-product. Acetate is the preferred substrate for the deposition of the subcutaneous fat layer while propionate is utilized by the liver for glucose production and further used in the deposition of intramuscular fat (marbling) and hence, more marbling is visible in beef that has been fed with grains (Lunn, 2020). It is thus essential to analyse the effect of the feeding system on the lipid content and profile to bring about a nutritional balance to the meat product without compromising nutritional and sensory properties (De la Fuente et al., 2009).

2.2 Post-Mortem Changes (Muscle to Meat)

When an animal is alive, the muscles undergo repetitive muscle contraction and relaxation. Both the actions require energy from adenosine triphosphate (ATP); the energy carrying molecule. The presence of oxygen, which circulates in the bloodstream, helps in the efficient production of ATP. Once the animal is dead the supply of nutrients, oxygen and energy for the muscle structure ceases, but metabolism continues, creating metabolite accumulation in the tissues that causes various alterations to muscle proteins and their structure (Lonergan et al., 2018; Vaclavik & Christian, 2014).

Complex biochemical events such as glycolysis and proteolysis with other physical and energetic changes take place in the muscle tissue to convert it into meat that is palatable (Gagaoua et al., 2018). During slaughter or immediately post-mortem, the circulation of blood within the animal ceases, and in turn the oxygen supply is exhausted. The muscles can no longer use oxygen to generate ATP, hence, the glycogen stored in muscle tissues is utilized to produce ATP via anaerobic glycolysis (a process that involves the break-down of molecules in the absence of oxygen). Anaerobic glycolysis produces energy from glycogen and promotes the accumulation of lactic acid in the muscles. As the lactic acid is not circulated throughout the system in the post-mortem period, the build-up in the muscles releases hydrogen ions that aids in the contraction of muscles and decreases the muscle pH (usually 5.4 to 5.8). When the glycogen supply is depleted, the regeneration of ATP finally ceases which causes actin and myosin to remain in a state of permanent contraction called *rigor mortis* (Boland et al., 2018; Cobos & Diaz, 2014). Cattle raised on concentrates or grain have high levels of muscle glycogen, but during post-mortem glycolysis several metabolic alterations such as impaired insulin sensitivity and increase in acute stress levels have been observed. On the contrary, lower muscle glycogen storage and fewer metabolic alterations were noted in pasture-raised cattle (Pighín et al., 2015).

Rigor mortis causes the muscle structure to stiffen and become inextensible and the decrease of energy causes the release of calcium ions from the mitochondria and sarcoplasmic reticulum. This is an important process during post-mortem aging of meat. Aging the carcass in appropriate conditions allows the activity of endogenous proteolytic enzymes such as cathepsins, μ- and m-calpains (activated by calcium ions), caspases, metalloproteases, etc, which help to break down most of the overlapping proteins and in turn makes the meat tender (Calkins & Sullivan, 2007; Guo & Greaser, 2017).

There are various factors that influence the post-mortem quality of meat. Muscle pH and temperature interaction, influx of stress hormones that regulate vasoconstriction of muscle and exsanguination of outermost tissues are some of the peri-mortem events that invariably lead to post-mortem biochemical changes (Listrat et al., 2016; Pighín et al., 2015). Post-mortem biochemical changes mainly cause immobilized water to purge out of the fibres. The water holding capacity of fresh meat is one of the important quality parameters that is measured under external factors like heating, gravity, pressing, etc. (England et al., 2017; Toldrá, 2017; Vaclavik & Christian, 2014).

2.3 Meat Quality Parameters

For a consumer, organoleptic attributes like texture and colour are important factors that are considered during the purchase of meat.

2.3.1 Tenderness

An essential textural factor is tenderness of meat. It plays a significant role in the palatability and marketability of the meat to consumers, as it provides satisfaction in eating, acceptability of the purchase and desire to repurchase meat products (Calkins & Sullivan, 2007; Lee et al., 2017; Listrat et al., 2016). The tenderness of meat from any animal depends on factors such as gender, age, diet, species, genetic background, type of muscle, location of the muscle on the carcass, the contraction of muscles, the amount of collagen, the amount of marbling caused by intramuscular fat deposits, and changes induced post-mortem (storage conditions) (Bekhit et al., 2014; Jung et al., 2016; Shen et al., 2012).

Skeletal muscles in beef animals that are less exercised like the back muscles and the loins are the most tender. Muscles that are used regularly for locomotion like the thoracic and pelvic limbs of the leg muscles, under belly and neck (fore-shank, flank, brisket, short plate, etc.) are the least tender as they have more connective tissue than muscles found on the back portion of the animal. Collagen is an important determinant that contributes to the tenderness of the meat and muscle cut. Collagen fibres can cross link intramolecularly or intermolecularly and are relatively insoluble during aging of meat. As the animal ages the number of cross links increases and so does the toughness of meat. This is also known as background effect in meat (Calkins & Sullivan, 2007). Different cuts have variable amounts of collagen as is it not uniformly distributed throughout the animal. As the tenderness is not uniform on the carcass it creates a demand for prime cuts like steaks, which are more palatable and tender and are usually found in less than 10% of the carcass, while tough cuts are sold in the form of minced meat or meat sub-products (Guzek et al., 2016; Scozzafava et al., 2016; Vaclavik & Christian, 2014).

2.3.1.1 Measuring meat tenderness

For years the tenderness of the meat has been measured by either sensory analysis (especially using trained panellists and/or volunteers/consumers) or mechanical instruments (which is a measure of the shear force). Warner-Bratzler shear force is the widely used mechanical method while the slice shear force method has also been gaining popularity as a faster method to determine the tenderness of the muscle cut. A score of below or equal to 4.00 kg for Warner-

Bratzler shear force and/or a score of below or equal to 20.00 kg for slice shear force are the standard range for samples that are deemed tender. Post-mortem sarcomere length affects the textural properties of both raw and cooked meat. Hence, it is crucial to measure the length of the sarcomere to estimate the tenderness of the meat samples. Microscopy and laser diffraction are methods used to measure the sarcomere length of the contracted myofibrils (Battaglia et al., 2020).

2.3.2 Colour

In a broader sense colour is the physical interaction of light with the meat, registered by the human eye and processed by the brain, and hence colours are interpreted subjectively. The colour of beef is an important attribute that governs the consumer's decision for purchase and shows its marketability. Any deviation from standard colour can lead to product rejection (Jukna et al., 2017; Salim et al., 2019). The colour of meat depends on the concentration of mainly myoglobin and its degree of oxidation over time (Saláková, 2012). Meat colour is also influenced by factors such as ultimate pH, animal age, fatness, and intermuscular content of the carcass (Priolo et al., 2001).

During slaughter and storage of meat a rapid decrease in temperature and relatively higher ultimate muscle pH can lead to the formation of darker beef colour and cold muscle shortening with a low concentration of glycogen to produce tougher meat (Della Rosa et al., 2018). The muscle source is also found to be an intrinsic factor that influences the colour stability. Certain muscles are characterized based on their contraction speed (slow-twitch or fast-twitch) and their preferred metabolic pathway for the degradation of glycogen (oxidative or glycolytic). For example, skeletal muscle fibres in meat from cattle are red, slow-twitch and undergo oxidative metabolism. These muscles are used in extended periods of activity such as walking or standing and hence require a consistent source of energy (Cobos & Diaz, 2014). The meat protein myoglobin stores oxygen in the skeletal muscle cells. This oxygen is utilized to provide energy for constant activity. The amount of myoglobin in the muscle cells determines the redness of the meat, the metmyoglobin reducing activity, colour on the surface of the muscle, oxidation of the lipids and the ATPase activity (Boland et al., 2018). In the case of the *Longissimus* muscle, studies have shown that it is colour-stable and has greater surface redness. Extrinsic factors like diet also play an important role in the colour stability of fresh meat as diet has the potential to affect the oxidative components. The breed of the cattle is said to also affect

colour stability. Pure bred varieties show pronounced redness compared to that of animals that have been cross bred (Salim et al., 2019; Vaclavik & Christian, 2014).

The fat colour is also an important quality parameter that is affected by the fatty acid composition. Solidified fats have higher melting points and can appear whiter in colour than liquid fats, which contain fatty acids that confer lower melting points (Wood et al., 2004).

2.3.2.1 Measuring meat colour

CIE (Commission Internationale de l'Eclairage) is responsible for promoting international colour standardization. The CIE generates defined colour spaces, standard methodologies, and illumination parameters to measure the colour of an object. The CIELAB colour space (Figure 2.3) is mostly used to record values in terms of different chromaticity coordinates in three-dimensional space (Warriss, 2001). Surface lightness (L*) corresponds to brightness and is measured in a scale of 0 to 100 where, 100 = all the light is reflected while 0 = all light is absorbed. The neutral colours like black, grey, and white pass through the centre of the colour space. Coordinate a* measures positive red, negative green and hence denotes redness of the object while the coordinate b* measures positive yellow, negative blue and denotes the yellowness of the object (Priolo et al., 2001; Saláková, 2012).

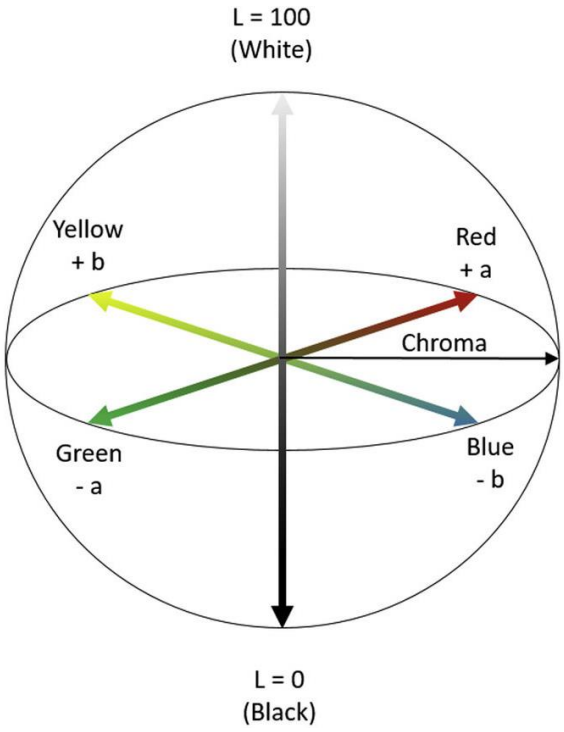


Figure 2.3: CIELAB colour space (Reproduced from Ly et al., 2020, with permission from Elsevier).

2.3.3 Oxidation

Oxidation of meat lipids and proteins can lead to detrimental effects of the quality aspects like colour, odour, flavour, and nutritional value (Promeyrat et al., 2010). In the presence of atmospheric oxygen, the myoglobin changes to a reddish colour, oxygenated oxymyoglobin, on further oxidation of the iron molecule the colour changes to metmyoglobin, an undesirable brownish-red discolouration, with the onset of bacterial contamination that shows that the meat is no longer fresh (Vaclavik & Christian, 2014). Thus, it becomes essential for muscle tissue to contain a minimum concentration of antioxidants, such as fat-soluble vitamins A and E that help to delay the process of lipid oxidation and water-soluble vitamin C that helps to protect the haem and decreases metmyoglobin production. However, vitamin C is destroyed in the process of cooking meat (De la Fuente et al., 2009).

Iron, the transition metal found in meat, is a strong pro-oxidant. Diet is a main factor that can affect the balance between antioxidant and pro-oxidant concentrations in meat. In cattle, extensive pasture feeding promotes an increased deposition of natural antioxidants in the skeletal muscles of the animals when compared to concentrate-fed cattle. This gives a superior oxidative stability in pasture-fed beef compared to concentrate-fed beef. It has been reported that a high concentrate diet that maximizes the performance of the cattle can also lead to metabolic stress and disorders that can reduce the resistance to certain infectious and respiratory diseases in grain-fed animals (Fruet et al., 2018; Li et al., 2015).

Lipid oxidation directly affects the nutritional value, organoleptic properties and product quality of meat and other meat products. There is lower availability of desirable fatty acids as oxidation keeps progressing once it is initiated by stress compounds arising due to both internal and external factors. The unsaturated fatty acids, especially the PUFAs present in meat, are easily susceptible to oxidation. It is thus, essential to consider the PUFA content, the amount of reactive oxygen species including free radicals, peroxides, and level of antioxidants naturally present in meat (Terevinto et al., 2019).

During the process of digestion, the lipids in the meat matrix are exposed to pro-oxidant conditions in the gastro-intestinal tract which include the low pH of gastric juice, presence of reactive species or metallic ions released from meat components like metalloproteins into the bolus and incorporation of oxygen during mastication which result in the unavoidable occurrence of oxidative degradation of lipids in meat (Nieva-Echevarría et al., 2020).

The primary product of lipid oxidation is hydroperoxide which is unstable in nature and readily decomposed to form a myriad of other products such as hydrocarbons, alcohols, aldehydes and ketones and their derivatives. Malondialdehyde is one such product of lipid oxidation that gives a rancid off-flavour and can prove to be toxic to consumers (Horcada et al., 2020). It has been found that pasture-fed meat contains higher levels of natural antioxidants such as vitamins A, C and E and phytochemicals like flavonoids and carotenoids when compared to grain-fed meat. This higher antioxidant content exerts a protective effect against free radical attack and oxidation of lipids and enhances the redness of meat (Horcada et al., 2020; Salim et al., 2019; Terevinto et al., 2019).

On the other hand, protein oxidation produces carbonyls and pronounced cross links between muscle fibres which decreases tenderness and solubility (Fruet et al., 2018). During the cooking of meat, the free radicals produced react with cysteine, basic and aromatic amino acids. Further thermal treatment causes the hydrogen bonds of the meat proteins to break thereby exposing the hydrophobic amino acids on the surface of the protein molecule. These favour the formation of aggregates which in turn affects the quality of the meat (Promeyrat et al., 2010; Santé-Lhoutellier et al., 2008). Certain antioxidant enzymes like catalase, superoxide dismutase and glutathione peroxidase act as the primary barrier and protect the muscle tissues from oxidative damage. These enzymes are relatively stable in refrigeration conditions and offer protection during post-mortem changes (Terevinto et al., 2019).

2.4 Cooking of Meat

2.4.1 Effect of cooking on meat proteins

When meat is subjected to heating methods the proteins present in the meat lose their native conformation (denaturation of proteins). Heating increases the kinetic energy of the polypeptides and in turn ruptures other weak intramolecular forces that hold the protein molecules together. As the temperature increases the protein molecule unwinds and unfolds and further loses its secondary and tertiary structure. The unfolded protein then forms aggregates which may have modifications to their side chains, disordered disulphide bonds, and crosslinking with other polypeptides. Water is exuded or squeezed out and the protein molecules coagulate. Aggregation ultimately causes nonpolar interactions between proteins that have been heat-denatured and have their hydrophobic groups turned outward to surrounding water to adopt a lower energy state (Yu et al., 2017).

Cooking denatures and coagulates the meat proteins and improves the palatability, extends the storage life, and inactivates endogenous proteolytic enzymes (Oz et al., 2017). A vital component during the cooking process is the loss of water, known as cook loss (Pighin et al., 2016). Cooking of meat causes exudation of fluid from within the meat structure and evaporation from the surface of the meat, which causes shrinkage of dimensions and loss of volume and mass due to aggregation of myofibrillar and sarcoplasmic proteins. The muscle fibres shrink both in diameter and in length on heating and during moist-heating, collagen softens and solubilizes to water-soluble gelatine (Figure 2.4). There is a loss of some vitamins due to their lower thermostability that contributes to loss of nutritional value in cooked meat (Oz et al., 2017). However, the process of cooking the meat enhances the flavour, appearance and tenderness while destroying pathogenic microorganisms. Cooking enhances meat protein digestibility up to 94% (Pighin et al., 2016; Toldrá, 2017; Yu et al., 2017).

The colour of meat is also affected by cooking temperature and cooking time, it has been reported that the native red pigment myoglobin denatures at a temperature of 65-80 °C with 70% denaturation at 73 °C. The denaturation of myoglobin is also dependent on the cooking method and time. The myoglobin denatures on cooking at high temperatures and Figure 2.5 shows the series of interconversions of myoglobin through oxygenation, oxidation and reduction reactions which ultimately results in alterations of appearance of meat colour (García-Segovia et al., 2007).

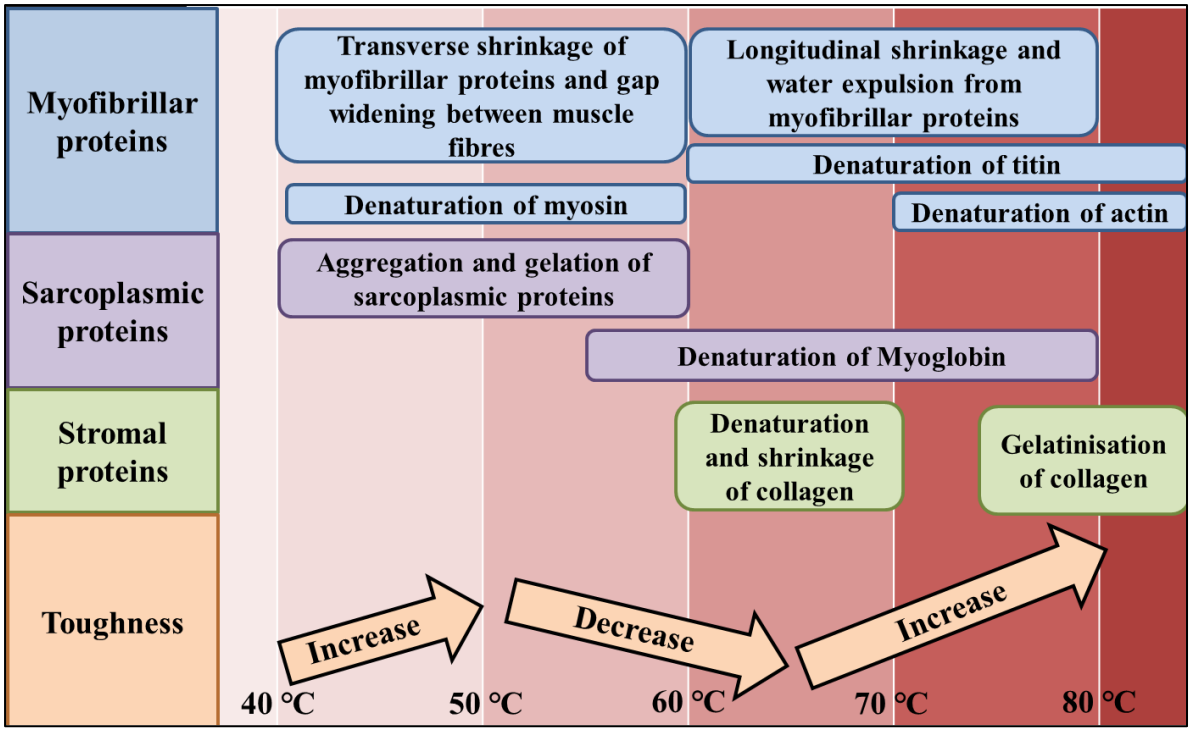


Figure 2.4: The effect of cooking temperature on meat protein structures (Chian, 2021)

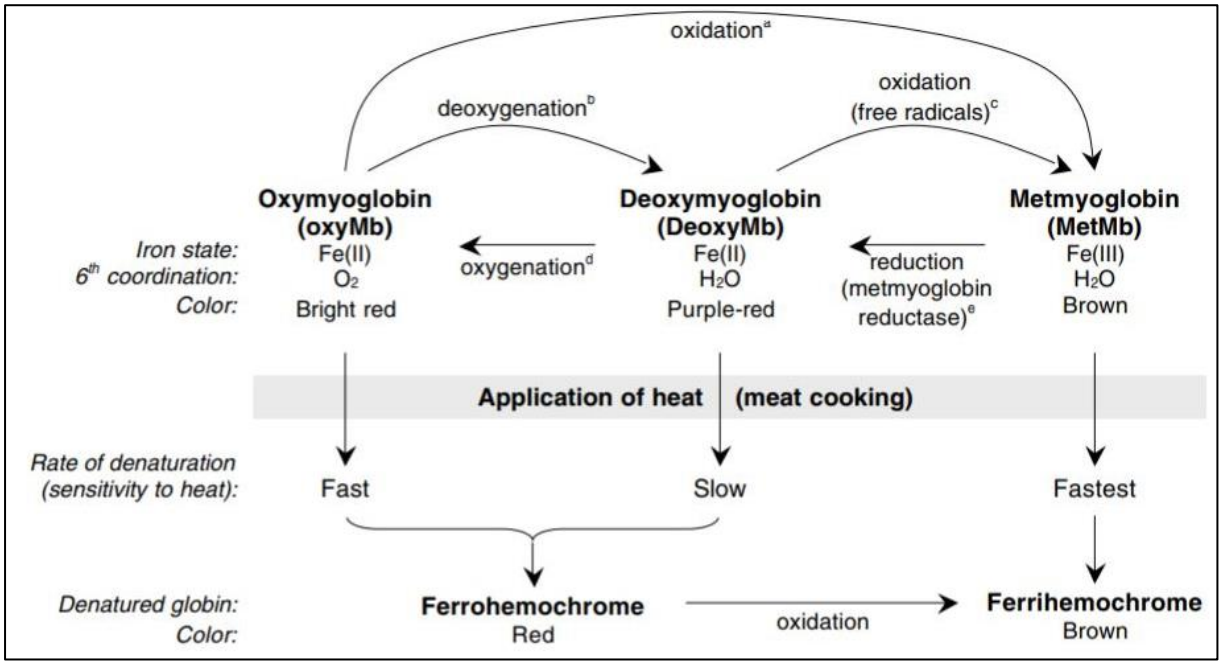


Figure 2.5: Denatured products of myoglobin formed during cooking (Reproduced from King & Whyte, 2006 with permission from John Wiley and Sons).

2.4.2 Effect of cooking on meat fats

Fat provides lubrication between the muscle fibres and within the connective tissues, thus reducing the bulk density and force required to cut a piece of cooked meat. This helps to improve the perception of tenderness of the meat cut and provides some protection from overcooking. Fat in meat is also an important precursor in the generation and release of flavour during cooking and consumption. Some studies have shown that irrespective of production system or breed, higher levels of intramuscular fat have demonstrated a greater flavour intensity and thus a critical role in the beef eating experience, as the link between IMF and palatability has been well established (Calkins & Sullivan, 2007; Guo & Greaser, 2017; Toldrá, 2017).

During grilling of beef, a combination of thermally generated volatile compounds derived from lipids, proteins and reducing sugars are subjected to two sets of reactions: Maillard browning between amino acids and reducing sugars and oxidative degradation of the lipid molecules, which in turn affects the aroma profile (Watkins et al., 2013). Non-volatile compounds such as free amino acids, organic acids found in the muscle matrix, connective tissue (collagen), partially dissolved fat content and other warm meat juices give meat the essential beef flavour and characteristic grilled feature (Frank et al., 2017).

2.5 Characteristics of Beef from Different Production Systems

It is well known that meat is fit for human consumption because it contains nutrient dense and health beneficial macro-and micro-nutrients. A 100-gm serving of beef is found to have about 25% of the recommended dietary intake of protein and contains all the essential amino acids, a variety of healthy fatty acids, vitamins A, E, B6 and B12, niacin, iron, zinc, riboflavin, selenium, approximately 10% recommended dietary intake of phosphorus and antioxidants (Horcada et al., 2020; Mwangi et al., 2019).

Because cattle are ruminants, i.e., foregut fermenters, the nutrients that are available for uptake (bioaccessible) comprise the products of fermentation and the remaining undigested or partially digested components of their forage. Unlike the protein in meat, where the total amount may vary according to the lifestyle of the animal, but the actual composition of the proteins is fixed by the genetics of the animal. In most studies, the nutrition intake and genetic makeup of the animals do not influence the protein and amino acid content of the beef (Duckett et al., 2013; Van Elswyk & McNeill, 2014). The following Table 2.2 contains the main amino acids found in meat along with their respective functions that are beneficial to the human body (Pighin et al., 2016).

Table 2.1: Major amino acids in beef and their functions.

Amino acid		Function
Isoleucine	Essential	Regulates blood glucose level; aids in growth and repair of muscle tissue; haemoglobin production and energy regulation.
Leucine	Essential	Blood glucose level regulation; muscle tissue growth and repair; hormone production, wound healing, and energy regulation.
Valine	Essential	Growth and repair of muscle tissues and energy regulation.
Lysine	Essential	Development and regulation of collagen, hormones, various enzymes, and antibodies; promotes the absorption of calcium; lowers serum triglycerides level and helps in growth and repair of muscle tissue.

Arginine	Non-essential	Stimulation and release of hormones; increased immune cells production; neutralization of ammonia in the liver; ameliorate tissues
Alanine	Non-essential	Regulation of energy
Aspartic acid	Non-essential	Glycoprotein synthesis
Threonine	Essential	Antibody production
Methionine	Essential	Helps to prevent arterial fat clogging; act as an antioxidant promotes the synthesis of collagen and helps to remove harmful metals
Glutamic acid	Non-essential	Regulation of excitatory neurotransmitters and regulator of inflammation
Tryptophan	Essential	Precursor for serotonin and helps in production of niacin

The common variety of pasture-feed found in New Zealand includes ryegrass and clover and a pasture-finished diet is considered less costly than finishing on grain. Inclusion of legumes (such as clover) enhances the protein values of the forage and promotes the digestibility in ruminants. Herbaceous forage or pasture-fed beef has healthier fatty acid profile with optimized oxidative stability and appears to be darker than grain-fed beef (Jukna et al., 2017). However, some studies report negative effects of pasture feeding on meat colour and texture (Hajji et al., 2016).

Most cattle in the world feed on pasture and the composition of the fat in an animal is very much affected by what it has been eating and the results of rumen fermentation. Pasture- or silage-fed beef is often considered to be leaner meat with a lower glycogen content in the muscles and a more desirable fatty acid composition than that of grain-fed beef (Huuskonen et al., 2010). Several studies indicate pasture-fed beef to be leaner in comparison to grain-finished beef in terms of intramuscular fat composition. It has been observed that forage or pasture-based diets increase the omega-3 polyunsaturated fatty acid content and reduce the saturated fatty acid and cholesterol content in meat than concentrate-based diets (Bermingham et al., 2018). But some studies report that the intramuscular fat content is low in pasture-fed beef,

which plays an important role in juiciness, flavour, and tenderness of meat. If the intramuscular fat amount is reduced there can be negative impact on consumer satisfaction. Hence, a supplementation of corn grain can increase subcutaneous fat thickness, intramuscular fat, and muscle glycogen content (Della Rosa et al., 2018).

On the other hand, a grain feed like maize as an energy source improves the growth performance of the cattle giving more meat yield or muscle volume and greater fat content (Fruet et al., 2019; Jukna et al., 2017). Traditionally a concentrate-based diet included combined cereal grains and cereal straw or hay from grass as a fibre source. Nowadays it is preferred to include a total mixture of pasture with grains and hay to help reduce the presence of atherogenic and thrombogenic fatty acids (lauric, myristic and palmitic acid) and increase the amount of conjugated linoleic acid and other essential n-3 polyunsaturated fatty acids (Horcada et al., 2016; Purchas et al., 2014).

Antioxidants in meat include vitamins A and E. Beta-carotene, a precursor of vitamin A has contributed to the integrity of mucous membrane and skin and helps in the protection against viral and bacterial infection. Vitamin A has shown to modulate the immune system functions by influencing white blood cells. While incorporating pasture-feeding in the cattle's diet the PUFA content is said to be increased as well, which could also lead to instances of lipid oxidation but α-tocopherol, the major biologically active form of Vitamin E is also found in higher concentration in pasture-fed beef and has been shown to positively affect dietary protein, lipid content, and immune genes (Fruet et al., 2018; Li et al., 2015).

2.5.1 Influence of feed type on deposited fatty acids in beef

Most of the fat in meat is made up of triglycerides – each triglyceride is made up of 3 fatty acids esterified with a molecule of glycerol. The fatty acid composition of the triglycerides dictates their physical characteristics and the health benefits of consuming the fat. Cattle obtain the precursors for fat synthesis from their diet. Fat that is taken in, for example from plant origin, is broken down to fatty acids in the rumen, and a degree of saturation (removal of double bonds) and breakdown of the fat occurs in the rumen. Anaerobic metabolism in the rumen (notably of carbohydrates), leads to the production of short-chain fatty acids, mostly acetic (C2) and propionic (C3) acids. Starch that is taken in (mostly in the grain-fed animals) is metabolised to short-chain fatty acids. The animal is able to synthesise longer-chain fatty acids from C2, but only up to C16, thus starch cannot be a precursor for longer-chain fatty acids. Longer-chain fatty acids must be derived from the food source, although they can be modified

by elongation and desaturation reactions. Thus, the source of fatty acid precursors in the diet and as a result of rumen metabolism very much dictates the composition of the body fat.

2.5.1.1 Saturated fatty acids (SFAs)

Long chain fatty acids such as myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) contribute to the SFA content in beef. Beef containing less palmitic acid may be perceived as more beneficial in the human diet to lower serum cholesterol. Regardless of feeding regime, the major SFA found in beef is stearic acid (C18:0), a neutral fatty acid that has been widely studied and reported to have a neutral effect on the plasma lipid or cholesterol level in the human body (Vahmani et al., 2020). It has also been found that pasture-fed beef has a higher concentration of stearic acid than grain-fed beef especially in the polar lipids of the intramuscular fat. Several studies report that pasture-fed beef has a lower total fat content, but studies suggest that pasture-feeding and finishing has shown to deposit more neutral stearic acid in certain beef cuts. Hence more studies on the contribution of pasture-feeding and the fat deposition in a variety of beef cuts are important to understand the role of SFAs in the diet (Van Elswyk & McNeill, 2014).

2.5.1.2 Monounsaturated fatty acids (MUFAs)

Beef is one of the primary sources of MUFAs in western countries and the most common source of oleic acid (C18:1 n-9), which is also regarded as a heart-healthy fatty acid for humans (Vahmani et al., 2020). Hence, a greater concentration of oleate, may be perceived as a more beneficial component. However, the source and origin of MUFA in the animal diet should be considered to evaluate the potential benefits of this type of fatty acids. Oleic acid is found mostly in the intramuscular fat cells. It has been reported that different muscles respond differently to feed intake and fat deposition of MUFAs, and studies have shown that pasture-fed beef has 30-70% less MUFA content when compared to grain-fed/finished beef (Duckett et al., 2013).

2.5.1.3 Polyunsaturated fatty acids (PUFAs)

Concentrate-fed beef contains omega-6 (n-6) PUFAs. The quantity of n-6 polyunsaturated fatty acids has been found to be at least four times more in concentrate-fed beef than in pasture-fed beef (Van Elswyk & McNeill, 2014). Linoleic acid (C18:2 n-6) is the primary omega-6 PUFA found in pasture-fed and grain-finished beef. It constitutes 60 to 85% of the overall PUFA content. (Carabante et al., 2018; De la Fuente et al., 2009; Ibáñez et al., 2017; Mwangi et al., 2019). Hence it is essential to reduce the feed of concentrate in the diet of beef cattle as it

enhances the n-6 PUFA content and a particular isomer, C18:1 10t, seems to have detrimental effects on human health (Ibáñez et al., 2017). Incorporating pasture-feed to primarily grain-fed beef can help in increasing trace amounts polyunsaturated fatty acids like linoleic, linolenic, arachidonic, and dietary essential long-chain fatty acids eicosapentaenoic, docosapentaenoic and docosahexaenoic acid (Carabante et al., 2018; Huuskonen et al., 2010).

There have been various studies conducted on the omega-6 and omega-3 PUFAs. The precursor for n-6 PUFAs is linoleic acid while the precursor for n-3 PUFAs is α-linoleic acid. These essential fatty acids that act as precursors are not naturally synthesized by ruminants and they must obtain these through dietary means. However, it has also been found that higher linoleic acid feeding can interfere with α-linoleic acid conversion and deposition (Vahmani et al., 2020). Omega-6 PUFAs are commonly found in high energy foods like cereals and grains hence, grain- or concentrate-finished beef contains more omega-6 PUFAs (Gupta et al., 2013). The conversion of α-linoleic acid by the elongation-desaturation pathway to long chain n-3 PUFAs like eicosapentaenoic acid (EPA, C20:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3) is controlled by several biological factors (Scollan et al., 2006). The conversion is a slow and sometimes inefficient process. Hence it is necessary to incorporate a diet that contains EPA, and DHA to be readily and directly absorbed by the digestive system of the ruminant to meet requirements for effective metabolic activities that can also aid in disease prevention (Vahmani et al., 2020).

2.5.1.4 Conjugated linoleic acids (CLAs)

Intramuscular fat deposits contain beneficial CLAs besides MUFAs and PUFAs. In recent years conjugated linoleic acids have been studied for their potential benefits when consumed by humans. Ruminant fats are one of the richest sources of conjugated linoleic acids particularly (C18:2) cis-9 trans-11 isomer (which accounts for more than 80% of the total CLA content) and (C18:2) trans-10 cis-12 isomer (which comprises 3-5%) of the naturally occurring CLAs (Shokryazdan et al., 2017; Scollan et al., 2014). However, studies show that total CLA isomers rarely exceed 2% of the total lipid content in beef (Hajji et al., 2016). The two primary CLA isomers have dissimilar biological effects. The cis-9 trans-11 CLA isomer has been associated with modification and improvement of immune response and inhibition of carcinogenesis in cell lines (~ 60% decreases in viable cell numbers) while the trans-10 cis-12 CLA isomer also demonstrated anticarcinogenic effect but only up to 15% decrease in viable cell numbers. However, numerous studies suggest that the trans-10 cis-12 isomer is the

biologically active isomer to impart anti-obesity effects in many animal models while cis-9 trans-11 isomer has shown to cause opposing effects such as increasing the accumulation of triglycerides in cell cultures. The trans-10 cis-12 isomer is also important for repartitioning body fat and reduction of atherosclerosis (Churruca et al., 2009; Jukna et al., 2017; Smith et al., 2009).

Studies have shown that PUFAs, especially linoleic acid (C18:2), which is abundant in livestock feed and pasture, are toxic to many rumen bacteria. The process of biohydrogenation is speculated to be a defence mechanism of the rumen bacteria. The CLAs are produced in the rumen of the animal as an intermediate product in the microbial biohydrogenation of linoleic acid to stearic acid (den Hartigh, 2019). The occurrence of these bioactive fatty acid isomers is dependent on factors such as feed type, breed, age of animal and rumen pH. Grain-feeding tends to reduce the rumen pH, which is unfavourable for rumen bacteria isomerase activity, thus affecting linoleic acid conversion. Pasture-feeding incorporates higher levels of PUFAs and promotes a more favourable rumen conditions for CLA-producing bacteria, which yields higher CLA content in the animal's tissue (De la Fuente et al., 2009; den Hartigh, 2019). Hence it has become crucial to increase pasture feeding and supplement the animal's diet with oil seeds rich in PUFAs to obtain maximum CLA biosynthesis (Smith & Smith, 2014; Vahmani et al., 2017).

2.6 Plant-Based Meat Analogues

There has been immense pressure and environmental stress on the meat production industries for many years and over the years many studies have reported controversial health impacts of meat consumption. These factors have motivated some consumers to reduce their intake of muscle-based food in their diet. Hence, plant-based meat alternatives have been developed and introduced into the market this past decade to address the growing consumer demands and the sustainability of food supply in the future (Kyriakopoulou et al., 2019; Sha & Xiong, 2020). Despite the benefits of a plant-based diet, a large section of consumers, especially in Western societies, do not willingly shift to a complete plant-based diet or reduce the consumption of meat (Graça et al., 2015).

Meat analogues are largely derived from legumes (pea, soy, etc), cell-based (*in vitro* cultured muscle tissues) and fermented products (mycoprotein). Recently, more protein sources such as insects and microalgae have been utilized to produce meat alternatives (Lee et al., 2020; Sha & Xiong, 2020). Plant-based sources are currently very popular due to their direct utilization and lower production costs as opposed to the other protein options. To be a successful product, it is essential for the plant-based meat analogue to have similar sensory properties to those of meat. The texture, flavour, appearance, and mouthfeel influence a consumer's perception and acceptability of meat analogues (Bohrer, 2019; Sha & Xiong, 2020). In recent times reports show that plant-based meat alternatives have negative sides in terms of having (i) higher costs; (ii) a long list of ingredients; (iii) highly processed and not natural ingredients; and (iv) unpleasant flavour and taste of cooked products (Jahn & Strässner, 2021).

The product development of plant-based meat analogues has been limited to restructured (or reconstructed) products that aim to create the texture that resembles meat fibres. The formation of fibrous structures from plant-based proteins and various other functional ingredients requires intensive processing methods such as thermo-extrusion, cross-linking, shearing, and fibre spinning. These methods enable the native protein structures to unfold and denature, which promotes the interaction of the transformed structures with other proteins and carbohydrate polymers. However, the extrusion process is carried out at very high temperatures, which can result in reduced moisture content of the end product (Chiang et al., 2020).

Additives such as red pigments are added to improve meat-like aesthetics. Trace amounts of vitamins and minerals are supplemented to enhance the nutritive value and make it comparable to meat (Kyriakopoulou et al., 2019; Sha & Xiong, 2020). To make the lipid content of meat

analogues equivalent to meat, a variety of vegetable and plant (seed and grain) oils and fats are used during processing. It is to be noted that most of the fat sources used in the processing of meat analogues are rich in SFAs. Some of the commercially available meat alternative brands include, Beyond Meat™, Gardein, Impossible burger, MorningStar burger, Boca burger, etc. (Bohrer, 2019).

The following Table 2.2 summarises the ingredient list, and purpose of the ingredients that go into making meat analogues (Lee et al., 2020).

Table 2.2: Ingredients used from plant-based meat alternative production (Lee et al., 2020).

Ingredient used	Amount used (%)	Purpose of ingredient used
Water	50-80	Distribution of ingredients; Emulsification and to incorporate juiciness
Textured vegetable protein	10-25	Source of protein and insoluble fibre; texture and water-binding
Non-textured proteins	4-20	Source of protein; emulsification, texture, and water-binding
Fats	0-15	Enhancing flavour, and texture, incorporates succulence and Maillard reaction
Binding agents	1-5	Improve texture, water-binding, may contribute to fibre content
Flavours and spices	3-10	Flavour enhancement (salty, savoury, meaty) and masking plant-based notes
Colouring agents	0-0.5	Improves appearance; can be natural or artificial

It has been reported widely in the literature that approximately 60 % of dietary protein consumed is derived from plant sources. It is important to note that many plant sources lack sulphur-containing essential amino acids like methionine and cysteine and are also deficient in other essential amino acids like leucine and lysine. This compromises the availability of a complete amino acid profile, which is important for protein synthesis. Plant-based proteins exhibit lower digestibility due to the molecular interactions that occur within the protein structure during processing. The structure affects cooking conditions and protein digestibility when compared to meat related products (Gorissen & Witard, 2018). There have been some undesirable effects relating to flavour, uniformity, texture, and acceptability when producing

plant-based alternatives to meat (Baugreet et al., 2019). Based on available scientific evidence it can be concluded that animal-derived proteins have a higher anabolic potential than plant-based protein sources (Gorissen & Witard, 2018).

2.7 Digestion of Food

The digestion of food is a complex process, which involves mechanical and chemical breakdown of food into smaller components and the extraction of bioavailable nutrients from the food matrix to be absorbed into the bloodstream. Digestion of food is crucial for maintaining proper health and welfare in human beings.

2.7.1 Physiology of Digestion

The gastro-intestinal digestion in humans can be segregated into three phases:

2.7.1.1 Oral phase

The first stage of digestion begins with the introduction of the food into the oral cavity or mouth. The structure of the food is altered in the mouth by mastication or chewing (mechanical forces). This breaks down the food matrix into small particles. Saliva is a complex biochemical fluid which helps in optimizing the food to reach body temperature. It contains salivary proteins and enzymes such as α-amylase which can partially hydrolyse starch-based foods. The masticated food with the help of saliva is converted into a lubricated mass called a bolus. The bolus is then swallowed and passes through the oesophagus and reaches the stomach for initiation of gastric digestion (Singh et al., 2014).

2.7.1.2 Gastric phase

The stomach is a muscular organ that mixes the food with gastric juices. The contents in the stomach are mixed by peristaltic movements and rhythmic contractions. During gastric digestion a thick liquid called chyme is formed. The gastric juices in the stomach mainly constitute hydrochloric acid, pepsin, and gastric lipase. The acidic pH in the stomach inhibits microbial growth, causes aggregation of protein structures, and destabilizes lipid droplets. The enzyme pepsin partially breaks down protein molecules into smaller peptides (Astruc, 2014). On the other hands gastric lipase hydrolyses 10-30% of lipids in food into free fatty acids. The gastric emptying rate depends on the viscosity of the chyme that is formed during the digestion process (Singh et al., 2014).

2.7.1.3 Small intestinal phase

The process of digestion continues well into the small intestine where the nutrients that are released get converted to be easily absorbed. The gastric chyme progresses into the duodenum and is further mixed with pancreatic secretions containing enzymes such as proteases,

peptidases, glucosidases, and lipases. Proteins that were resistant to gastric digestion are hydrolysed to a greater extent in the small intestine by the enzymes – trypsin, chymotrypsin, and carboxypeptidases. Carbohydrates are digested by amylases and glucosidases into monosaccharides. While the triglycerides are digested by pancreatic lipase into two free fatty acid molecules and one 2-monoacylglycerol. Bile salts produced by the gall bladder help in stabilizing and emulsifying lipid droplets to form micelles which are then efficiently absorbed by the cells of the small intestine (Singh et al., 2014).

2.7.2 Assessing digestion of foods with digestion models

To understand the fate of food during gastro-intestinal digestion several models have been developed for observation of the dynamic process by stimulating the digestive conditions. There are two methods that are used to assess the digestion of foods: (i) *In vivo* systems, which utilise human or animal subjects (especially pigs and rats) to elucidate accurate results that focus on comprehensive food digestibility and interactions. However, this method is time consuming and has huge experimental costs and several ethical issues that can prove to be challenging when analysing results. (ii) *In vitro* systems are more effective for collecting data when compared to *in vivo* systems as they are easily set up in laboratories. They are equipped to simulate different phases of digestion using artificial systems and appropriate digestive conditions. However, certain processes and phenomena during digestion that involve adaptations to external stimuli are hard to replicate in *in vitro* systems (Astruc, 2014; Hur et al., 2011). Keeping that in mind, scientists have been developing simple static and more complex dynamic *in vitro* models according to their research objectives.

2.7.2.1 Static *in vitro* digestion model

The INFOGEST protocol was proposed and developed in 2014 and was updated in 2019 by an international group of scientists working on food digestion (Minekus et al., 2014; Brodkorb et al., 2019). The protocol outlines experimental conditions that closely mimic human digestion physiology (Kaur et al., 2014). Preparation of digestive juices for the three different phases (oral, gastric, and intestinal), sample to simulated digestive juice ratio, required enzyme amount to be added and appropriate digestion conditions are summarised in the protocol. The advantages of the static model include running a number or samples at the same time and ease of use. However, the limitations of the static *in vitro* model are the evolution of physicochemical changes occurring at different times and in different compartments of the gastro-intestinal tract during the course of digestion (Dupont et al, 2019).

2.7.2.2 Semi-dynamic *in vitro* digestion model

Several multi-compartmental dynamic models have be developed around the world. Relevant physiological parameters are set up within the different parts of the gastro-intestinal tract to closely mimic the fate of food during the process of digestion which involves many dynamic processes. The results have also been validated against *in vivo* data. To be physiologically relevant the *in vitro* semi dynamic models need to be properly programmed. Harmonizing the parameters for digestion of different food families at an international level is also necessary for the future of understanding the mechanisms of food digestion. Limitation of the dynamic systems currently includes lack of devices to evaluate the simulation of intestinal absorption (Dupont et al, 2019).

2.7.3 Digestion of meat and its nutrient bioavailability

In the case of meat, the structure and mechanical properties influence the overall rate of digestion and absorption. Mastication helps to initially break down the structure, reduces the particle size and increases the surface area. This enhances the penetration of acid and various enzymes that continue disintegration of the meat during gastro-intestinal digestion (Baugreet et al., 2019). The bioavailability of nutrients is defined as the amount of ingested nutrient that is absorbed and utilized by the human body for physiological processes (H. Singh et al., 2014).

2.7.3.1 Amino acid availability in meat

Skeletal tissues in the human body are constantly remodelled through protein synthesis and breakdown. Several studies report higher satiety levels after consuming high quality dietary proteins such as meat (McNeill, 2014). Protein ingestion into the human body delivers amino acids that serve as precursors that stimulate protein synthesis. Postprandial protein synthesis encompasses various processes that utilize ingested protein digestion and amino acid absorption into the body to carry out regular metabolic functions (Trommelen et al., 2021). Several proteolytic enzymes are required to breakdown dietary proteins into smaller peptides or amino acids. Each enzyme has specificity for different peptide bonds of the dietary protein structure. The following table summarises protein digestive enzymes.

Table 2.3: Protein digestive enzymes and their amino acid specificity (adapted from Wildman & Medeiros, 2019).

Enzyme	Peptidase type	Organ of origin	Amino acid hydrolysed
Pepsin	Endopeptidase	Stomach	Arginine, phenylalanine, tyrosine, tryptophan
Trypsin	Endopeptidase	Pancreas	Lysine, arginine (basic amino acids)
Chymotrypsin	Endopeptidase	Pancreas	Phenylalanine, tyrosine, tryptophan (aromatic amino acids)
Carboxypeptidase A	Exopeptidase	Pancreas	Alanine, threonine, valine, leucine, isoleucine (aliphatic amino acids) and aromatic amino acids
Carboxypeptidase B	Exopeptidase	Pancreas	Arginine, lysine

Certain proteins are rapidly digestible and release peptides to a greater extent in a short period of time, whereas other slowly digested proteins stimulate amino acid synthesis over a longer period. Thus, different types of protein when ingested are shown to have varying protein digestion rates and hence have different amino acid absorption rates. This ultimately affects the postprandial muscle protein synthesis (Gorissen et al., 2015).

Meat contains high-quality protein and bioavailable haem iron that is proven to increase vitality when consumed moderately over time. Meat in general is a consistent source of indispensable amino acids and the nutritional quality of protein is defined by the rate of release of peptides or amino acids during digestion which is correlated to amino acid uptake in the intestinal phase. According to Ciuris et al. (2019), meat protein had the highest calculated true illeal digestibility value of 1.00 which corresponds to 100 % digestibility rate of most amino acids in meat while plant-based proteins sources had a lower true illeal digestibility which ranged from 0.75 to 0.92. Protein ingestion can strongly influence and accelerate protein synthesis rates, which is mainly attributed to stimulatory effects of amino acids such as isoleucine, leucine, and valine. Several studies have reported the effectiveness of high-quality proteins in promoting weight loss, maintaining muscle mass and in some case regaining of lost muscle due to sarcopenia (degenerative loss of skeletal muscle mass) in older adults (Berrazaga et al., 2019; Dangin et al., 2003). For the elderly population and fitness enthusiasts, studies suggest incorporating

rapidly digestible protein sources into the diet, as they can prove to be beneficial with shorter absorption times and limited protein absorption losses (Dangin et al., 2003).

Recent studies conducted using *in vitro* digestion models have highlighted that cooking temperature, cooking time, and chewing time of meat can greatly affect the protein digestion and absorption rates (Bax et al., 2012; Pennings et al., 2013; Rémond et al., 2007). During the cooking of meat, coagulation of proteins occurs, and tightly coiled polypeptides tend to unfold and form intermolecular crosslinks, which result in the formation of large aggregates. The aggregates formed have lower rates of proteolysis due to reduced enzyme or protease susceptibility under gastro-intestinal conditions and hence the release of amino acids and their bioavailability during digestion is reduced (Bhat et al., 2021; Kaur et al., 2014; Santé-Lhoutellier et al., 2008). Moreover, non-hydrolysed peptides are extensively fermented in the colon into potential mutagenic products that increase the risk of colon cancer (Santé-Lhoutellier et al., 2008). However, grilling beef steaks at high temperature for lower time has been reported to give 95% protein digestibility, which is measured in terms of hydrolysed peptides in the small intestine, with improved amino acid absorption to maintain proper metabolic functions of the body (Bax et al., 2012; Kaur et al., 2014).

2.7.3.2 Free fatty acid availability in meat

The lipids in meat are largely in the form of triacylglycerols. The triacylglycerols present in the intramuscular fat of the meat are predominantly surrounded by multilayers of soluble meat proteins. Hence, for digestion of meat lipids to take place, it is crucial for the meat protein structures to be disintegrated to release the lipid molecules in order to be accessible by enzymes which carry out lipolysis. The physical transformations and degradation of the meat matrix during gastro-intestinal digestion is a key factor which facilitates the release of triacylglycerols in various forms. The protein-lipid interactions in meat contributed by the high protein content and low to medium lipid content can affect the rate of lipolysis which has been observed to range from (45% – 100%) at pH 7 gastro-intestinal conditions (Calvo-Lerma et al., 2018; Asensio-Grau et al., 2019).

Triacylglycerols are insoluble in aqueous conditions while lipases are water soluble, hence the lipolysis takes place in the oil-water interfaces. The surface area of the interface, emulsification by bile salts and the structure of the triacylglycerol molecules influence the rate of lipid digestion (H. Singh et al., 2014).

Postprandial lipemia is caused by the elevated levels of triacylglycerols in the blood serum after the consumption of lipid foods. Postprandial lipemia depends on the food matrix, quantity, and the type of ingested lipids. Saturated fatty acid (SFAs) induced lipemia is longer in duration and more pronounced when compared to lipemia induced by PUFAs. In meat, most of the fatty acids are long chain fatty acids (> C12). The digestion of long chain fatty acids and absorption in the small intestine promotes satiety when compared to the quick absorption of medium chain fatty acids (Goodman, 2010).

2.8 Health Impacts of Dietary Lipids

The biological value of beef is determined in part by the lipid profile present in the meat and so it is essential to understand, ration and balance the feed for cattle to ensure optimized meat production (Jukna et al., 2017). It is well established that the genetics and the feed regime/diet of the animal plays an important role of type and extent of deposition of lipid in the carcass. Lately the focus on meat lipids has extended beyond quality aspects. There have been increasing studies on their effects on human health.

2.8.1 Lipidomic profile

2.8.1.1 SFA levels

Red meat lipids predominantly contain myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0). It has been reported that some saturated fatty acids, such as (C12:0) and (C14:0), have been linked to vascular and coronary diseases in humans while research has shown that (C18:0) has no effect on plasma cholesterol levels (Troy et al., 2016). The fat in red meat is often erroneously considered as high in fat especially the SFAs. The intramuscular fat is usually in the range of 3-5% in lean beef and 5-10% in grain-finished beef (Frank et al., 2016). However, it has become essential to eradicate vascular and coronary disease-causing risk factors by utilizing feeding systems to manipulate the intramuscular fat content and produce leaner meat (Bermingham et al., 2018).

The free fatty acid (FFA) composition in the human body is a very important determinant of the health benefits in the body. The free fatty acids are mainly released into the bloodstream from the adipose tissue of consumed food materials during lipolysis. The free fatty acids provide energy to the body and serve as a substrate for re-esterification into triacylglycerols. It is highly recommended to have a low proportion of saturated fatty acids in the serum and a high proportion of unsaturated fatty acids as an elevated and prolonged lipemia is associated with increased levels of SFAs and enhances the risks of diabetes, atherosclerotic CVD, and obesity (Warensjö et al., 2009). In recent times reducing the SFA intake has been one of the key nutritional recommendations worldwide to prevent the risk of chronic diseases. However, it is important to note that individual SFAs affect human health differently and are also influenced by various food matrices (Vahmani et al., 2020).

2.8.1.2 PUFA content

It is recommended to consume n-3 PUFAs as these contain vaccenic acid (11t-18:1 isomer). This fatty acid is linked to prevention of cardiovascular diseases, depression and obesity and is also beneficial in improving brain development and better cognitive function in humans. Among the n-3 PUFAs, EPA and DHA have been demonstrated to have important roles in proper brain functioning and reduction in the risk of CVD (Scollan et al., 2006). The difference between n-3 and n-6 fatty acid-derived eicosanoids is that most of the mediators formed from EPA are anti-inflammatory, whereas those from arachidonic acid (derived from Linoleic acid) are pro-inflammatory or show other disease propagating effects.

2.8.1.3 Desaturase indices (DI)

Delta-9 desaturases also known as stearoyl CoA desaturases (SCD) are crucial enzymes in the metabolism of fatty acids (Mwangi et al., 2019). Many SCD related studies were conducted on animal models. The enzymes aid by catalysing the conversion of SFAs to MUFAs by introducing a cis bond in their delta-9 position (Warensjö et al., 2009). SFAs such as myristic C14:0, palmitic C16:0 and stearic C18:0 acids are the major substrates which are converted to myristoleic C14:1, palmitoleic C16:1(n-7) and oleic C18:1(n-9) acids respectively. A higher desaturase index (DI) or ratio of DI-16 and DI-18 indicates the possible development of various pathological diseases ranging from diabetes, insulin resistance, CVD, obesity, hypertension, immune disorders, and other neurological diseases (Alarcón et al., 2016; Ponnampalam et al., 2006).

It has been found that increased activity of SCD can also play an important role in the development of fatty liver and influence abnormal fatty acid partitioning by promoting the synthesis of MUFAs which serve as mediators for cellular signal transduction in humans. Hence, an imbalance in the fatty acid levels can also be implicated in carcinogenesis (Chajès et al., 2011).

2.8.1.4 Fatty acid ratios

A high MUFA/SFA ratio (above 1) in the human diet can also lead to various complications, hence studies indicate that the inclusion of pasture-fed beef into the human diet could help lower the MUFA/SFA ratio and significantly reduce the low-density lipoprotein content and increase the high-density lipoprotein content in the blood plasma of humans. This can help maintain cholesterol levels and prevent bodily complications (Van Elswyk & McNeill, 2014).

A study conducted on rats showed that when the (PUFA+MUFA)/SFA ratio of the diets was lower than 2 it helped to maintain a low plasma cholesterol level (Chang et al., 2004).

There has been a reduced intake of omega-3 PUFAs recently, which is due to the intake of high energy foods. This has resulted in a higher omega-6/omega-3 fatty acids ratio (Alarcón et al., 2016). Many studies conducted all over the world report different n-6/n-3 ratios. In countries such as the, UK, European nations, USA, and urban India the values have been found to be 15-74 while in Japan it is reported to be around 4. Studies recommend an omega-6/omega-3 ratio ideally ≤ 4 (Alfaia et al., 2010; Simopoulos., 2011). A higher ratio is a risk marker for the pathogenesis of chronic diseases like CVD. Increasing the intake of omega-3 fatty acids can lower adverse health risks (Gupta et al., 2013).

Chapter 3: Research Significance and Hypotheses

3.1 Significance of the Research

This research project is a part of the programme called "The New Zealand Pasture-Raised Advantage" funded by the Meat Industry Association, Beef + Lamb New Zealand Ltd, the High-Value Nutrition National Science Challenge and the Ministry of Business, Innovation and Employment. The project aims to understand the nutritional and health implications of consuming pasture-fed beef (cattle that has predominantly grazed on forage, hay and/or silage) and grain-finished beef (cattle that has been raised on pasture and also fed a concentrate/grain-based diet during later phases before slaughtering) from New Zealand. For this study the striploin, tenderloin, and topside were chosen as the three commercially relevant meat cuts. Beyond Burger® from Beyond Meat™ was chosen as the plant-based meat alternative after discussion with industry professionals and scientific partners. This research study is the second part of the programme. Part one was undertaken by Crown Research Institute AgResearch, analysing the overall nutritional profiles of the meat. Researchers from The University of Auckland will then oversee the final two stages, clinical studies investigating both the short-term and long-term well-being and health effects of red meat consumption.

3.2 Significance of Samples Used

The striploin cut is derived from the *Longissimus dorsi* muscle. This cut is found towards the middle to rear part of the cattle in the loin region behind the ribs of the animal. The striploin steaks were chosen, as they have relatively less connective tissue hence producing one of the most tender cuts with good marbling and strong beef flavour. The fat on this cut is easier to trim and there are no large pockets of fat, which favours a faster cooking time and makes it easier to cut (Lopez-Alt, 2011).

Beef tenderloin is primarily made up of the *Psoas major* muscle. This muscle extends along the rear portion of the spine, from the hip bone to the thirteenth rib of the animal. As the muscle is seldom exercised this cut is regarded as the most tender cut and has a milder beef flavour than other muscle cuts. The tenderloin is encased in a layer of crumbly fat, and a smaller chain muscle called the *Psoas minor* runs along the tenderloin (Lopez-Alt, 2011). Tenderloin steaks are usually sold defatted with the chain muscle removed.

Topside in beef is a large and relatively lean cut. It is taken from the *Semimembranosus* muscle which is found at the rear of the inner thigh in the hind legs of the animal. It is slightly tougher in texture when compared to striploin or tenderloin which ends up making it cheaper on the price scale. Topside however, has a more pronounced beef flavour. The topside muscle is mostly preferred for slow cooking methods such as braising and roasting (Lopez-Alt, 2011).

The Beyond Burger® from Beyond Meat™ was chosen as the plant-based meat alternative used for the study based on the following criteria: 1) nutrition matched to beef (macronutrients), 2) readily available in the retail space in New Zealand in the year 2020, and 3) matched the appearance of beef.

Table 3.1: The nutritional composition of 100g of uncooked Beyond Burger® and 100g of uncooked premium beef mince (90 % lean and 10 % fat) found in a New Zealand supermarket (Woolworths New Zealand Limited., 2022).

Nutritional composition	Energy (kcal)	Protein (gm)	Fat (gm)	Saturated fat (g)	Carbohydrate (g)	Fibre (gm)	Salts (mg)
Beyond Burger®	252	17.7	19.0	5.6	3.5	1.3	750
Beef mince (90 % lean and 10 % fat)	180	22.4	10.0	4.1	0.0	0.0	400

3.3 Research Hypotheses

This research addresses two hypotheses:

- Different feeding regimes (pasture-fed or grain-finished beef) will affect the enzyme kinetics and digestibility of complex proteins and lipids that have been hypothesised to be beneficial for maintaining overall good health.
- The different rates of amino acids released during digestion of meat and plant-based meat alternative affect the anabolic effects in muscle and metabolism while the different amounts of free fatty acids released and absorbed after meat and plant-based meat alternative digestion affect heart related health and diseases.

3.4 Research Objectives

- The first objective of this study was to determine the nutritional value of three New Zealand pasture-fed cooked beef cuts and compare it with three grain-finished cooked beef cuts and with a cooked plant-based meat alternative.
- The second objective was to quantify and compare the rate of protein digestion during *in vitro* digestion of cooked beef from different production systems and cooked plant-based meat alternative using ninhydrin assay and SDS-PAGE.
- The third objective was to quantify and compare the amount of individual free fatty acids released during static *in vitro* lipid digestion of cooked beef from different production systems and cooked plant-based meat alternative, using gas chromatography.

Chapter 4: Materials and Methods

4.1 Materials

In late 2019, fifteen pasture-fed steers and heifers were sourced from three farms that supply Silver Fern Farms and were slaughtered over 1 day at the Silver Fern Farms (Whakatu plant). Fifteen grain-finished steers were sourced from the Canterbury feedlot of Five Star Beef where they were finished an average of 122 days (range 118-125 days) on maize silage/barley wheat/potato starch and straw, producing an average daily gain of 1.4 kg. They were slaughtered at the ANZCO Foods Ashburton plant.

The carcasses from fifteen cattle each for pasture-fed beef and grain-finished were collected and chilled for 24 hours post slaughter and the meat cuts were collected from the left side of the carcasses, vacuum packaged and aged for 21 days at -1.5 °C before sub-dividing into separate steaks. The striploin and tenderloin samples were cut into 1-inch-thick steaks and vacuum packaged individually in labelled bags (**Figures 4.1 and 4.2**). The topside samples were cut into 1-inch cubes and vacuum packaged individually in labelled bags (**Figure 4.3**). Samples were shipped in chiller boxes to Massey University (Palmerston North, New Zealand) by AgResearch, Ruakura campus (Hamilton, New Zealand) and stored at -20 °C in a temperature-controlled room until they were used for analysis. Out of the fifteen carcasses, five carcasses each from pasture-fed beef, and grain-finished beef were selected for this study, based on carcass live weight, pH, and marbling score, in consultation with the team at AgResearch. The MSA and AUS-MEAT marbling scores are independent of each other as each of their assessment criteria are different (*The Effect of Marbling on Beef Eating Quality*, 2018). However, an AUS-MEAT marbling score of 3 denotes beef with light steaks of fat and can be related to a MSA marbling score range of 500 to 600. While most pasture-fed beef which have a MSA marbling score between 300 and 400 can be compared to AUS-MEAT marbling score 1. More details on the selected carcasses are given in **Table 4.1.**

The plant-based meat alternative chosen for this study was Beyond Burger® from Beyond Meat™ (Lot code-V1B0112209) (**Figure 4.4**). Samples were bought frozen from Countdown supermarket (Palmerston North, New Zealand) in June 2020. The ingredients in the Beyond Burger include water, pea protein isolate, rice protein, mung bean protein, expeller-pressed canola oil, refined coconut oil, cocoa butter, methylcellulose, potato starch, sunflower lecithin,

apple extract, pomegranate fruit powder, beet juice extract, potassium chloride, vinegar, lemon juice concentrate, and salt (Beyond Meat™., 2021).

Five animals each from pasture-fed and grain-finished production systems were chosen to conduct the following experiments and to account for biological variability. Whereas the Beyond Burger® is a consistent formulated product hence triplicate experiments were conducted for reproducibility.

All chemicals and reagents used in the study were of analytical grade and their sources have been elaborated further in the methods section.

Table 4.1: Details of carcasses used for this study

Production	Carcass ID	Type	Carcass Live Weight	Graded weight	Side weight L	Side weight R	pH	Marbling
Pasture	253	Steer	618	343	171	172	5.57	340
Pasture	250	Steer	615	342	171	171	5.55	400
Pasture	252	Steer	611	341	170	171	5.55	320
Pasture	255	Steer	609	343	172	171	5.80	300
Pasture	256	Steer	606	335	168	168	5.57	330
Grain	103	Steer	616	326	165	162	5.47	3
Grain	116	Steer	612	333	166	167	5.45	3
Grain	112	Steer	604	337	170	167	5.45	3
Grain	124	Steer	612	340	172	167	5.46	3
Grain	118	Steer	598	324	163	162	5.46	3

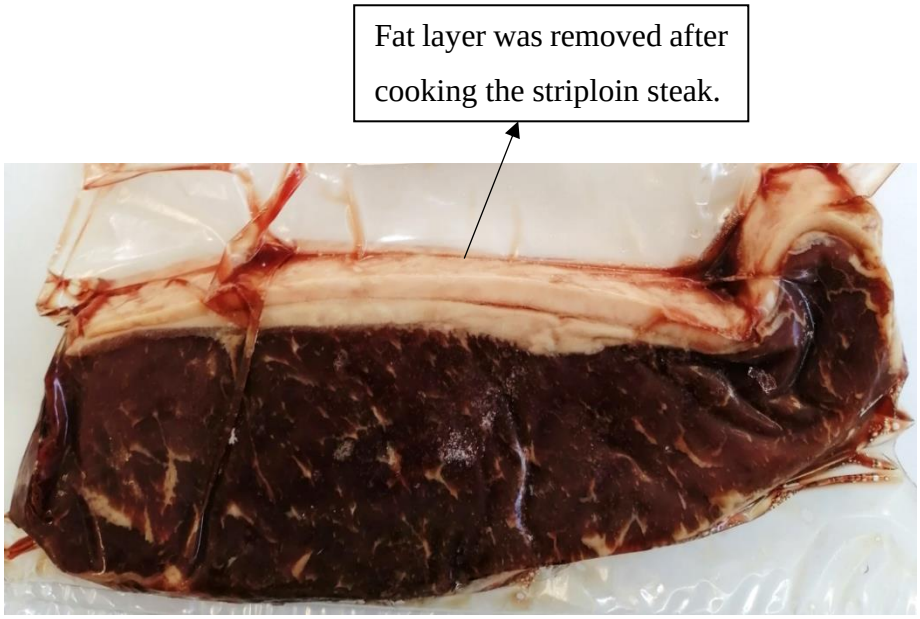


Figure 4.1: Striploin steak (16 cm x 7 cm x 2.5 cm) individually vacuum packaged.

Figure 4.2: Tenderloin steak (9 cm x 7 cm x 2.5 cm) individually vacuum packaged.

Figure 4.3: 300 gm of topside cubes (2.5 cm x 2.5 cm x 2.5 cm) in separate vacuum sealed bags.

Figure 4.4: Plant-based meat alternative (Beyond Burger ™) used for this study.

4.2 Methods

4.2.1 Cooking of Samples

The meat was cooked using the methods of Purchas and Wilkinson (2013). The meat alternative was cooked by slightly modifying the instructions on the package.

4.2.1.1 Cooking of Striploin and Tenderloin Steaks

The selected steak with 2.5 cm thickness was submerged in water at room temperature for 1 min to thaw the surface for easier cutting of the frozen meat. Approximately 6.0 cm x 5.0 cm x 2.5 cm frozen meat was cut and immediately vacuum packaged and placed at 4 ℃ to thaw for 16 to 20 h. The thawed meat was then kept at room temperature for 5 to 10 min, pat dried and weighed. The meat was cooked with an electric skillet (ZIP non-stick electric skillet 26 cm dia.) with a surface temperature of 220-230 °C. The meat was cooked for 2.5 min on each side initially and additionally for 1 min on each side or to an internal temperature of 65 to 70 °C. The cooked meat was rested for 10 min, pat dried and weighed to estimate cook loss. The subcutaneous fat layer of the striploin was removed. The meat was cut into 5 mm cubes, individually vacuum packaged in separate bags to be used for determination of moisture content, crude fat, crude protein, freeze drying and *in vitro* digestibility studies. The vacuum sealed sample for *in vitro* digestibility studies was used within 2 hours of cooking and stored in an ice bath post-cooking until added into the digestion reactors.

4.2.1.2 Cooking of Topside cubes

Approximately 70 to 100 g of the topside 1-inch cubes weighing approximately 15-20 g per cube were vacuum packed and thawed at 4 ℃ for around 16 to 20 h. The thawed meat was brought to room temperature, pat dried, and weighed. The meat was browned using an electric skillet (ZIP non-stick electric skillet 26 mm dia.) with a surface temperature of 220-230℃ for 2 min. The browned meat cubes were then put in a casserole (1.35 L, 22cm diameter). Water was added into the casserole until 50 % of the cubes were submerged. The casserole was sealed with two layers of aluminium foil and baked in an oven (OB60SCEX1, Fisher & Paykel Ltd, Dunedin, New Zealand) at 160 °C using the fan forced function. After 2 h of cooking, the water was discarded and the meat was allowed to cool down, followed by weighing after pat drying to determine cook loss. The meat was then cut into 5 mm cubes which were then vacuum packed and placed in an ice bath until *in vitro* digestion experiments were performed.

4.2.1.3 Cooking of Meat Alternative

Three different boxes, each containing 2 patties, were used for replication purposes. One frozen patty weighing approximately 110 to 113 g (8 cm diameter x 2 cm thickness) was cut in half, vacuum packaged immediately and thawed at 4 °C for 16 to 20 h. The thawed sample was pat dried and weighed before cooking. An electric hot skillet (ZIP non-stick electric skillet 26 cm dia.) was heated to 85 to 95 °C surface temperature. The sample was cooked for 2.5 min per side, twice on each side, for a total of 10 min or until the internal temperature was 60 °C. The cooked sample was rested for 10 min, pat dried and weighed again to estimate cook loss. The meat alternative sample was then cut into 1 cm cubes, and vacuum packaged in separate bags to be used for moisture content, crude fat, crude protein, freeze drying and *in vitro* digestibility studies. The vacuum sealed sample for *in vitro* digestibility studies was used within 2 h of cooking and stored in an ice bath post-cooking until added into the digestion reactors.

4.2.2 Physicochemical Analysis of Meat

The following analyses were carried out on both cooked and raw meat and cooked meat alternative samples.

4.2.2.1 Cook Loss Measurements

The cook loss was estimated as the difference in weight of the samples before and after cooking according to method described in section 4.2.1 for each meat cuts and meat alternative respectively. It was expressed in percentage of the weight before cooking.

$$\% \text{ Cook Loss} = \frac{\text{wt of sample before cooking (g)} - \text{wt of sample after cooking (g)}}{\text{wt of sample before cooking (g)}} \times 100 \quad \text{....Eq.1}$$

4.2.2.2 Moisture Analysis

The moisture content in cooked samples was analysed using AOAC 950.46 method (Lawrence, 2010). A conventional mechanical oven was set at a temperature of 105 ºC with consistent airflow and heat distribution. The cooked and vacuum packaged samples used for moisture content were removed from -18 ºC and thawed in room temperature for 2 h. Two to three grams of meat and meat alternative samples were added into an aluminium dish and weighed. The samples were placed in the oven for air drying for 16 h. Afterwards, the aluminium dishes were cooled in a desiccator and weighed after cooling for 20 min. The loss in weight was reported as moisture content.

$$\%\text{Moisture (w/w)} = \frac{\text{wt of wet sample (g)} - \text{wt of dry sample (g)}}{\text{wt of wet sample (g)}} \times 100 \quad \text{.....Eq.2}$$

4.2.2.3 % Nitrogen and Crude Protein Analysis

The nitrogen content in cooked meat and meat alternative was analysed using the Kjeldahl method (AOAC, 1981). The cooked and vacuum packaged samples for crude protein analysis were removed from -18 ºC and thawed at room temperature for 2 h. The test was performed in triplicate for *n*=5 pasture fed sample, *n*=5 grain-finished sample and *n*=3 plant-based meat alternative. 6.25 was used as the nitrogen to protein conversion factor for meat related samples (Benedict., 1987).

Digestion: ~0.5 g of cooked sample was weighed and added to a digestion tube along with 2 Kjeltabs (Foss North America, MN, USA) followed by 18 mL of concentrated H_2SO_4 (95-98%). A blank digestion was carried out at the same time without any sample but containing all other reagents. The digestion tubes were placed in the digestor unit (DT 208 Foss Digestor, MN, USA) with the water aspirator fully turned on. The samples were digested at 420 °C until the contents of the tubes turned to a clear liquid. The tubes were removed from the heating unit and placed on the exhaust manifold to be cooled. Approximately 70 mL of distilled water was added to each tube and gently mixed.

Distillation: 25 mL of 4 % boric acid solution was added into a 250 mL conical flask and placed in the receiver platform of the distillation unit. The tube containing the digested sample was placed in the pre-warmed distillation chamber of the unit (Kjeltec™ 8100 distillation unit, Foss). 80 mL of NaOH solution was automatically added to the tube once the safety door was closed.

Titration: After the distillation process, the solution in the receiver conical flask was titrated against 0.1 M HCl until the colour of the solution reached a grey-mauve colour. The volume of HCl used at the end point was noted down.

Calculation: the nitrogen percentage in the sample was calculated as follows:

$$\%\text{ Nitrogen} = \frac{\text{vol of HCl (mL)} \times \text{molarity of HCl} \times 14}{1000 \times \text{wt of the sample initially used (g)}} \times 100 \quad \text{.....Eq.3}$$

$$\%\text{ Crude Protein} = \%\text{ Nitrogen} \times 6.25 \text{ (conversion factor for meat)} \quad \text{.....Eq.4}$$

4.2.2.4 % Crude Fat Content

The fat content of the cooked meat samples was estimated using the Soxtec, (Meat), AOAC 991.36 method. The fat content of the cooked meat alternative samples was estimated using the Mojonnier (acid) method, AOAC 922.06 for Flour, Baked, Extruded products. The fat content of all the cooked samples was analysed by the Nutrition Laboratory at Massey University.

4.2.2.5 Colour Measurements

The colour of the meat samples was measured before and after cooking the meat cuts. For each animal one replicate or steak weighing approximately 200 g was chosen and divided into two portions (Raw and cooked). The colour was measured using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing, Singapore) using a D65 illuminant and a 10 ° standard observer. Colour readings were expressed according to the CIELAB system.

The raw samples were pat dried and cut horizontally through the grain leaving a thickness of 10 to 15 mm for enough opacity. The samples were covered with cling wrap and bloomed at 4 °C for 30 min. A minimum of 6 readings at different locations on the surface of each of the sample was recorded (Khliji et al., 2010). The second portion of the samples was cooked according to section 4.2.1 for meat cuts and meat alternative respectively. The samples were then rested for 10 min, pat dried and cut horizontally through the grain of the meat leaving a thickness of 10 to15 mm for opacity. The samples were covered with cling wrap and bloomed at 4 °C for 30 min. A minimum of 6 readings at different locations on the surface of each of the cooked sample was recorded (Khliji et al., 2010).

4.2.3 *In vitro* oral-gastro-small intestinal digestion experiments

4.2.3.1 *In Vitro* Digestion Protocol

The *in vitro* digestion of the cooked meat samples and meat alternative was conducted as described by Chian et al. (2019) and Minekus et al. (2014) with some modifications. In total, three replicates were done for the plant-based sample and triplicate digestions for each meat cut of each selected animal. For each replicate, two separate *in vitro* digestion experiments were performed in individual double jacketed glass reactors at 37 °C. In the previous protocols each time point was done in each jacketed reactor. The pepsin amount was calculated based on 8 U/mg meat protein for this study while the INFOGEST protocol uses 2000 U/ml of digestion mixture (Chian et al., 2019; Kaur et al., 2014).

Eight grams (8 g) of meat or meat alternative was ground using a mortar and pestle for 30 sec and added into first reactor followed by incubating the samples with 8 mL simulated salivary fluid (maintained at pH 7 ± 0.1) containing 1.25 x 10^{-6} katal /mL bolus of α-amylase ((enzyme activity of 31.1 units/mg; 10065, Sigma Aldrich, Saint Louis, MO, USA) for 2 min to mimic mastication and oral digestion. Gastric digestion was initiated by the addition of 32 mL of simulated gastric juice (maintained at pH 3 ± 0.1) containing 1.33 x 10^{-7} katal/mg protein of porcine pepsin (enzyme units of 466 units/mg solid; P7000, Sigma Aldrich, Saint Louis, MO, USA). A magnetic stir-bar (30 mm length and 7 mm dia.) along with some glass balls (3 to 5 mm dia.) were added to stir at 100 revolutions per minute and mimic maceration in the gastric phase. Although 10 and 30 min of gastric digestion were conducted, they did nort show any significant differences. Hence, only the 30 sec (considered as t=0 min) and 60 min of gastric digestion were reported in the present study.

In the second reactor with 8 g of ground sample, *in vitro* small intestinal digestion was initiated after 60 min of gastric digestion as described above. Small intestinal digestion was commenced by adding 48 mL simulated small intestinal fluid (maintained at pH 7 ± 0.1), pancreatin (4x USP specifications; P1750, Sigma Aldrich, Saint Louis, MO, USA) at 1:100 pancreatin to protein ratio and 6 mL of 10 mmol/L bile extract (B8631, Sigma-Aldrich, Saint Louis, MO, USA) into the reactor containing 48 mL of gastric chyme. Sampling was done after 10, 60 and 120 min of small-intestinal digestion. The pH was adjusted according to each phase using 6M hydrochloric acid (HCl) and 1N sodium hydroxide (NaOH) solution.

After every sampling time the digestive enzymes were inhibited by adding 12 µL of Pepstatin A (ab141416, Abcam, UK) (0.5mg/mL in methanol) to every 1 mL of the gastric digests while 0.45 mL of SIGMAFAST^{TM} protease inhibitor cocktail solution (S8820, Sigma Aldrich, USA) (one tablet in 50 mL milliQ water) was mixed to every 1 mL of small-intestinal digest (Ménard et al., 2018). The mixture of digests and enzyme inhibitors was homogenised for 30 sec using a homogeniser with a 5mm diameter disperser (T 10 basic ULTRA-TURRAX® Homogenizer, IKA, Germany) at setting 3. The digests were transferred to 2 mL centrifuge tubes and immediately stored at -20 °C until further analysis.

The protein and lipid digestibility referred in further sections correspond exclusively to *in vitro* digestibility.

4.2.3.2 Ninhydrin Analysis

The degree of proteolysis of meat protein can be estimated as the concentration of free amino nitrogen in the digests using the ninhydrin assay (Chian, 2021). The ninhydrin assay is convenient to use, utilises inexpensive equipment and can be used to analyse large number of samples (Sun et al., 2006). Ninhydrin forms a purple chromophore known as Ruhemann's purple when it reacts with the α-amino group of primary amino acids on heating to boiling temperatures (Chian, 2021; Friedman, 2004).

Digested samples were thawed at 4 °C for 30 min and centrifuged at 14,100 x *g* for 3 min (Eppendorf Minispin plus, Enfield, CT, USA) at room temperature. The supernatant was then filtered through a 0.45µm PVDF filter (Millex®, Merck, Darmstadt, Germany) before analysing for ninhydrin reactive amino N (%) using the method described by Moore (1968) using 2% ninhydrin reagent (N7285, Sigma Aldrich, Saint Louis, MO, USA) (Sigma, 2003).

The supernatant of the digests was made up to 1 mL using pH adjusted milli Q water and then 0.5 mL of the ninhydrin solution was added. The Kimax tubes were placed in boiling water for 10 min. The tubes were cooled down to room temperature before adding 2.5 mL of 95 % ethanol and vortexed to mix the contents. The absorbance was measured at 570 nm using a UV-Vis spectrophotometer (Thermo Scientific Helios Epsilon, WI, USA). A standard curve using a series of concentrations (0, 0.0125, 0.025, 0.0375 and 0.05 µmol/mL) was prepared using a stock solution of 50 µM glycine in 0.05 % glacial acetic acid in Kimax tubes and made up to 1 mL using milli Q water. It was followed by the addition of ninhydrin and ethanol as described above. The standard curve was plotted as absorbance (y-axis) vs glycine content (x-axis). The amino N concentration was determined by using the equation from the standard curve. The percentage of ninhydrin reactive amino nitrogen released at different digestion time points was calculated using the equation below:

Ninhydrin-reactive amino nitrogen released (%) = Ninhydrin-reactive amino nitrogen in the digests / total nitrogen content present in the sample x 100……... Eq.5

Three replications with two absorbance readings were recorded for each digestion time point.

4.2.3.3 Tricine-Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was first described by Laemmli in 1970 and since then it has been used for polypeptide separation and molecular weight quantification. The SDS-PAGE is widely used due to its convenience, simplicity, and rapid reproducibility (Matsumoto et al., 2019).

For meat protein digests, which contain fragmented proteins (1-30 kDa), it is recommended to use Tricine-SDS-PAGE which allows separation of smaller peptides with high resolution (Haider et al., 2012).The homogenised digests were examined for breakdown of proteins using reduced-Tricine-SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Chian et al, (2019). The digests were mixed with tricine sample buffer (Bio-Rad Laboratories, Hercules, CA, USA) containing 2% β-mercaptoethanol (M6250, Sigma Aldrich, Saint Louis, MO, USA), in a 1:1 ratio and heat treated for 5 min in a 100 °C water bath. 25 µL of each sample was loaded into individual wells (16.5% gradient Tricine gels, Criterion™ Precast Gel, Bio-Rad Laboratories, Hercules, CA, USA) at a protein concentration of 1 mg/mL. 10 µL of Precision Plus Protein™ All Blue Prestained Protein Standard (Bio-Rad Laboratories, Hercules, CA, USA) was loaded into the wells as reference.

The gel was run using 1x Tricine running buffer (Bio-Rad Laboratories, Hercules, CA, USA) in a Criterion™ cell (Bio-Rad Laboratories, Hercules, CA, USA) at a constant voltage of 125 V for at least 2 h. The gel was fixed using the fixation solution (40% methanol, 10% glacial acetic acid and 50% Milli-Q water) for 30 min before staining with Bio-safe™ Coomassie blue stain (Bio-Rad Laboratories, Hercules, CA, USA) for 1 hr and rinsed and stored in Milli-Q water at 4°C for at least 24 hours until observation. The gel was scanned with a Gel Doc XR + Gel Documentation System (Bio-Rad Laboratories, Hercules, CA, USA), followed by analysis using Image Lab™ software (version 6.1.0, Bio-Rad Laboratories, Hercules, CA, USA).

4.2.4 Free fatty acid analysis of digests

The meat samples were trimmed of all external fat prior to *in vitro* lipid digestion; thus, the only source of fat was the intramuscular fat. The *in vitro* lipid digestions of meat samples and meat alternative were conducted according to section 4.2.3.1. However, sampling was only done at the end of 180 min of gastro-small intestinal digestion. 96 mL of digesta after 180 min was inhibited with 43 mL of SIGMAFAST™ protease inhibitor cocktail solution (S8820, Sigma Aldrich, USA) (one tablet in 50 mL milliQ water stock). The mixture of digest and enzyme inhibitors was homogenised for 30 sec using a homogeniser with a 5mm diameter disperser (T 10 basic ULTRA-TURRAX® Homogenizer, IKA, Germany) at setting 3. The homogenised digests were transferred into individual Ziploc bags (30 cm x 20 cm) and stored at -20 °C to be freeze-dried. The freeze-dried digests were ground to a fine powder and used for subsequent analysis.

4.2.4.1 Methylation of total fatty acids

The total amount of fatty acids was analysed in the ground freeze-dried digests using the method described by Zhu et al. (2013).

Freeze-dried (100 mg) digested meat sample was weighed in a glass tube. An internal standard was prepared by making a stock solution of 1 mg of methyl tricosanoate/mL of heptane. The standard solution (1 mL) was added to the glass tubes containing the sample. Potassium hydroxide (0.7 mL of 10 M) and 5.3 mL of methanol were then added, and the contents were vortexed. The tubes were then incubated for 90 min at 55 °C in a water bath and vortexed individually every 20 minutes. The tubes were then cooled to room temperature and 0.58 mL of 12 M H_2SO_4 was then added and a precipitate of K_2SO_4 was formed. The tubes were again incubated for 90 min at 55 °C water bath and vortexed every 20 min. The tubes were cooled, and the fatty acid methyl esters (FAMEs) were extracted as follows: 3 mL of heptane was added and vortexed until well mixed and centrifuged at 3500 rpm for 10 min. The top heptane layer was collected in another glass tube containing 1 mm bed of anhydrous Na_2SO_4. Finally, the heptane layer containing methylated total fatty acids was collected in a separate 2 mL GC vial and stored at -20 °C until GC analysis.

4.2.4.2 Methylation of ester forms of fatty acids

Ester forms of the fatty acids was determined in the digests by the sodium methoxide transesterification method as described by Zhu et al, (2013).

Ground freeze-dried (100 mg) samples of meat digest were weighed in glass tubes and 1mL of internal standard (1 mg of methyl tricosanoate/mL of heptane) was added. Then 1mL of sodium methoxide solution was added and the contents were well mixed using a vortex mixer and incubated for 60 min at 55°C in a water bath without shaking. The tubes were then cooled to room temperature and 0.1 mL of glacial acetic acid, 5 mL of saturated NaCl solution and 3 mL of heptane were added and the mixture was vortexed until the contents were well mixed and then centrifuged at 1000 x *g* for 10 min. The organic phase was separated from the aqueous phase. The top heptane layer was transferred into tubes containing anhydrous Na_2SO_4 to remove any traces of water. The top layer was finally collected in 2 mL GC vials and stored at -20°C until GC analysis.

4.2.4.3 GC analysis of FAMEs

Chromatography helps to separate different volatile molecules in a sample by passing it through a column with a matrix that can selectively retard the flow based on the molecule's matrix affinity. The stronger the affinity the slower the molecule will pass through the column. Hence, the separation of different molecules relies on the strength of interaction with the matrix (Srigley & Mossoba, 2016).

The fatty acid compositions of the samples were determined using a gas chromatograph system (GC-2010 Plus, Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector and an AOC-20I auto injector. The column used was RTX®-2330 GC column (Restek, Bellefonte, PA, USA; 60 m in length, 0.25 mm in diameter and 0.10 µm film thickness).

Intact triacylglycerols and free fatty acids formed as products during lipolysis are not very volatile and hence need to be derivatised and volatilized before GC analysis. Triacylglycerols are esterified and methylated to form fatty acid methyl esters (FAMEs) that are highly volatile (Figueiredo et al., 2016). The FAMEs were dissolved in a suitable organic solvent and injected into the GC and heated. The injection volume was set at 1 µL and the carrier gas used was hydrogen gas at a linear velocity of 40 cm/s. The split ratio was set at 50:1. The initial oven temperature was set as 125 ℃ for 3 min, that was increased to 220 ℃ at the rate of 2 ℃/ min and then maintained for 5 min. The temperatures of the injector and the detector were set at 260 ℃ and 265 ℃, respectively. The heated carrier gas carries the volatilized FAMEs into the separating column. The FAMEs are separated into numerous peaks of individual fatty acids and then identified, and quantified based on their different polarities, molecular weights and retention time using an internal standard (C23:0) (T9900, Sigma Aldrich, Saint Louis, MO, USA), an external standard (Supelco FAME mix C4-C24), Sigma Aldrich, Saint Louis, MO, USA), and theoretical flame ionization detector response factors. An internal standard facilitates calculation of FAMEs on mg/g basis. The FAMEs are then converted to individual fatty acids using conversion factors to estimate the total fat content of the sample, SFA levels, desaturase indices, PUFA ratios, etc. The equations for generating the response and conversion factors to quantify individual fatty acids from the Fatty Acid Methyl Esters (FAMEs) were obtained from American Oil Chemists' Society (AOCS Ce 1f-96, Ce 1h-05 and Ce 1i-07).

Unresolved fatty acids were not reported.

4.2.4.4 Calculation of free fatty acids (FFA)

The amounts of individual free fatty acids (mg fatty acid/g cooked meat) were determined in the digests after 180 min of *in vitro* gastro-small intestinal digestion using the Eq.1 described by Zhu et al, (2013).

Individual FFA after 180 min of simulated digestion (mg/g cooked meat)

= individual Total fatty acid after 180 min of simulated digestion – individual EFA (fatty acid in ester form) after 180 min of simulated digestion……Eq.6

4.2.4.5 Calculation of SFA, MUFA and PUFA

The sum of all calculated saturated fatty acids (SFAs), the sum of all calculated monounsaturated fatty acids (MUFAs) and the sum of all calculated polyunsaturated fatty acids (PUFAs) for both methylated total fatty acids and methylated ester forms of fatty acids were reported separately and used for calculating relevant health indicator ratios.

4.2.4.6 Desaturase index and n-6/n-3 fatty acids ratio

The Stearoyl-CoA desaturase index has been reported as product/precursor ratios of individual FA as DI_{16} = C16:1/C16:0 and DI_{18} = C18:1 c9/C18:0 (Alarcón et al., 2016).

The n-6/n-3 FA ratio was calculated as:

Linoleic acid + arachidonic acid / linolenic acid + eicosapentaenoic acid + Docosapentaenoic acid + docosahexaenoic acid…….Eq.7

4.2.5 Statistical Analysis

All the data were recorded and reported as means of at least three measurements. Most of the experiments were carried out in triplicates. Analysis of variance (ANOVA) was carried out using the OriginPro software (OriginLab Corporation, Northampton, MA, USA) with Tukey's test for estimating significance of difference ($p < 0.05$). Principle component analysis (PCA) was done using Minitab® 19 Statistical Software (Minitab LLC, State College, Pennsylvania, USA). Results obtained from the statistical analysis are reported as means ± standard deviation of means. Standard deviation (SD) has been represented as errors bars in some figures.

Chapter 5: Results and Discussion

5.1 Physico-chemical analysis results

5.1.1 Cook Loss (%)

The percentage cook loss in meat cuts and the meat alternative was significantly different as seen in Tables 5.1 and 5.3. It is important to mention that striploin was cooked with the intact subcutaneous fat layer which could partly explain the observed differences in cook loss between tenderloin and striploin, which were cooked using the same method. The cooking of topside cubes using moist heating method in a casserole at a very high temperature for a longer period resulted in significantly higher cook loss. However according to normal practice, the liquid phase or cook loss in the casserole would also be consumed. There was no significant difference noted between meat from different production systems (pasture-fed and grain-finished) when considering the cook loss percentages within the three meat cuts. The meat alternative showed a lower percentage of cook loss which could imply that networks of structured plant proteins have a stronger ability to bind water molecules with the help of the binders added during manufacturing (Kamani et al., 2019).

During cooking, the molecules in meat undergo combinations of thermal processing that includes dry and moist heating techniques. Cooking loss is the phenomenon that causes the meat to lose volume and weight by the process of fluid exudation during the cooking process. The type of cooking method along with cooking temperature and cooking time are crucial in exudation of fluid and the denaturation of the protein's native conformation. Cook loss measures the ability of a food matrix to bind water and fat after the denaturation and aggregation of protein molecules. This change in fluid content, along with modifications of texture-forming properties of the proteins and fats in meat, leads to variation of its quality attributes (Yu et al., 2017,Jiang et al., 2018). The overall cook loss results for meat cuts specifically showed a similar trend as seen in the report of Purchas and Wilkinson (2013). Jiang et al (2018), reported an upward trend between cook loss and higher temperature. Schönfeldt and Strydom (2011) also reported differences in cook loss among different meat cuts due to variation in sample dimensions and spatial distribution of fat or lean on the meat and other meat surface properties. This is believed to affect meat proteins or fat when exposed to heat, shrinkage, protein denaturation and melting of fat (Jeremiah & Gibson, 2003).

5.1.2 Moisture content (%)

There were significant differences among the meat cuts and different production systems for moisture content as seen in Tables 5.1 and 5.3. Pasture-fed beef striploin and tenderloin had a higher moisture content when compared to grain-finished striploin and tenderloin. However, the difference was significant only for the striploin meat cut. The moisture content of topside and the meat alternative were similar which could be due to the higher cooking temperature and time for topside and high temperature and/or pressure during manufacturing of plant-based meat alternative which resulted in loss of moisture content

The water content in meat is mostly bound to protein molecules and are usually found between and within muscle cells and muscle bundles. Cooking can cause water molecules to exude from the meat matrix as increasing the kinetic energy of the polypeptide molecules through heating results in rupturing of weak intramolecular forces that hold the protein molecules together. As the temperature increases the tertiary and secondary structures unfold, lose their disulphide bridges, undergo modifications in their side chains, cross link with other polypeptides and lose the surrounding water (Yu et al., 2017). The moisture content of cooked meat and plant-based meat alternative is usually associated with the sensory attribute of juiciness (Schönfeldt and Strydom 2011). The moisture content of the cooked meat depends on factors such as cooking temperature, final internal temperature, and portion size. Research indicates braised cuts such as topside show higher moisture loss compared to grilled cuts like striploin and tenderloin (Roseland et al., 2015).

5.1.3 Protein content (%)

The protein content of all meat cuts was found to be not significantly different between different production systems (pasture-fed and grain-finished in Tables 5.1 and 5.3). Different meat cuts showed significant differences in protein content. However, when comparing striploin and tenderloin cuts which were cooked using the same method, no significant differences in protein content were observed. Higher cook loss during braising of topside, led to an increase in the dry matter (protein content) when compared to the other two meat cuts.The protein content of the meat alternative was significantly lower when compared to the meat cuts as the commercial product is formulated and contains many other added non-protein ingredients approximately 16 % fat content and 4 % carbohydrates respectively.

A similar protein content was noted in Purchas and Wilkinson (2013) for New Zealand striploin and tenderloin meat cuts. Many researchers have reported a similar trend, where lower moisture content due to cooking at higher temperatures for longer time showed an inverse relationship between moisture and other nutrient components like protein content (Roseland et al., 2015; Smith et al., 2011).

Animal-based food sources are known to contain high quality protein, which is directly related to the composition of nutritionally indispensable amino acids (Edge & Garrett, 2020). Plant-based food sources require consumption of greater quantities or extraction and concentration of plant derived proteins to achieve similar protein content to that of animal food sources (Nosworthy & House, 2017). In the case of the plant-based meat alternative used in this study, the major protein component is pea protein isolate which contains limited amounts of sulphur-containing amino acids, including cysteine and methionine that are important with respect to human nutrition (Lefranc-Millot & Teichman-Dubois, 2018). The amino acid composition and protein content of the meat alternative is also significantly altered based on the protein source and the processing method (Thavamani et al., 2020).

5.1.4 Fat content

The three different meat cuts showed significant differences in the fat content (Tables 5.1 and 5.3). The meat cuts from grain-finished animals had a higher fat content than the meat cuts from pasture-fed animals. But the values were significantly different between the production systems for the tenderloin meat cut. In this study the fat content of cooked Beyond Burger® was found to be significantly higher than all the meat samples from both production systems. The higher fat content in the plant-based meat alternative sample is due to inclusion of fat sources such as coconut, cocoa butter, and canola during processing.

The meat cuts used in this study were chosen based on their relatively leaner meat and lower fat content. Several studies have shown differences in fat content among retail beef cuts, while in red meat the loin is regarded as the leanest portion of the carcass (Pereira & Vicente, 2013). Cuts like striploin have been reported to have varying fat content, which could be due of the inclusion of the subcutaneous fat layer (Purchas et al., 2014; Roseland et al., 2015). However, the subcutaneous fat layer was removed after cooking in this study to follow common eating practice, thereby reducing the fat content.

The plant-based meat alternative is usually assumed by consumers to be lower in fat content. The total fat content reported is very similar to that reported by the manufacturer (Beyond Meat™., 2021).

5.1.5 Colour of meat (raw and cooked)

The colour or lightness (denoted by L*) of raw beef showed significant differences between the three different cuts as well as between the different production systems (Tables 5.2 and 5.3). Since the grain-finished beef was initially pasture-fed then finished on grains before slaughter there was no significant difference between meat cuts and production systems in terms of redness (a*) and yellowness (b*) of the raw meat samples.

The cooked meat cuts showed significant differences in L*, a* and b* between different meat cuts, however there were no significant differences between cooked meat from grain-finished and pasture-fed beef. The significant differences in cooked meat redness and yellowness are mainly attributed to different cooking methods of the meat cuts (fast fry method for steaks and braising for topside cubes).

The muscle cut from extensively pasture-fed beef is darker in colour according to the literature (Hernández et al., 2016; Raes et al., 2003). The colour opacity increases as the proteins myosin and actin start to denature above 50 °C. Cooked meat samples have more colour brightness or lightness than raw samples due to increased reflection of light (also known as scattering) from denatured proteins (Pathare & Roskilly, 2016). Many researchers concluded that major colour changes occur in beef at temperatures above 75 °C. This is the case for the topside samples, which showed a decrease in a* value due to the denaturation of oxymyoglobin, the pigment which imparts bright red colour in meat samples (Brewer & Novakofski, 1999).

Table 5.1: % cook loss, % moisture, % protein and % fat of cooked meat cuts and plant-based meat alternative used in this study.

Production	Meat cut	% Cook loss	% Moisture	% Protein	% Fat
Pasture-fed*	Striploin	14.6 ± 1.3 ^{a}	66.7 ± 1.9 ^{a}	28.1 ± 0.4 ^{ab}	5.0 ± 1.3 ^{bc}
	Tenderloin	18.7 ± 1.3 ^{b}	64.1 ± 1.1 ^{ab}	28.4 ± 1.4 ^{ab}	6.8 ± 1.1 ^{bc}
	Topside	45.4 ± 1.0 ^{d}	53.4 ± 0.4 ^{c}	40.2 ± 1.1 ^{c}	4.1 ± 0.4 ^{c}
Grain-finished*	Striploin	15.8 ± 1.7 ^{ac}	63.5 ± 2.3 ^{b}	29.4 ± 1.0 ^{a}	7.7 ± 1.7 ^{ab}
	Tenderloin	18.0 ± 1.9 ^{bc}	61.5 ± 2.1 ^{b}	27.1 ± 1.1 ^{b}	10.0 ± 2.8 ^{a}
	Topside	44.6 ± 0.8 ^{d}	52.8 ± 0.6 ^{c}	39.2 ± 1.5 ^{c}	7.0 ± 1.4 ^{abc}
Beyond Burger™^		13.7 ± 1.4 ^{a}	55.4 ± 0.9 ^{c}	21.9 ± 0.4 ^{d}	18.1 ± 1.4 ^{d}

Results are represented as mean ± standard deviation. Values with different (a-d) superscripts in the same column differ significantly ($p < 0.05$).

* N =5, all measurements taken for 5 carcasses in triplicate.

^ N =3, all measurements taken for 1 sample in triplicate.

Table 5.2: Colour analysis of raw and cooked meat cuts using CIELAB.

Production	Meat cut	Raw samples			Cooked samples		
		L*	a*	b*	L*	a*	b*
Pasture-fed	Striploin	38.0 ± 0.5 ^{b}	14.3 ± 1.3 ^{a}	8.9 ± 1.1 ^{a}	48.9 ± 1.3 ^{b}	17.5 ± 1.0 ^{c}	12.2 ± 0.7 ^{b}
	Tenderloin	41.0 ± 1.5 ^{a}	15.3 ± 1.0 ^{a}	8.6 ± 0.7 ^{a}	52.6 ± 0.9 ^{a}	20.4 ± 1.7 ^{b}	13.8 ± 0.6 ^{a}
	Topside	39.7 ± 0.9 ^{ab}	14.9 ± 1.5 ^{a}	9.0 ± 0.4 ^{a}	52.0 ± 1.2 ^{a}	6.2 ± 0.3 ^{d}	9.9 ± 0.5 ^{c}
Grain-finished	Striploin	40.0 ± 1.9 ^{ab}	15.5 ± 0.6 ^{a}	9.7 ± 0.8 ^{a}	51.1 ± 1.1 ^{ab}	17.8 ± 1.1 ^{c}	13.7 ± 1.1 ^{a}
	Tenderloin	41.5 ± 1.4 ^{a}	16.7 ± 0.4 ^{a}	8.9 ± 0.3 ^{a}	52.0 ± 1.0 ^{a}	22.9 ± 1.8 ^{a}	14.1 ± 0.6 ^{a}
	Topside	42.3 ± 1.5 ^{a}	15.6 ± 2.9 ^{a}	9.5 ± 1.4 ^{a}	51.5 ± 1.3 ^{a}	6.3 ± 0.2 ^{d}	9.5 ± 0.4 ^{c}

Results are represented as mean ± standard deviation. Values with different superscripts (a-d) in the same column differ significantly ($p < 0.05$).

*N=5, all measurements taken for 5 carcasses in triplicate.

Table 5.3: Two-way ANOVA results between cooked meat cuts and production system for physicochemical analysis.

Analysis	Significance		
	Meat Cut	Production system	Interaction
Cook loss %	*		
Moisture content %	*	*	
Protein content %	*		*
Fat content %	*	*	
Raw L value	*	*	
Raw a value			*
Raw b value			
Cooked L value	*		*
Cooked a value	*		*
Cooked b value	*		*

'*' shows significant difference ($p < 0.05$)

5.2 Estimation and characterization of in vitro protein digestibility

5.2.1 Ninhydrin-reactive free amino nitrogen

The rate of protein hydrolysis was analysed by estimating the amount of reactive amino nitrogen released at specific digestion time points during gastro-small intestinal *in vitro* digestion (Figures 5.1 - 5.4). No large effects of the animal feeding system could be seen for any of the digests from all three meat cuts on the overall release of free amino N values during simulated digestion. From the results it can be concluded that during the gastric digestion there was minimal increase in free amino groups. However, within the first 10 mins of the small intestinal digestion a steep rise in free amino groups was observed, mainly in striploin and tenderloin digests (Figures 5.1 and 5.2). The striploin and tenderloin digests had a higher ninhydrin reactive amino N (%) throughout the digestion course when compared to the free amino N (%) of digests from topside meat cuts and Beyond Burger™. The lower values of free amino N (%) for topside digests (Figure 5.4) when compared to the digests from the other two meat cuts could be due to prolonged cooking at high temperatures (>100 °C) which resulted in aggregation of meat proteins and loss of soluble nitrogen content in the cook loss (Table 5.1). For the plant-based meat alternative digests the lower free amino N (%) values were likely to be related to protein aggregation during high temperature and high-pressure extrusion processing during manufacturing. In Figure 5.3 the striploin and tenderloin digests were compared to see which of them was better digested. The results showed significant differences between the two meat cuts. Tenderloin showed higher digestibility than striploin in terms of free amino N released during 0, 60 and 180 mins of gastro-small intestinal digestion (Table 5.4).

The food matrix containing proteins were broken down during digestion into smaller peptides. Pepsin cleaves protein molecules into smaller peptides and is responsible for about 15% of protein hydrolysis during gastro-small intestinal digestion (Smith & Morton, 2010). The acidic pH of the simulated gastric fluid might have induced the formation of gastric chyme with coagulated proteins that were resistant to further protein hydrolysis by pepsin but in the small intestinal phase greater protein solubilization was observed probably due to the alkaline pH environment (Kaur et al., 2014). Moreover, the pancreatic enzymes present during small intestinal digestion contain peptidases such as trypsin, chymotrypsin, and carboxypeptidase which help to cleave polypeptides, produced as pepsin hydrolysates, into smaller peptides. The specificity of the pancreatic proteases for peptide bonds of amino acids (in Table 2.3) makes

the small intestinal digestion more efficient, resulting in products with only 6-8 amino acids (Ganapathy & Martindale, 2006).

According to the literature, thermal unfolding of proteins exposes hydrophobic sites for proteolytic activity. Some studies suggest that addition of certain kinds of dietary fibre and other added components in the product during meat alternative processing can alter the rate of protein digestion and absorption (Zhou et al., 2021). Aggregated proteins generally have decreased susceptibility to digestive enzymes, which can contribute negatively to bioavailability and the nutritional quality of proteins. Poor amino acid availability and reduced protein digestion can affect human health as non-hydrolysed proteins can be fermented by colon bacteria into mutagenic products (Bax et al., 2012; Kaur et al., 2014; Santé-Lhoutellier et al., 2008).

Free Amino N % analysis after gastro-small intestinal digestion of striploin and tenderloin	Significance		
	Meat Cut	Production system	Interaction
0 min	*		
60 min	*	*	
180 min	*		

Table 5.4: Two-way ANOVA results of Free Amino N % values of striploin and tenderloin meat cuts after gastro-small intestinal digestion.

'*' shows significant difference ($p < 0.05$)

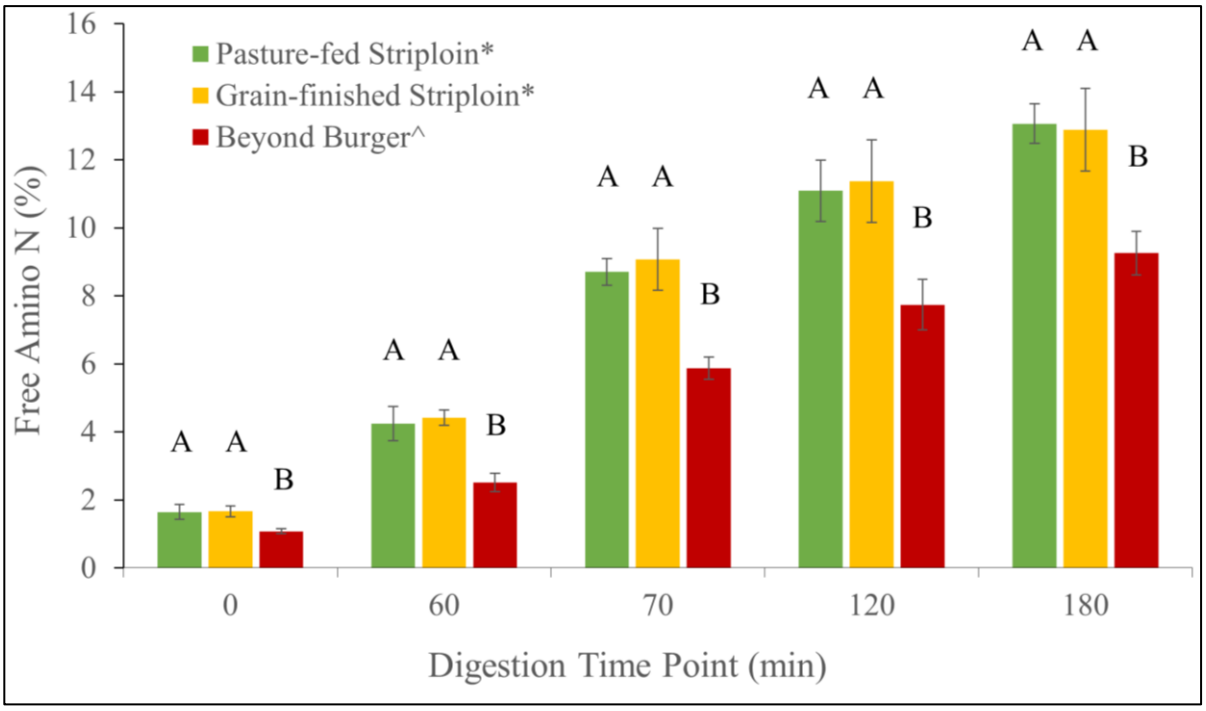


Figure 5.1: Striploin & Beyond Burger: Free amino N (%) release during simulated gastro-small intestinal digestion of grain-finished striploin, pasture-fed striploin and Beyond Burger.

Digestion times 0 & 60 are the gastric digestion times following 2 min of oral digestion. 70, 120 or 180 are the total digestion times in gastro-small intestinal digestion following 2 min of oral digestion. The error bars represent standard deviation of means. Values with different letters (A & B) within the same digestion time point differ significantly ($p < 0.05$).

* N =5, all measurements taken for 5 carcasses in triplicate.

^ N =3, all measurements taken for 1 sample in triplicate.

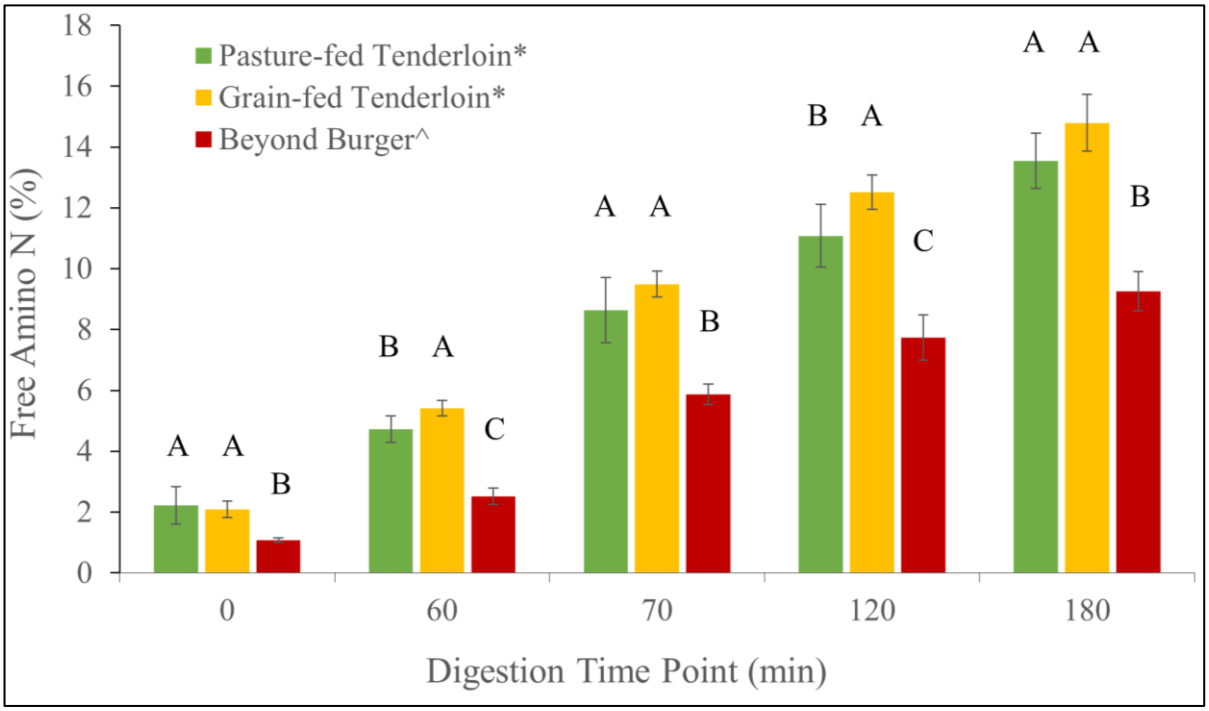


Figure 5.2: Tenderloin & Beyond Burger: Free amino N (%) release during simulated gastro-small intestinal digestion of grain-finished tenderloin, pasture-fed tenderloin and Beyond Burger.

Digestion times 0 & 60 are the gastric digestion times following 2 min of oral digestion. 70, 120 or 180 are the total digestion times in gastro-small intestinal digestion following 2 min of oral digestion. The error bars represent standard deviation of means. Values with different letters (A-C) within the same digestion time point differ significantly ($p < 0.05$).

* N =5, all measurements taken for 5 carcasses in triplicate.

^ N =3, all measurements taken for 1 sample in triplicate.

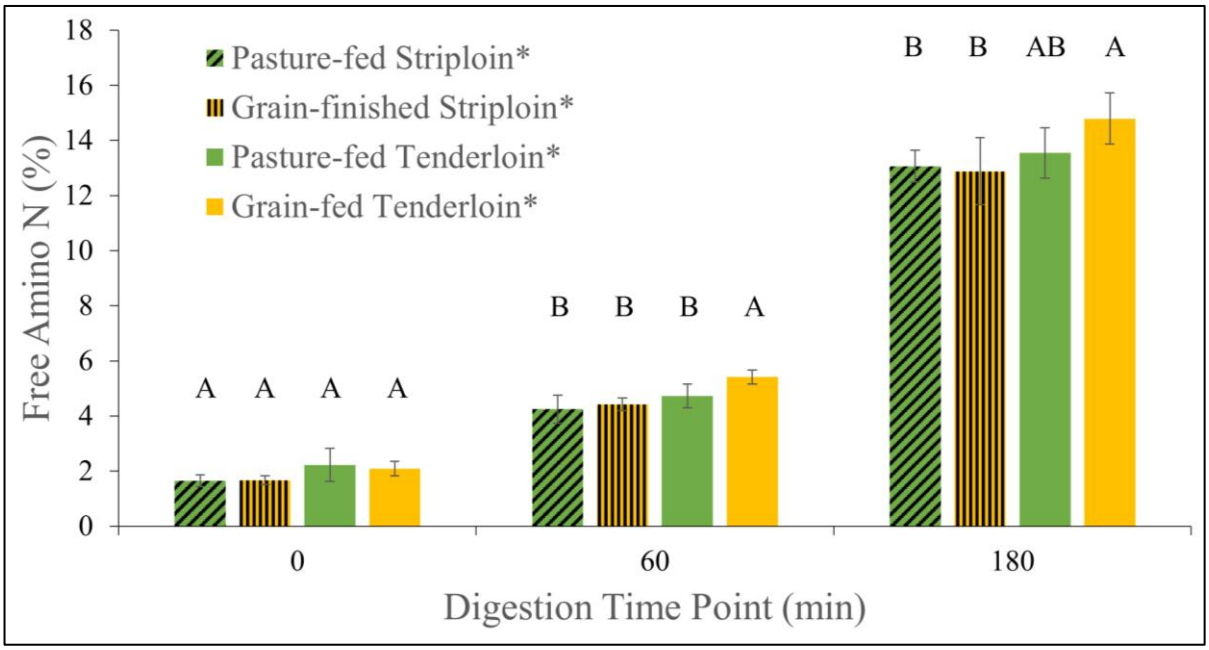


Figure 5.3: Striploin & Tenderloin: Free amino N (%) release during simulated gastro-small intestinal digestion of pasture-fed striploin, grain-finished striploin, pasture-fed tenderloin, and grain-finished tenderloin.

Digestion times 0 & 60 are the gastric digestion times following 2 min of oral digestion. 180 min is the total digestion time in gastro-small intestinal digestion following 2 min of oral digestion. The error bars represent standard deviation of means. Values with different letters (A & B) within the same digestion time point differ significantly ($p < 0.05$).

* N =5, all measurements taken for 5 carcasses in triplicate.

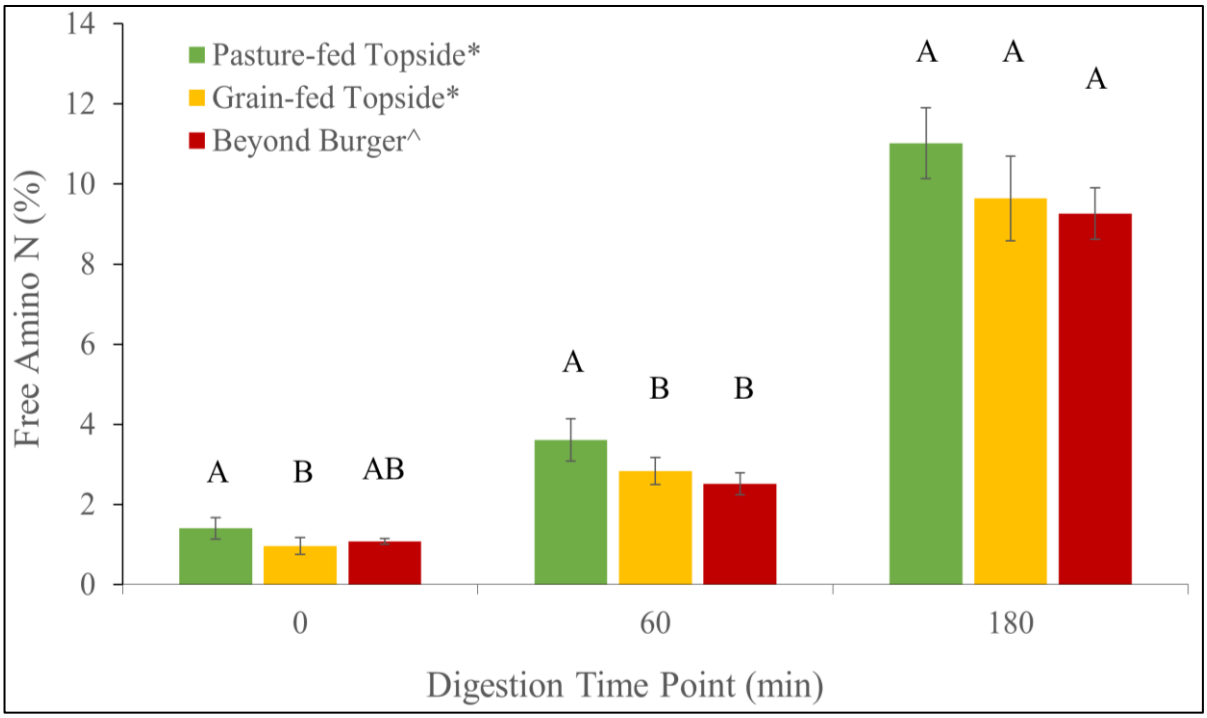


Figure 5.4: Topside & Beyond Burger: Free amino N (%) release during simulated gastro-small intestinal digestion of grain-finished topside, pasture-fed topside, and Beyond Burger.

Digestion times 0 & 60 are the gastric digestion times following 2 min of oral digestion. 70, 120 or 180 are the total digestion times in gastro-small intestinal digestion following 2 min of oral digestion. The error bars represent standard deviation of means. Values with different letters (A & B) within the same digestion time point differ significantly ($p < 0.05$).

* N =5, all measurements taken for 5 carcasses in triplicate.

^ N =3, all measurements taken for 1 sample in triplicate.

5.2.2 Tricine SDS-PAGE

Figures (5.5 - 5.8) provide information regarding the breakdown of meat and meat alternative proteins by proteolytic enzymes. The meat digests from pasture-fed beef and grain-finished beef from all three meat cuts did not show noticeable differences in protein breakdown profiles and peptide release patterns. Striploin and tenderloin digests were observed to have greater protein breakdown when compared to topside and Beyond Burger™ digests. The higher molecular weight (HMW) proteins which correspond to myosin heavy chain (220 kDa) at 0 mins gastric digestion were observed to be digested during 60 min of gastric digestion in both striploin and tenderloin. Some of the other meat proteins such as actin (43 kDa), tropomyosin (39 kDa), troponin (35 kDa), and myosin light chain (23 kDa) were identified in Figures 5.5 and 5.6 at t=0 min of gastro-intestinal digestion. Small peptides with low molecular weight (< 25 kDa) were also formed during 60 mins of gastric digestion.

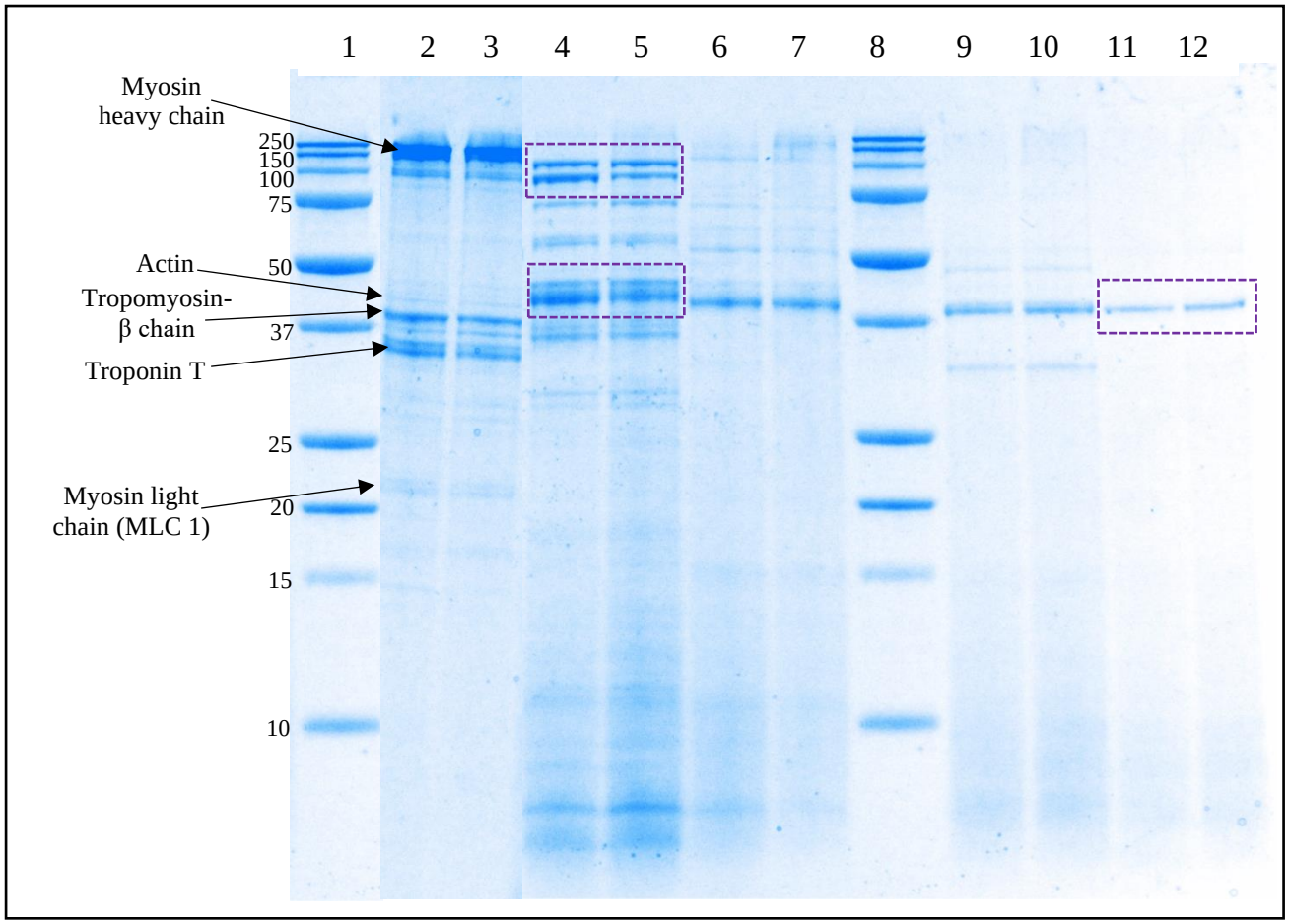
Small intestinal digestion was marked by rapid decrease in band intensity of large proteins and peptides with molecular weight > 100 kDa. By the end of 180 mins of gastro-small intestinal digestion most of the HMW proteins were digested and peptides with molecular weight < 10 kDa were formed. New bands were formed in Figures 5.5 and 5.6 after 180 min of gastro-small intestinal digestion for both striploin and tenderloin digests. Noticeable changes in band intensity (marked in purple) were observed in tenderloin samples when compared to striploin samples at 0, 60 and 180 min which indicated better digestibility.

In the SDS-PAGE protein electrophoretograms of topside high molecular weight aggregates (> 250 kDa) that did not enter the gel (marked in red in Figure 5.7) were observed. Aggregation of protein due to high temperature and longer cooking time resulted in very slow and low protein digestion with non-uniform peptide formation, which can be observed as streaks, through the course of 180 minutes gastro-small intestinal digestion. Similar high molecular aggregates were observed in the meat alternative gel profile (marked in red in Figure 5.8). The protein aggregates were observed even after 180 mins of simulated digestion for both topside and meat alternative suggesting their resistance to digestion. The results are consistent with the lower free reactive amino N % for topside and plant-based meat alternative (Beyond Burger®).

Among the three meat cuts tenderloin samples from both production systems (pasture-fed and grain-finished) were observed to have better overall protein breakdown. The above results agreed with the two-way ANOVA results in Table 5.4 which showed significant differences

between the free amino N % of tenderloin and striploin meat cuts at 0, 60 and 180 min of gastro-small intestinal digestion.

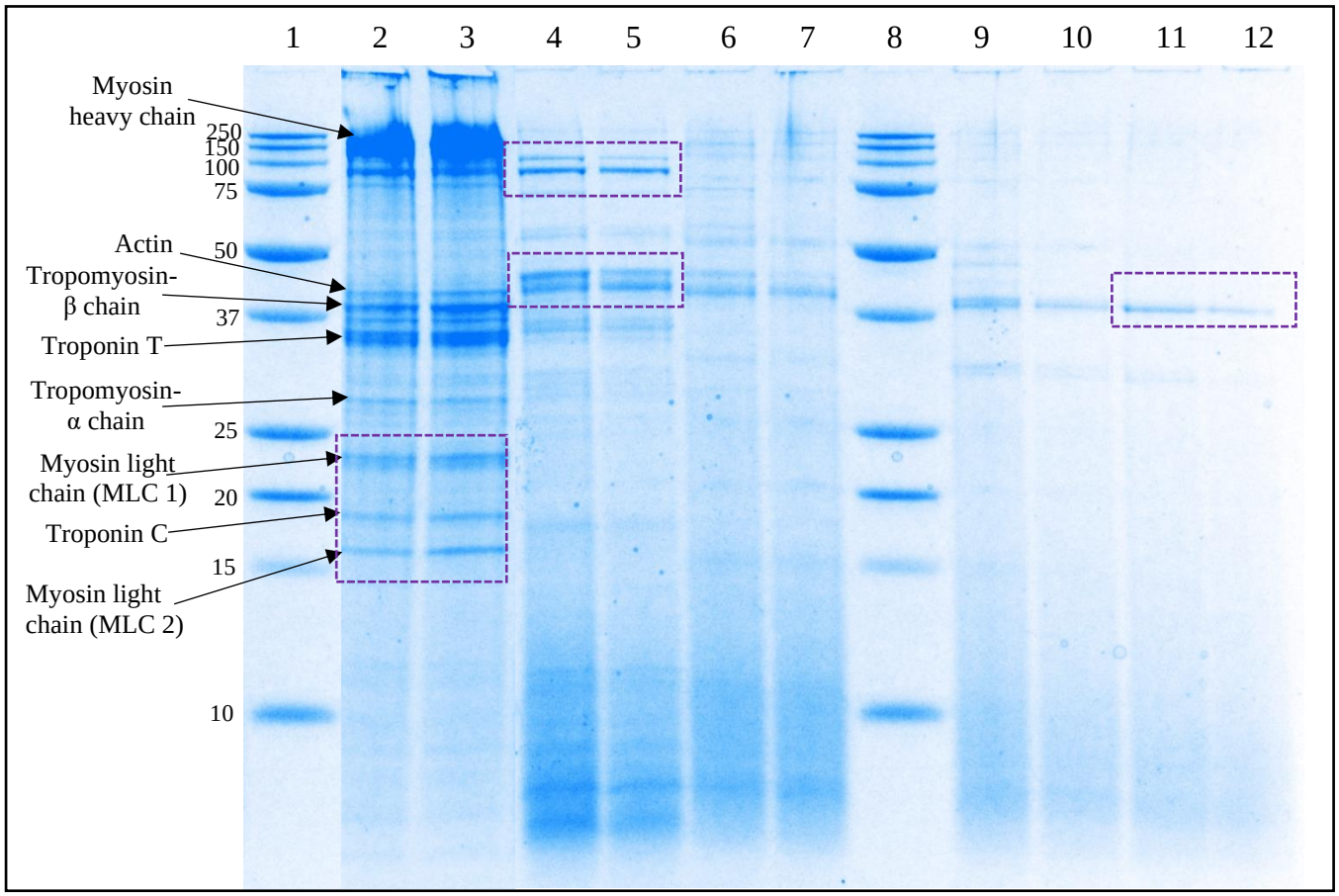
Studies suggest that actin and some proteins in the myosin light chains are hydrolysed at a slower rate in the gastro-small intestinal digesta (Kaur et al., 2010a). Similar results for digested beef muscle were observed by Denis et al., (2016) and Farouk et al., (2019). The alkaline pH in the simulated small intestinal digestion could be the reason for increased protein solubility of HMW proteins in tenderloin and striploin meat cuts (Kaur et al., 2014). The SDS-PAGE protein electrophoretograms of topside and plant-based meat alternative (Beyond Burger®) point towards structural changes in dietary proteins such as cross linking, protein aggregation and loss of solubility during processing at high temperature, shear and/or pressure for plant-based meat alternative and cooking at high temperatures for topside.



Well Number	Sample ID
1	Protein standard
2	SLG – 0 min
3	SLP – 0 min
4	SLG – 60 min
5	SLP – 60 min
6	SLG – 70 min
7	SLP – 70 min
8	Protein standard
9	SLG – 120 min
10	SLP – 120 min
11	SLG – 180 min
12	SLP – 180 min

Figure 5.5: Tricine SDS-PAGE electrophoretogram of striploin grain-finished (SLG) and striploin pasture-fed (SLP) meat digests after 0, and 60 min of simulated oral-gastric digestion and 70, 120 and 180 min of simulated gastro-small intestinal digestion following initial 2 minutes of oral digestion.

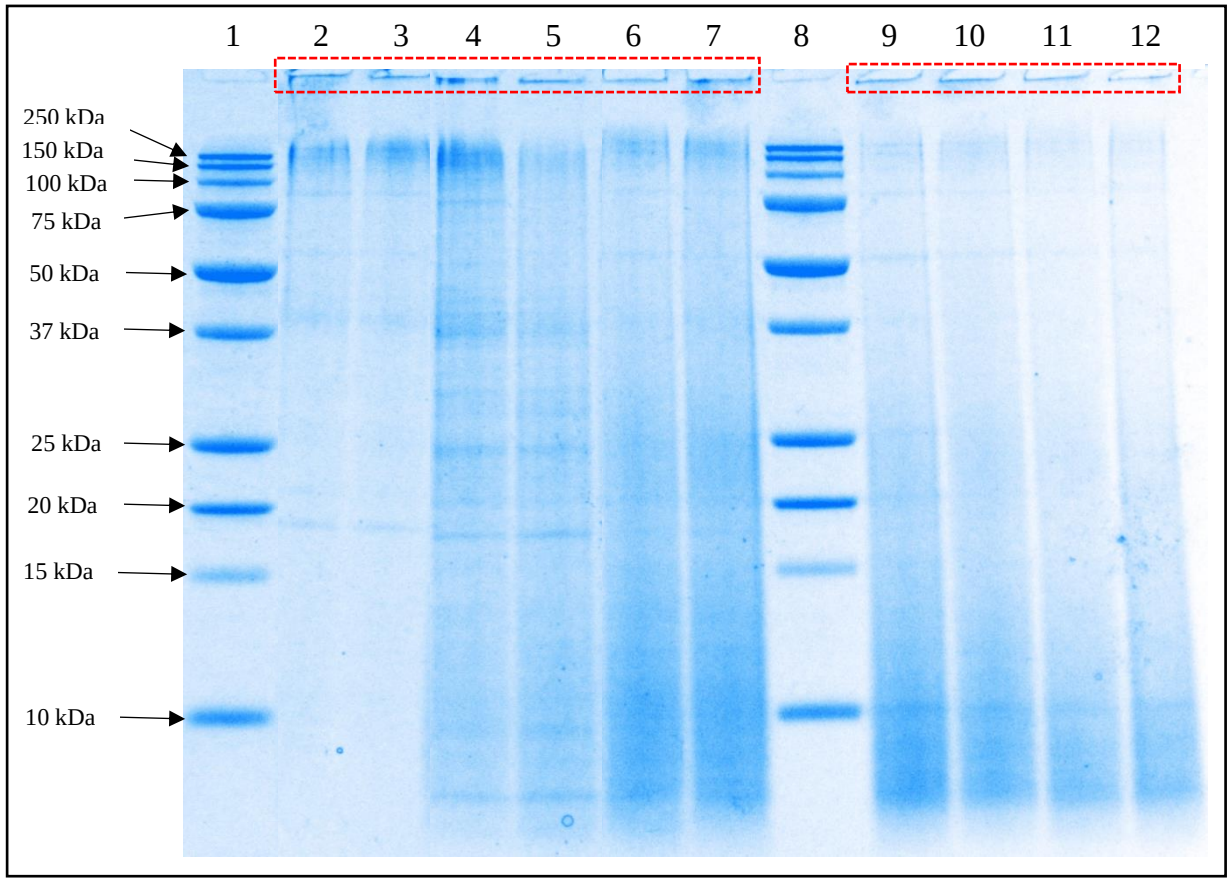
Bands in the molecular weight protein standard lane correspond to molecular weights 250, 150, 100, 75, 50, 37, 25, 20, 15 and 10 kDa.



Well Number	Sample ID
1	Protein standard
2	TLG – 0 min
3	TLP – 0 min
4	TLG – 60 min
5	TLP – 60 min
6	TLG – 70 min
7	TLP – 70 min
8	Protein standard
9	TLG – 120 min
10	TLP – 120 min
11	TLG – 180 min
12	TLP – 180 min

Figure 5.6: Tricine SDS-PAGE electrophoretogram of tenderloin grain-finished (TLG) and tenderloin pasture-fed (TLP) meat digests after 0, and 60 min of simulated oral-gastric digestion and 70, 120 and 180 min of simulated gastro-small intestinal digestion following initial 2 minutes of oral digestion.

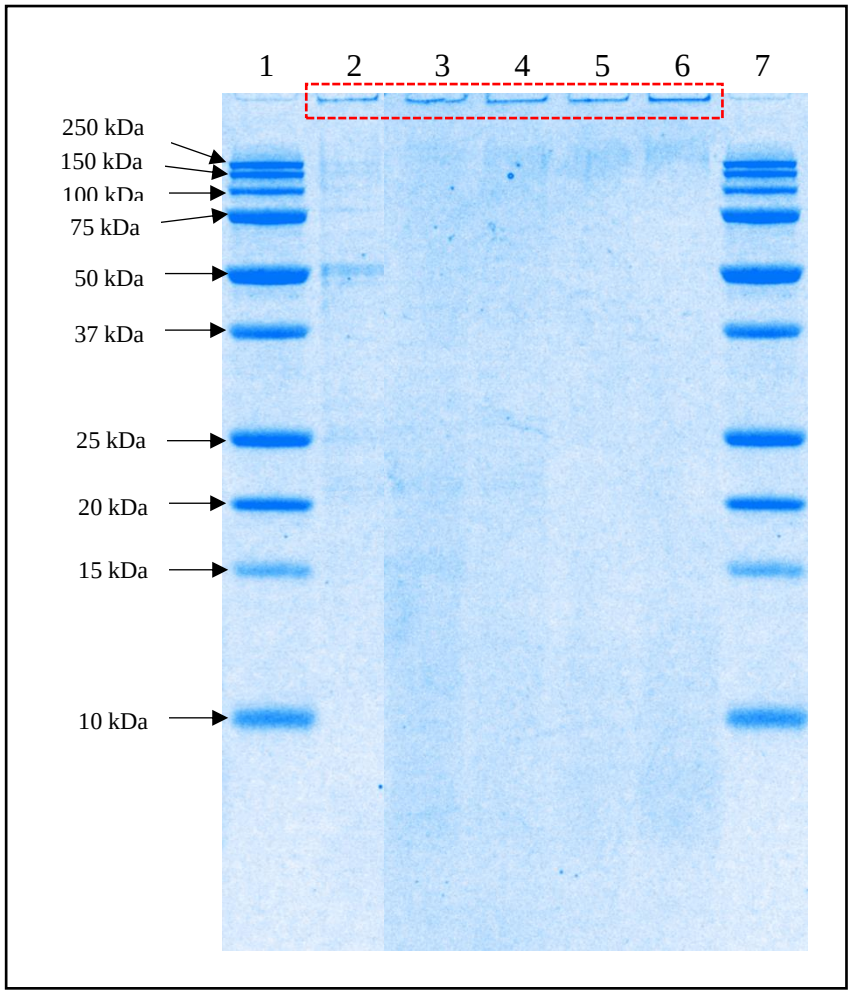
Bands in the molecular weight protein standard lane correspond to molecular weights 250, 150, 100, 75, 50, 37, 25, 20, 15 and 10 kDa.



Well Number	Sample ID
1	Protein standard
2	TSG – 0 min
3	TSP – 0 min
4	TSG – 60 min
5	TSP – 60 min
6	TSG – 70 min
7	TSP – 70 min
8	Protein standard
9	TSG – 120 min
10	TSP – 120 min
11	TSG – 180 min
12	TSP – 180 min

Figure 5.7: Tricine SDS-PAGE electrophoretogram of topside grain-finished (TSG) and topside pasture-fed (TSP) meat digests after 0, and 60 min of simulated oral-gastric digestion and 70, 120 and 180 min of simulated gastro-small intestinal digestion following initial 2 minutes of oral digestion.

Bands in the molecular weight protein standard lane correspond to molecular weights 250, 150, 100, 75, 50, 37, 25, 20, 15 and 10 kDa.



Well Number	Sample ID
1	Protein standard
2	BB – 0 min
3	BB – 60 min
4	BB – 70 min
5	BB – 120 min
6	BB – 180 min
7	Protein standard

Figure 5.8: Tricine SDS-PAGE electrophoretogram of Beyond Burger™ (BB) digests after 0, and 60 min of simulated oral-gastric digestion and 70, 120 and 180 min of simulated gastro-small intestinal digestion following initial 2 minutes of oral digestion.

Bands in the molecular weight protein standard lanes correspond to molecular weights 250, 150, 100, 75, 50, 37, 25, 20, 15 and 10 kDa.

5.3 Estimation of lipid digestibility

5.3.1 Free fatty acid profiles and fatty acid ratios of digests

The results for total initial fatty acid profiles, total SFA, MUFA, PUFA and health indicating fatty acids ratios of all meat samples and meat alternative at 0 min of simulated gastro-small intestinal digestion are reported in Appendix A. The meat samples used in this study were trimmed of all external fat prior to *in vitro* lipid digestion; thus, the only source of fat was the intramuscular fat. As seen in Tables 5.1 and 5.3, no significant effect of the beef production system could be seen on the total fat content of cooked striploin and topside samples, while tenderloin showed significantly higher fat content for the grain-finished cooked meat samples.

The individual free fatty acid profiles of all meat samples and meat alternative after 180 min of simulated gastro-small intestinal digestion are shown in Table 5.5. From the results it is observed that oleic acid was released the highest per gram of meat followed by palmitic and stearic acids. In addition to oleic, linoleic, palmitic, and stearic acids, higher amounts of lauric acid were released from the plant-based meat alternative.

Digested samples from grain-finished tenderloin meat cut had significantly higher concentrations of individual free palmitic acid and oleic acid than digests from pasture-fed tenderloin meat cut. The concentration of free α-linoleic acid was higher and significantly different in meat digests from pasture-fed striploin and topside meat cuts than digests from grain-finished striploin and topside meat cuts. The amounts of almost all the monounsaturated (MUFA) fatty acids released after digestion were significantly higher for grain-finished meats than for their pasture-fed meat counterparts. Higher amounts of free long chain PUFAs were observed after 180 min of lipid digestion in most pasture-fed meat cuts.

The above results agree with the Figures 5.9 and 5.10 which show the PCA score and loading plots of the important fatty acids indicating that the pasture-fed digested meat cut clusters are compact with lower free SFA and MUFA and higher free EPA+DHA. The grain-finished meat cut clusters were more spread apart and observed to have higher free SFA and MUFA and lower EPA+DHA in both Figures 5.9 and 5.10.

The plant-based meat alternative samples were observed to be significantly different and contained the highest levels of total SFAs, MUFAs, n-6 PUFAs and n-3 PUFAs (Table 5.6 and Figure 5.9), however the free long chain n-3 PUFAs which includes EPA, DPA and DHA were

not detected in plant-based meat alternative, thus preventing their contribution to the human diet (Table 5.5).

The results point towards the advantages of consuming pasture-fed meat over grain-finished meat and the plant-based meat alternative, as it provides lower total amounts of free SFAs and higher total amounts of free long chain n-3 PUFAs after 180 min of lipid digestion.

The free fatty acid ratios calculated and presented in Table 5.6 serve as health indication markers.

The MUFA/SFA ratio was found to be significantly different between the two animal production systems in the digests of each of the three meat cuts. However, the highest ratio was observed in the digests of the plant-based alternative followed by grain-finished beef cuts.

The results in this study show the plant-based meat alternative to have a significantly different and higher (PUFA+MUFA)/SFA ratio than both grain-finished and pasture-fed meat cuts. There were significant differences in the animal production systems as well.

The results in the study showed significantly higher n-6/n-3 ratio (≥ 4) for digests from grain-finished meat cuts while the digests from pasture-fed meat cuts reported significantly lower n-6/n-3 ratios.

The DI-16 was found to be significantly different between the digests from the two different production systems. The pasture-fed meat digests had lower ratio values than grain-finished meat digests of each meat cut.

The plant-based meat alternative digests contained the highest DI-18 ratio while the digests pasture-fed meat cuts were observed to have the lowest DI-18 ratio in this study.

Table 5.5: Individual free fatty acids released after 180 min simulated gastro-small intestinal digestion for striploin grain-finished (SLG), striploin pasture-fed (SLP), tenderloin grain-finished (TLG), tenderloin pasture-fed (TLP), topside grain-finished (TSG), topside pasture-fed (TSP), and plant-based meat alternative (BB).

Fatty acids		Free fatty acids (FFA, mg/g cooked meat)						
		SLG*	**SLP***	**TLG***	**TLP***	**TSG***	**TSP***	**BB^**
Individual SFA								
C8:0	Caprylic acid	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	1.31 ± 0.15 ^{a}
C10:0	Capric acid	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	1.15 ± 0.14 ^{a}
C12:0	Lauric acid	0.01 ± 0.01 ^{b}	0.01 ± 0.00 ^{b}	0.03 ± 0.01 ^{b}	0.01 ± 0.00 ^{b}	0.02 ± 0.00 ^{b}	0.01 ± 0.00 ^{b}	9.30 ± 1.42 ^{a}
C14:0	Myristic acid	0.47 ± 0.08 ^{bc}	0.22 ± 0.04 ^{c}	0.90 ± 0.48 ^{b}	0.41 ± 0.08 ^{bc}	0.65 ± 0.16 ^{bc}	0.34 ± 0.10 ^{c}	3.93 ± 0.60 ^{a}
C15:0	Pentadecylic acid	0.10 ± 0.02 ^{b}	0.06 ± 0.01 ^{bc}	0.23 ± 0.12 ^{a}	0.14 ± 0.03 ^{ab}	0.14 ± 0.03 ^{ab}	0.10 ± 0.03 ^{bc}	0.03 ± 0.00 ^{d}
C16:0	Palmitic acid	5.48 ± 0.67 ^{bc}	3.75 ± 0.46 ^{c}	10.47 ± 4.11 ^{a}	5.61 ± 0.88 ^{bc}	7.98 ± 2.26 ^{ab}	5.30 ± 1.24 ^{bc}	8.70 ± 1.50 ^{ab}
C17:0	Heptadecanoic acid	0.33 ± 0.09 ^{bc}	0.14 ± 0.03 ^{c}	0.67 ± 0.31 ^{a}	0.27 ± 0.04 ^{bc}	0.44 ± 0.12 ^{ab}	0.21 ± 0.05 ^{bc}	0.10 ± 0.02 ^{c}
C18:0	Stearic acid	2.50 ± 0.59 ^{c}	2.33 ± 0.51 ^{c}	6.35 ± 2.40 ^{a}	4.43 ± 0.68 ^{abc}	3.66 ± 1.33 ^{bc}	3.78 ± 1.21 ^{abc}	5.63 ± 1.18 ^{ab}
Individual MUFA								
C14:1	Myristoleic acid	0.13 ± 0.02 ^{ab}	0.05 ± 0.01 ^{c}	0.15 ± 0.02 ^{ab}	0.06 ± 0.02 ^{c}	0.18 ± 0.03 ^{a}	0.04 ± 0.01 ^{c}	Not detected
C16:1	Palmitoleic acid	0.70 ± 0.08 ^{ab}	0.31 ± 0.04 ^{c}	1.03 ± 0.33 ^{a}	0.41 ± 0.11 ^{bc}	1.11 ± 0.31 ^{a}	0.47 ± 0.11 ^{b}	0.16 ± 0.02 ^{d}
C17:1	cis-10 Heptadecanoic acid	0.30 ± 0.07 ^{ab}	0.07 ± 0.01 ^{c}	0.49 ± 0.27 ^{a}	0.11 ± 0.03 ^{bc}	0.45 ± 0.12 ^{a}	0.10 ± 0.02 ^{bc}	0.08 ± 0.01 ^{bc}
C18:1 *c* 9	Oleic acid	8.92 ± 1.74 ^{bcd}	4.88 ± 0.71 ^{d}	16.76 ± 8.44 ^{b}	7.32 ± 1.39 ^{cd}	13.72 ± 4.81 ^{bc}	7.38 ± 1.65 ^{cd}	37.73 ± 6.27 ^{a}
C18:1 *c* 11	cis-Vaccenic acid	0.45 ± 0.06 ^{c}	0.18 ± 0.02 ^{e}	0.84 ± 0.34 ^{b}	0.29 ± 0.06 ^{d}	0.71 ± 0.22 ^{bc}	0.29 ± 0.06 ^{d}	1.67 ± 0.26 ^{a}
Individual PUFA								
C18:2 n-6 (LA)	Linoleic acid	0.68 ± 0.07 ^{bc}	0.56 ± 0.09 ^{bc}	1.14 ± 0.24 ^{b}	0.76 ± 0.13 ^{bc}	1.04 ± 0.18 ^{b}	0.80 ± 0.05 ^{bc}	12.30 ± 2.09 ^{a}
C18:2 *c* 9, *t* 11 (CLA)	Conjugated linoleic acid	0.05 ± 0.05 ^{b}	0.05 ± 0.01 ^{b}	0.12 ± 0.10 ^{b}	0.08 ± 0.03 ^{b}	0.06 ± 0.04 ^{b}	0.07 ± 0.02 ^{b}	0.59 ± 0.10 ^{a}
C18:3 n-3 (ALA)	α-Linolenic acid	0.05 ± 0.01 ^{d}	0.13 ± 0.02 ^{c}	0.19 ± 0.08 ^{bc}	0.28 ± 0.05 ^{b}	0.12 ± 0.02 ^{c}	0.26 ± 0.03 ^{b}	3.67 ± 0.57 ^{a}
C20:4 n-6	Arachidonic acid	0.19 ± 0.04 ^{bc}	0.22 ± 0.03 ^{b}	0.22 ± 0.05 ^{abc}	0.22 ± 0.04 ^{ab}	0.29 ± 0.03 ^{a}	0.28 ± 0.04 ^{ab}	0.15 ± 0.02 ^{c}
C20:5 n-3 (EPA)	Eicosapentaenoic acid	0.03 ± 0.01 ^{c}	0.05 ± 0.01 ^{bc}	0.05 ± 0.03 ^{bc}	0.08 ± 0.03 ^{ab}	0.07 ± 0.01 ^{b}	0.10 ± 0.01 ^{a}	Not detected
C22:5 n-3 (DPA)	Docosapentaenoic acid	0.06 ± 0.01 ^{c}	0.07 ± 0.01 ^{abc}	0.06 ± 0.02 ^{c}	0.10 ± 0.03 ^{ab}	0.09 ± 0.02 ^{ab}	0.12 ± 0.02 ^{a}	Not detected
C22:6 n-3 (DHA)	Docosahexaenoic acid	0.01 ± 0.01 ^{a}	0.02 ± 0.01 ^{a}	0.02 ± 0.00 ^{a}	0.02 ± 0.01 ^{a}	0.02 ± 0.00 ^{a}	0.02 ± 0.00 ^{a}	Not detected

Values of FFA with different (a-d) superscripts within the same row differ significantly ($p < 0.05$). * N =5, all measurements taken for 5 carcasses in triplicate. ^ N =3, all measurements taken for 1 sample in triplicate.

Table 5.6: Sum of released free saturated (SFA), mono- (MUFA) and polyunsaturated fatty acid (PUFA) amounts and fatty acid ratios for striploin grain-finished (SLG), striploin pasture-fed (SLP), tenderloin grain-finished (TLG), tenderloin pasture-fed (TLP), topside grain-finished (TSG), topside pasture-fed (TSP), and plant-based meat alternative (BB) after 180 min of digestion under simulated gastro-small intestinal conditions**.**

Free fatty acid (FFA mg/g cooked meat)							
mg/g cooked meat	**SLG***	**SLP***	**TLG***	**TLP***	**TSG***	**TLP***	**BB^**
Σ SFA	8.89 ± 1.32 ^{c}	6.50 ± 1.03 ^{c}	18.65 ± 7.35 ^{b}	10.87 ± 1.60 ^{c}	12.89 ± 3.89 ^{bc}	9.74 ± 2.56 ^{c}	27.70 ± 4.69 ^{a}
Σ MUFA	10.51 ± 1.88 ^{bcd}	5.49 ± 0.76 ^{d}	19.27 ± 9.62 ^{b}	8.19 ± 1.59 ^{cd}	16.16 ± 5.44 ^{bc}	8.28 ± 1.82 ^{cd}	39.64 ± 6.56 ^{a}
Σ PUFA	1.08 ± 0.11 ^{b}	1.09 ± 0.15 ^{b}	1.80 ± 0.35 ^{b}	1.54 ± 0.30 ^{b}	1.70 ± 0.26 ^{b}	1.66 ± 0.11 ^{b}	16.71 ± 2.76 ^{a}
Σ n-6 PUFA	0.87 ± 0.08 ^{b}	0.78 ± 0.11 ^{b}	1.35 ± 0.23 ^{b}	0.98 ± 0.16 ^{b}	1.33 ± 0.19 ^{b}	1.08 ± 0.08 ^{b}	12.45 ± 2.10 ^{a}
Σ n-3 PUFA	0.15 ± 0.02 ^{d}	0.27 ± 0.04 ^{c}	0.32 ± 0.07 ^{c}	0.47 ± 0.12 ^{bc}	0.31 ± 0.05 ^{c}	0.51 ± 0.05 ^{b}	3.67 ± 0.57 ^{a}
Σ LCn-3 PUFA	0.10 ± 0.01 ^{c}	0.14 ± 0.02 ^{bc}	0.13 ± 0.04 ^{bc}	0.19 ± 0.06 ^{ab}	0.19 ± 0.03 ^{ab}	0.25 ± 0.02 ^{a}	Not detected
FA ratios and DI							
MUFA/SFA	1.18 ± 0.14 ^{bc}	0.85 ± 0.06 ^{de}	1.00 ± 0.16 ^{cd}	0.75 ± 0.07 ^{e}	1.24 ± 0.08 ^{ab}	0.86 ± 0.06 ^{de}	1.43 ± 0.06 ^{a}
(PUFA+MUFA)/SFA	1.31 ± 0.13 ^{bc}	1.02 ± 0.06 ^{de}	1.11 ± 0.15 ^{cd}	0.89 ± 0.09 ^{e}	1.38 ± 0.07 ^{b}	1.04 ± 0.10 ^{de}	2.04 ± 0.11 ^{a}
n-6/n-3 PUFA	5.69 ± 0.35 ^{a}	2.88 ± 0.13 ^{d}	4.24 ± 0.20 ^{b}	2.11 ± 0.21 ^{e}	4.35 ± 0.43 ^{b}	2.13 ± 0.17 ^{e}	3.38 ± 0.05 ^{c}
DI_{16}*	0.13 ± 0.01 ^{a}	0.08 ± 0.01 ^{bc}	0.10 ± 0.02 ^{b}	0.07 ± 0.01 ^{c}	0.14 ± 0.01 ^{a}	0.09 ± 0.01 ^{bc}	0.02 ± 0.00 ^{d}
DI_{18}*	3.63 ± 0.62 ^{b}	2.14 ± 0.27 ^{cd}	2.56 ± 0.45 ^{c}	1.66 ± 0.23 ^{d}	3.75 ± 0.14 ^{b}	2.02 ± 0.28 ^{cd}	6.76 ± 0.66 ^{a}

Values of FFA with different superscripts (a-e) within the same row differ significantly ($p < 0.05$). * N =5, all measurements taken for 5 carcasses in triplicate. ^ N =3, all measurements taken for 1 sample in triplicate.

Studies have concluded that lauric acid along with palmitic acid raise plasma total cholesterol concentrations that can serve as a health marker for coronary artery diseases (Field & Robinson., 2019; Orsavova et al., 2015). Higher levels of lauric acid were found in plant-based meat alternative which is contributed by coconut oil added during processing. The role of SFAs in regard to increasing the risk of many diseases such as obesity and CVD; and the role of long chain n-3 PUFAs in regard to providing health benefits have been well established (Horman et al., 2020; Siriwardhana et al., 2012). Meat from animals that are fed grain-based diets have been reported to contain higher concentrations of n-6 PUFAs while pasture-fed animals have greater amounts of n-3 PUFAs (Daley et al., 2010).

According to epidemiological studies, a higher MUFA/SFA ratio tends to impart bodily complications in the form of various metabolic syndromes such as obesity, insulin resistance and cardiovascular diseases (Chang et al., 2004; Field & Robinson., 2019; Krishnan & Cooper., 2014; Van Elswyk & McNeill., 2014). Many studies show favourable effects of MUFA- rich diets however they are most effective when the level of SFAs is lower, in order to combat chronic diseases (Hammad et al., 2016; Schwingshackl, & Hoffmann., 2014).

According to Chang et al. (2004), a lower (PUFA+MUFA)/SFA ratio usually less than 2 is shown to lower cholesterol and reduce the chances of fatty liver in humans.

In recent times the n-6/n-3 ratio has become one of the important health indicating markers. Diets with low n-6/n-3 ratios (4:1) have been associated with better neurogenesis, reduced depression risks and imparting cognitive benefits (Gupta et al., 2013; Horman et al., 2020; Simopoulos, 2011).

The desaturase indices are an important health indicator. A very high DI-16 has been associated with dietary fat and obesity issues in humans. On the other hand, abnormally high ratios of DI-18 play a major role in several metabolic syndromes including diabetes and insulin resistance, CVD, hypertension, immune disorders, neurological disorders, and cancer (Alarcón et al., 2016; Sjögren et al., 2007; Vessby et al., 2013; Warensjö et al., 2009).

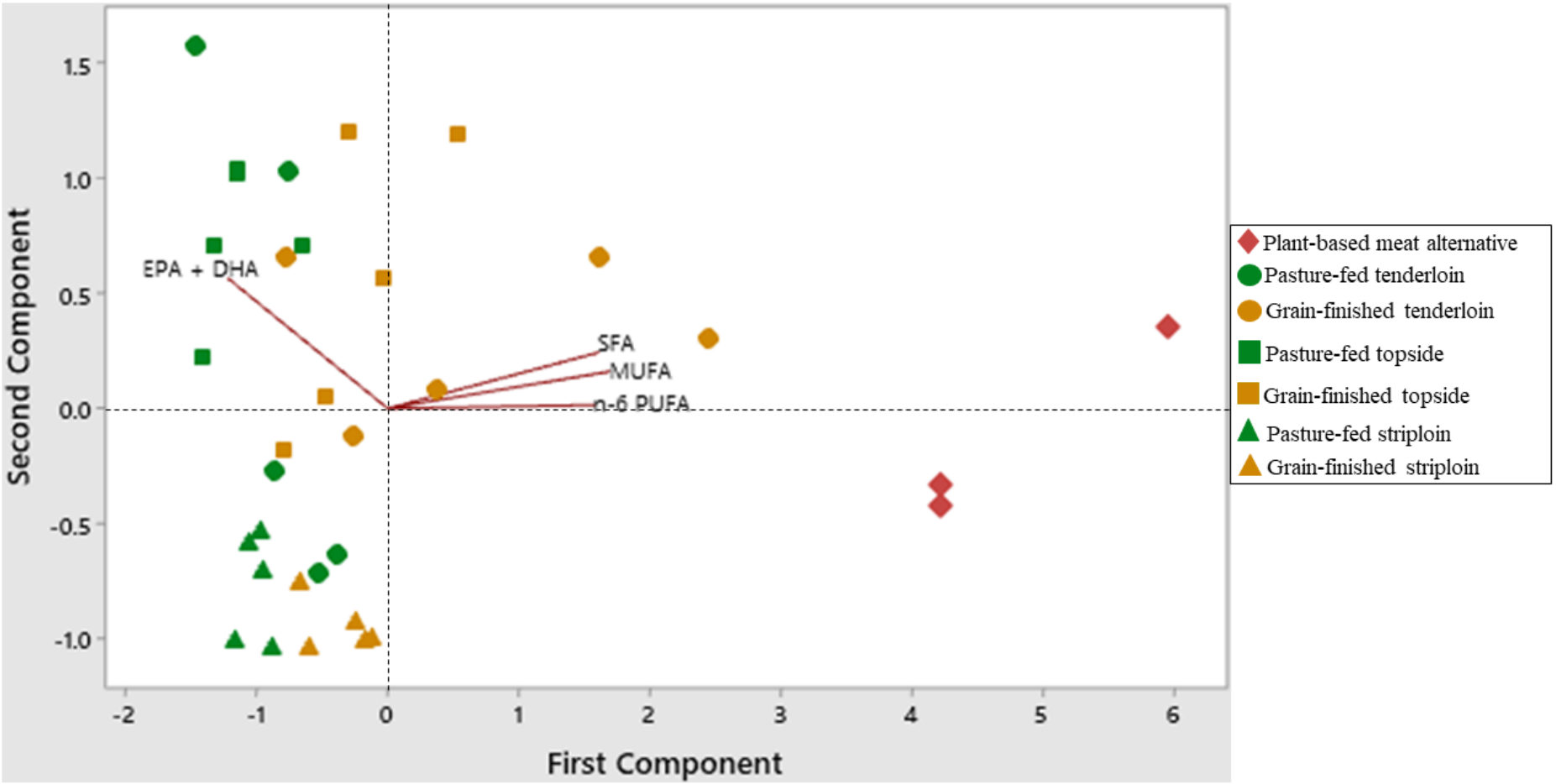


Figure 5.9: PCA score and loading plot of free SFA, MUFA, n-6 PUFA and EPA+DHA of digests from meat alternative and meat cuts from pasture-fed and grain-finished animals with 93% component extraction (PC1-77.1 % and PC2-15.9%).

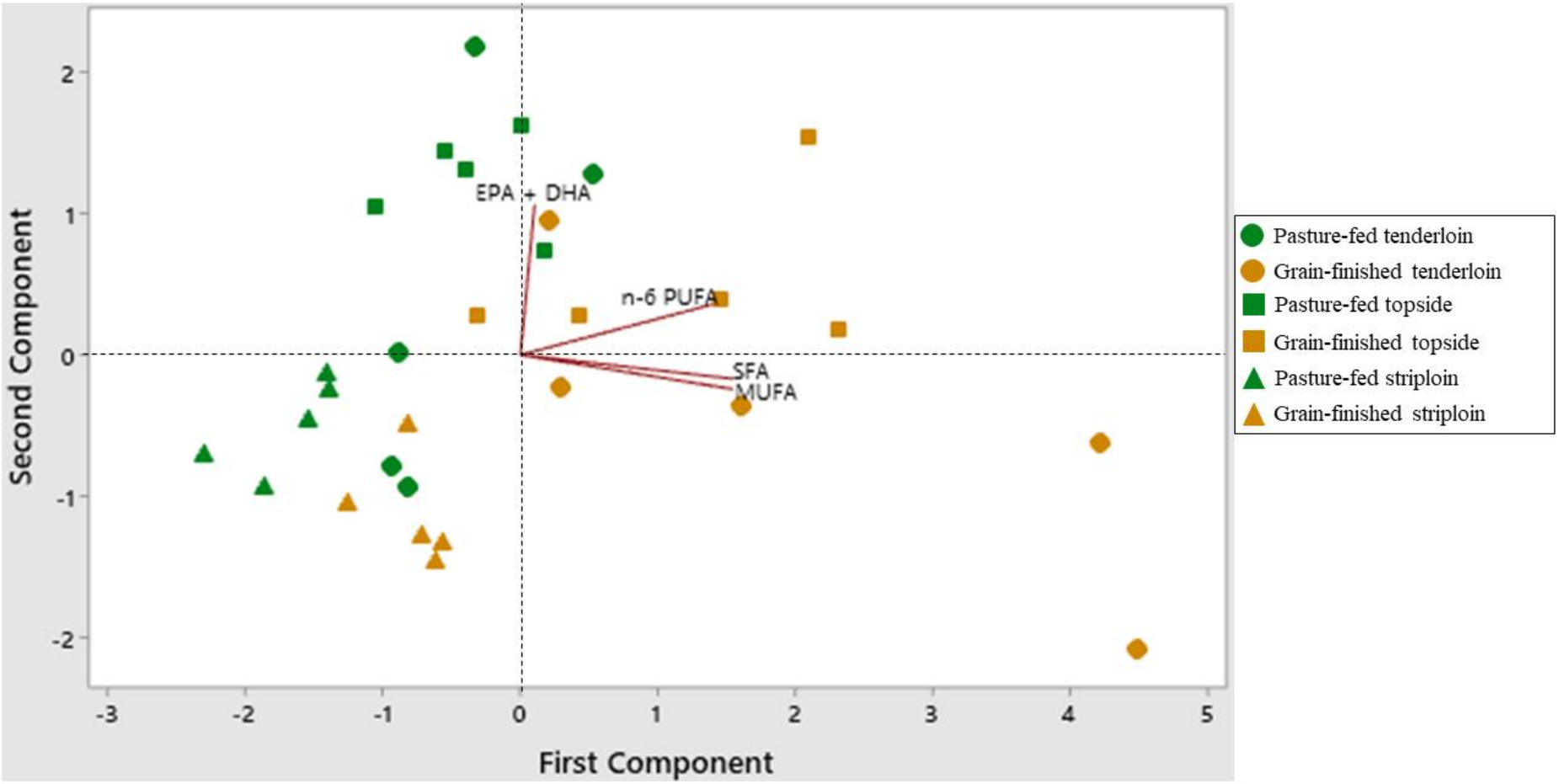


Figure 5.10: PCA score and loading plot of free SFA, MUFA, n-6 PUFA and EPA+DHA of digests from pasture-fed and grain-finished meat cuts with 94.6% component extraction (PC1-65.5% and PC2-29.1%)

Chapter 6: Conclusions

The objectives of this study were to determine the nutritional value, protein digestibility and free fatty acid release by utilizing *in vitro* digestion systems of a commercially available plant-based meat alternative (Beyond Burger™) and three types of meat cuts from grain-finished and pasture-fed production systems. This study compared the differences in protein and lipid digestibility within the meat cuts (which were cooked with different methods) and a meat alternative which constituted processed plant proteins and fat sources, along with fibre additives, colours, and flavour enhancers. The Beyond Burger® was chosen as the plant-based meat alternative sample as it contained a comparable nutrient profile to beef, was available in the retail space and was also similar in appearance to beef .

There were no significant differences ($p < 0.05$) in protein digestibility between pasture-fed and grain-finished meat digests in terms of the amounts of free amino N (%) released during digestion. However, the protein digestibility varied within meat cuts and the meat alternative. The striploin and tenderloin steaks which were fast fried to an internal temperature of 70 °C had better digestibility than the topside cubes which were braised in the oven for 2 hr at 160 °C and the plant-based meat alternative which had been extruded at high temperatures (Beyond Meat., 2021). Meat that has been cooked to an internal temperature of ~ 70 °C denatures the protein and exposes hydrophobic sites for efficient protein hydrolysis by digestive enzymes. The low protein digestibility of the topside cubes could be due to the higher cooking temperature and longer cooking time which might have resulted in loss of soluble proteins in the cook loss (which was discarded) and overall lower protein solubility due to the formation of large protein aggregates that were resistant to digestive enzymes. The plant-based meat alternative was also observed to have lower digestibility and large protein aggregates, which would have been possibly formed during high temperature extrusion processing that is commonly used to mimic the fibrous meat structure. The above observations were derived based on the ninhydrin-reactive free amino nitrogen results and the protein breakdown profile using SDS-PAGE.

The total amounts of free SFAs was higher in the plant-based meat alternative followed by grain-finished meat samples. This indicated that pasture-fed meat was likely to have lower risks of contributing to chronic diseases such as CVD which are related to high levels of individual SFAs (lauric, myristic and palmitic acids). The pasture-fed meat digests contained higher amounts of LCn-3 PUFAs which have been extensively studied for their beneficial effects in

combating various metabolic syndromes like CVD, diabetes, obesity, and other neurological diseases.

Meat has been proven to provide proteins of the highest quality when consumed in moderation than plant-based meat alternatives. The results from this study confirm that meat when cooked with appropriate methods provides highly digestible proteins. Higher protein digestibility rates have been linked to building muscle mass for fitness enthusiasts, athletes, and some elderly population suffering from sarcopenia.

This study has successfully addressed the digestibility of meat and a plant-based meat alternative and highlighted the potential health benefits of consuming pasture-fed beef cuts produced in New Zealand.

6.2 Limitations of this study

- Only one plant-based meat alternative was used for this study that may not be representative of other commercially available meat alternatives.
- Removal of the topside cook loss might have altered the soluble protein and lipid profile of the samples which could have affected the overall observations and significant effects of pasture-fed meat and grain-finished meat.

6.1 Recommendations for future work

- Further study to understand the effect of food structure on digestion can be undertaken.
- The results of this study need to be compared to a dynamic model for digestion of meat and plant-based meat alternatives.
- As more plant-based meat alternatives are available in the retail space nowadays, further protein and lipid digestibility studies need to be conducted on those products to compare it to meat

References

Alarcón, G., Roco, J., Medina, A., Van Nieuwenhove, C., Medina, M., & Jerez, S. (2016). Stearoyl-CoA desaturase indexes and n-6/n-3 fatty acids ratio as biomarkers of cardiometabolic risk factors in normal-weight rabbits fed high fat diets. *Journal of Biomedical Science*, *23*(1), 1-8.

Alfaia, C. M., Alves, S. P., Lopes, A. F., Fernandes, M. J., Costa, A. S., Fontes, C. M., ... & Prates, J. A. (2010). Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. *Meat Science*, *84*(4), 769-777.

AOAC. (1981). Official method 981.10, Crude protein in meat: block digestion method. *Official Methods of Analysis of AOAC*, *2*(37), 7-8.

AOAC Official Method 991.36, Fat (Crude) in Meats and Meat Products. *Official Methods of Analysis of AOAC International*. AOAC International, Gaithersburg, MD, 1997.

AOAC. (1990). Official method 922.06 fat in flour, baked and extruded product: Acid hydrolysis method. *Official Methods of Analysis of AOAC International*. AOAC International, Gaithersburg, MD. AOAC International.

Asensio-Grau, A., Calvo-Lerma, J., Heredia, A., & Andrés, A. (2019). Fat digestibility in meat products: influence of food structure and gastrointestinal conditions. *International journal of food sciences and nutrition*, *70*(5), 530-539.

Asgar, M. A., Fazilah, A., Nurul, H., Rajeev, B., & Karim, A. A. (2010). Nonmeat protein alternatives as meat extenders and meat analogs. *Comprehensive Reviews in FoodScience and Food Safety*, *9*(5), 513-529.

Astruc, T. (2014). Muscle structure and digestive enzyme bioaccessibility to intracellular compartments. In M. Boland, M. Golding, & H. Singh (Eds.), *Food Structures, Digestion and Health* (pp. 193-222). Academia Press.

Battaglia, C., Vilella, G. F., Bernardo, A. P. S., Gomes, C. L., Biase, A. G., Albertini, T. Z., & Pflanzer, S. B. (2020). Comparison of methods for measuring shear force and sarcomere length and their relationship with sensorial tenderness of longissimus muscle in beef. *Journal of Texture Studies*, *51*(2), 252-262.

Baugreet, S., Gomez, C., Auty, M. A., Kerry, J. P., Hamill, R. M., & Brodkorb, A. (2019). In vitro digestion of protein-enriched restructured beef steaks with pea protein isolate, rice

protein and lentil flour following sous vide processing. *Innovative Food Science and Emerging Technologies*, *54*, 152-161.

Bax, M.-L., Aubry, L., Ferreira, C., Daudin, J.-D., Gatellier, P., Rémond, D., & Santé-Lhoutellier, V. r. (2012). Cooking temperature is a key determinant of in vitro meat protein digestion rate: investigation of underlying mechanisms. *Journal of Agricultural and Food Chemistry*, *60*(10), 2569-2576.

Bekhit, A. E.-D. A., Carne, A., Ha, M., & Franks, P. (2014). Physical interventions to manipulate texture and tenderness of fresh meat: a review. *International Journal of Food Properties*, *17*(2), 433-453.

Belk, K. (2015). *Does muscle tissue contain different types of protein*. American Meat Science Association.

https://meatscience.org/TheMeatWeEat/topics/fresh-meat/article/2015/07/31/does-muscle-tissue-contain-different-types-of-protein

Benedict, R. C. (1987). Determination of nitrogen and protein content of meat and meat products. *Journal of the Association of Official Analytical Chemists*, *70*(1), 69-74.

Bermingham, E. N., Reis, M. G., Subbaraj, A. K., Cameron-Smith, D., Fraser, K., Jonker, A., & Craigie, C. R. (2018). Distribution of fatty acids and phospholipids in different table cuts and co-products from New Zealand pasture-fed Wagyu-dairy cross beef cattle. *Meat Science*, *140*, 26-37.

Berrazaga, I., Micard, V., Gueugneau, M., & Walrand, S. (2019). The role of the anabolic properties of plant-versus animal-based protein sources in supporting muscle mass maintenance: A critical review. *Nutrients*, *11*(8), 1825.

Bhat, Z. F., Morton, J. D., Bekhit, A. E. D. A., Kumar, S., & Bhat, H. F. (2021). Thermal processing implications on the digestibility of meat, fish and seafood proteins. *Comprehensive Reviews in Food Science and Food Safety*, *20*(5), 4511-4548.

Bohrer, B. M. (2019). An investigation of the formulation and nutritional composition of modern meat analogue products. *Food Science and Human Wellness*, *8*(4), 320-329.

Boland, M., Kaur, L., Chian, F., & Astruc, T. (2018). Muscle proteins. In Varelis, P., Melton, L., & Shahidi, F. (Eds.), *Encyclopedia of Food Chemistry* (pp. 164-179). Elsevier.

Brewer, M. S., & Novakofski, J. (1999). Cooking rate, pH and final endpoint temperature effects on color and cook loss of a lean ground beef model system. *Meat science*, *52*(4), 443-451.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., ... & Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature protocols*, *14*(4), 991-1014.

Brunelle, J. L., & Green, R. (2014). One-dimensional SDS-polyacrylamide Gel Electrophoresis (1D SDS-PAGE). *Methods in enzymology*, *541*, 151-159.

Calkins, C. R., & Sullivan, G. (2007). *Ranking of beef muscles for tenderness.* Univesity of Nebraska, (pp. 1-5). National Cattlemen's Beef Association.

Calvo-Lerma, J., Fornés-Ferrer, V., Heredia, A., & Andrés, A. (2018). In vitro digestion of lipids in real foods: influence of lipid organization within the food matrix and interactions with nonlipid components. *Journal of food science*, *83*(10), 2629-2637.

Carabante, K. M., Ardoin, R., Scaglia, G., Malekian, F., Khachaturyan, M., Janes, M. E., & Prinyawiwatkul, W. (2018). Consumer acceptance, emotional response, and purchase intent of rib-eye steaks from grass-fed steers, and effects of health benefit information on consumer perception. *Journal of Food Science*, *83*(10), 2560-2570.

Chajès, V., Joulin, V., & Clavel-Chapelon, F. (2011). The fatty acid desaturation index of blood lipids, as a biomarker of hepatic stearoyl-CoA desaturase expression, is a predictive factor of breast cancer risk. *Current Opinion in Lipidology*, *22*(1), 6-10.

Chang, N. W., Ten Wu, C., Chen, F. N., & Huang, P. C. (2004). High polyunsaturated and monounsaturated fatty acid to saturated fatty acid ratio increases plasma very low density lipoprotein lipids and reduces the hepatic hypertriglyceridemic effect of dietary cholesterol in rats. *Nutrition research*, *24*(1), 73-83.

Chian, F. M., Kaur, L., Oey, I., Astruc, T., Hodgkinson, S., & Boland, M.. (2019). Effect of Pulsed Electric Fields (PEF) on the ultrastructure and in vitro protein digestibility of bovine longissimus thoracis. *LWT*, *103*, 253–259.

Chian, F. M. (2021). *Effect of processing on muscle structure and protein digestibility in vitro* [Published Doctoral dissertation]. Massey University.

Chiang, J. H., Hardacre, A. K., & Parker, M. E. (2020). Extruded meat alternatives made from Maillard-reacted beef bone hydrolysate and plant proteins: part I–Effect of moisture content. *International Journal of Food Science & Technology*, *55*(2), 649-659.

Churruca, I., Fernández-Quintela, A., & Portillo, M. P. (2009). Conjugated linoleic acid isomers: differences in metabolism and biological effects. *Biofactors*, *35*(1), 105-111.

Ciuris, C., Lynch, H. M., Wharton, C., & Johnston, C. S. (2019). A comparison of dietary protein digestibility, based on diaas scoring, in vegetarian and non-vegetarian athletes. *Nutrients*, *11*(12), 3016.

Cobos, Á., & Díaz, O. (2015). Chemical composition of meat and meat products. In Cheung, P. C., & Mehta, B. M. (Eds.), *Handbook of Food Chemistry*, (pp. 471-510). Springer.

Daley, C. A., Abbott, A., Doyle, P. S., Nader, G. A., & Larson, S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition journal*, *9*(1), 1-12.

Dangin, M., Guillet, C., Garcia-Rodenas, C., Gachon, P., Bouteloup-Demange, C., Reiffers-Magnani, K., Fauquant, J., Ballèvre, O., & Beaufrère, B. (2003). The Rate of Protein Digestion affects Protein Gain Differently during Aging in Humans. *The Journal of Physiology*, *549*(2), 635–644.

De la Fuente, J., Diaz, M., Alvarez, I., Oliver, M., i Furnols, M. F., Sañudo, C., Campo, M., Montossi, F., Nute, G., & Caneque, V. (2009). Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. *Meat Science*, *82*(3), 331-337.

Della Rosa, M., Pouzo, L., & Pavan, E. (2018). Meat and fat quality traits of grazing steers supplemented with corn grain and increasing amounts of flaxseed. *Livestock Science*, *208*, 51-54.

den Hartigh, L. J. (2019). Conjugated linoleic acid effects on cancer, obesity, and atherosclerosis: a review of pre-clinical and human trials with current perspectives. *Nutrients*, *11*(2), 370.

Denis, S., Sayd, T., Georges, A., Chambon, C., Chalancon, S., Sante-Lhoutellier, V., & Blanquet-Diot, S. (2016). Digestion of cooked meat proteins is slightly affected by age as assessed using the dynamic gastrointestinal TIM model and mass spectrometry. *Food & function*, *7*(6), 2682-2691.

Duckett, S., Neel, J., Lewis, R. M., Fontenot, J., & Clapham, W. (2013). Effects of forage species or concentrate finishing on animal performance, carcass and meat quality. *Journal of Animal Science*, *91*(3), 1454-1467.

Dupont, D., Alric, M., Blanquet-Diot, S., Bornhorst, G., Cueva, C., Deglaire, A., ... & Van den Abbeele, P. (2019). Can dynamic in vitro digestion systems mimic the physiological reality?. *Critical reviews in food science and nutrition*, *59*(10), 1546-1562.

Edge, M. S., & Garrett, J. L. (2020). The Nutrition Limitations of Mimicking Meat. *Cereal Foods World*, *65*.

England, E., Matarneh, S., Scheffler, T., & Gerrard, D. (2017). Perimortal muscle metabolism and its effects on meat quality. In Purslow, P. P. (Ed.), *New aspects of meat quality* (pp. 63-89). Woodhead Publishing.

Farouk, M. M., Wu, G., Frost, D. A., Staincliffe, M., & Knowles, S. O. (2019). Factors affecting the digestibility of beef and consequences for designing meat-centric meals. *Journal of food quality*, *2019*.

Field, C. J., & Robinson, L. (2019). Dietary fats. *Advances in Nutrition*, *10*(4), 722-724.

Figueiredo, I. L., Claus, T., Júnior, O. O. S., Almeida, V. C., Magon, T., & Visentainer, J. V. (2016). Fast derivatization of fatty acids in different meat samples for gas chromatography analysis. *Journal of Chromatography A*, *1456*, 235-241.

Frank, D., Ball, A., Hughes, J., Krishnamurthy, R., Piyasiri, U., Stark, J., Watkins, P., & Warner, R. (2016). Sensory and flavor chemistry characteristics of Australian beef: influence of intramuscular fat, feed, and breed. *Journal of Agricultural and Food Chemistry*, *64*(21), 4299-4311.

Frank, D., Kaczmarska, K., Paterson, J., Piyasiri, U., & Warner, R. (2017). Effect of marbling on volatile generation, oral breakdown and in mouth flavor release of grilled beef. *Meat Science*, *133*, 61-68.

Friedman, M. (2004). Applications of the ninhydrin reaction for analysis of amino acids, peptides, and proteins to agricultural and biomedical sciences. *Journal of agricultural and food chemistry*, *52*(3), 385-406.

Fruet, A., De Mello, A., Trombetta, F., Stefanello, F., Speroni, C., De Vargas, D., De Souza, A., Júnior, A. R., Tonetto, C., & Nörnberg, J. (2018). Oxidative stability of beef from steers finished exclusively with concentrate, supplemented, or on legume-grass pasture. *Meat Science*, *145*, 121-126.

Fruet, A., Stefanello, F., Trombetta, F., De Souza, A., Júnior, A. R., Tonetto, C., Flores, J., Scheibler, R., Bianchi, R., & Pacheco, P. (2019). Growth performance and carcass traits of steers finished on three different systems including legume–grass pasture and grain diets. *Animal: An International Journal of Animal Bioscience*, *13*(7), 1552-1562.

Gagaoua, M., Picard, B., Soulat, J., & Monteils, V. (2018). Clustering of sensory eating qualities of beef: Consistencies and differences within carcass, muscle, animal characteristics and rearing factors. *Livestock Science*, *214*, 245-258.

Ganapathy, V., Gupta, N., & Martindale, R. G. (2006). Protein digestion and absorption. In *Physiology of the gastrointestinal tract* (pp. 1667-1692). Elsevier Inc.

Graça, J., Oliveira, A., & Calheiros, M. M. (2015). Meat, beyond the plate. Data-driven hypotheses for understanding consumer willingness to adopt a more plant-based diet. *Appetite*, *90*, 80-90.

García-Segovia, P., Andrés-Bello, A., & Martínez-Monzó, J. (2007). Effect of cooking method on mechanical properties, color and structure of beef muscle (M. pectoralis). *Journal of Food Engineering*, *80*(3), 813-821.

Goodman, B. E. (2010). Insights into digestion and absorption of major nutrients in humans. *Advances in physiology education*, *34*(2), 44-53.

Gorissen, S. H., Rémond, D., & Van Loon, L. J. (2015). The muscle protein synthetic response to food ingestion. *Meat Science, 109*, 96-100.

Gorissen, S. H., & Witard, O. C. (2018). Characterising the muscle anabolic potential of dairy, meat and plant-based protein sources in older adults. *Proceedings of the Nutrition Society*, *77*(1), 20-31.

Guo, Q., Ye, A., Bellissimo, N., Singh, H., & Rousseau, D. (2017). Modulating fat digestion through food structure design. *Progress in Lipid Research*, *68*, 109-118.

Guo, W., & Greaser, M. (2017). Muscle structure, proteins, and meat quality. In Purslow, P. P. (Ed.), *New aspects of meat quality* (pp. 13-31). Elsevier.

Gupta, R., Lakshmy, R., Abraham, R. A., Reddy, K. S., Jeemon, P., & Prabhakaran, D. (2013). Serum omega-6/omega-3 ratio and risk markers for cardiovascular disease in an industrial population of Delhi. *Food and Nutrition Sciences, 4(*9A), 94-97.

Guzek, D., Głąbska, D., Gutkowska, K., & Wierzbicka, A. (2016). Effect of carcass fat and conformation class on consumer perception of various grilled beef muscles. *Journal of Food Science and Technology*, *53*(10), 3778-3786.

Haider, S. R., Reid, H. J., & Sharp, B. L. (2012). Tricine-sds-page. In *Protein electrophoresis* (pp. 81-91). Humana Press, Totowa, NJ.

Hajji, H., Joy, M., Ripoll, G., Smeti, S., Mekki, I., Gahete, F. M., Mahouachi, M., & Atti, N. (2016). Meat physicochemical properties, fatty acid profile, lipid oxidation and sensory characteristics from three North African lamb breeds, as influenced by concentrate or pasture finishing diets. *Journal of Food Composition and Analysis*, *48*, 102-110.

Hammad, S., Pu, S., & Jones, P. J. (2016). Current evidence supporting the link between dietary fatty acids and cardiovascular disease. *Lipids*, *51*(5), 507-517.

Hernández, B., Sáenz, C., Alberdi, C., & Diñeiro, J. M. (2016). CIELAB color coordinates versus relative proportions of myoglobin redox forms in the description of fresh meat appearance. *Journal of food science and technology*, *53*(12), 4159-4167.

Horcada, A., Polvillo, O., González-Redondo, P., López, A., Tejerina, D., & García-Torres, S. (2020). Stability of fatty acid composition of intramuscular fat from pasture-and grain-fed young bulls during the first 7 d postmortem. *Archives Animal Breeding*, *63*(1), 45.

Horcada, A., Polvillo, O., Juárez, M., Avilés, C., Martínez, A., & Peña, F. (2016). Influence of feeding system (concentrate and total mixed ration) on fatty acid profiles of beef from three lean cattle breeds. *Journal of Food Composition and Analysis*, *49*, 110-116.

Horman, T., Fernandes, M. F., Tache, M. C., Hucik, B., Mutch, D. M., & Leri, F. (2020). Dietary n-6/n-3 Ratio Influences Brain Fatty Acid Composition in Adult Rats. *Nutrients*, *12*(6), 1847.

Hur, S. J., Lim, B. O., Decker, E. A., & McClements, D. J. (2011). In vitro human digestion models for food applications. *Food Chemistry*, *125*(1), 1-12.

Huuskonen, A., Jansson, S., Honkavaara, M., Tuomisto, L., Kauppinen, R., & Joki-Tokola, E. (2010). Meat colour, fatty acid profile and carcass characteristics of Hereford bulls finished on grazed pasture or grass silage-based diets with similar concentrate allowance. *Livestock Science*, *131*(1), 125-129.

Ibáñez, A. H., López, A., Polo, O. P., Pino, R., de la Vega, M. D. C., Barrado, D. T., & Torres, S. G. (2017). Fatty acid profile as a tool to trace the origin of beef in pasture-and grain-fed young bulls of Retinta breed. *Spanish Journal of Agricultural Research*, *15*(4), 14.

Jeremiah, L. E., & Gibson, L. L. (2003). Cooking influences on the palatability of roasts from the beef hip. *Food Research International*, *36* (1), 1-9.

Jahn, S., Furchheim, P., & Strässner, A. M. (2021). Plant-Based Meat Alternatives: Motivational Adoption Barriers and Solutions. *Sustainability*, *13*(23), 13271.

Jiang, Q., Han, J., Gao, P., Yu, L., Xu, Y., & Xia, W. (2018). Effect of heating temperature and duration on the texture and protein composition of Bighead Carp (Aristichthys nobilis) muscle. *International Journal of Food Properties*, *21*(1), 2110-2120.

Jukna, V., Jukna, Č., Prusevičius, V., Meškinytė-Kaušilienė, E., & Pečiulaitienė, N. (2017). Meat quality of different beef cattle breeds fed high energy forage. *Zemdirbyste-Agriculture*, *104*(3), 277-282.

Jung, E.-Y., Hwang, Y.-H., & Joo, S.-T. (2016). The relationship between chemical compositions, meat quality, and palatability of the 10 primal cuts from Hanwoo steer. *Korean Journal for Food Science of Animal Resources*, *36*(2), 145.

Kamani, M. H., Meera, M. S., Bhaskar, N., & Modi, V. K. (2019). Partial and total replacement of meat by plant-based proteins in chicken sausage: Evaluation of mechanical, physico-

chemical and sensory characteristics. *Journal of food science and technology*, *56*(5), 2660-2669.

Kaur, L., Rutherfurd, S. M., Moughan, P. J., Drummond, L., & Boland, M. J. (2010). Actinidin enhances gastric protein digestion as assessed using an in vitro gastric digestion model. *Journal of agricultural and food chemistry*, *58*(8), 5068-5073.

Kaur, L., Rutherfurd, S. M., Moughan, P. J., Drummond, L., & Boland, M. J. (2010). Actinidin enhances protein digestion in the small intestine as assessed using an in vitro digestion model. *Journal of agricultural and food chemistry*, *58*(8), 5074-5080.

Kaur, L., Maudens, E., Haisman, D. R., Boland, M. J., & Singh, H. (2014). Microstructure and protein digestibility of beef: The effect of cooking conditions as used in stews and curries. *LWT-Food Science and Technology*, *55*(2), 612-620.

King, N. J., & Whyte, R. (2006). Does it look cooked? A review of factors that influence cooked meat color. *Journal of Food Science*, *71*(4), 31-40.

Krishnan, S., & Cooper, J. A. (2014). Effect of dietary fatty acid composition on substrate utilization and body weight maintenance in humans. *European journal of nutrition*, *53*(3), 691-710.

Kyriakopoulou, K., Keppler, J. K., & Van Der Goot, A. J. (2021). Functionality of Ingredients and Additives in Plant-Based Meat Analogues. *Foods*, *10*(3), 600.

Lawrence, J. (2010). Meat and meat products. In W. Horwitz & G. W. Lantimer (Eds.), *Official Methods of Analysis of AOAC International* (18th ed., pp. 1-6). AOAC International.

Lee, H. J., Yong, H. I., Kim, M., Choi, Y. S., & Jo, C. (2020). Status of meat alternatives and their potential role in the future meat market—A review. *Asian-Australasian Journal of Animal Sciences*, *33*(10), 1533-1543.

Lee, K.-W., Hwang, Y.-H., & Joo, S.-T. (2017). Meat tenderness characteristics of ten major muscles from Hanwoo steers according to quality grades of carcasses. *Korean Journal for Food Science of Animal Resources*, *37*(4), 593-598.

Lefranc-Millot, C., & Teichman-Dubois, V. (2018). Protein from vegetable sources: a focus on pea protein. In M. Hayes (Ed.), *Novel Proteins for Food, Pharmaceuticals and Agriculture* (197-216). John Wiley and Sons.

Li, Y., Carrillo, J. A., Ding, Y., He, Y., Zhao, C., Liu, J., Liu, G. E., Zan, L., & Song, J. (2015). Transcriptomic profiling of spleen in grass-fed and grain-fed Angus cattle. *Plos one*, *10*(9), e0135670.

Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., Picard, B., & Bugeon, J. (2016). How muscle structure and composition influence meat and flesh quality. *The Scientific World Journal*, *2016*.

Lonergan, S. M., Topel, D. G., & Marple, D. N. (2018). *The Science of Animal Growth and Meat Technology*. Academic Press.

Lucas-González, R., Viuda-Martos, M., Pérez-Alvarez, J. A., & Fernández-López, J. (2018). In vitro digestion models suitable for foods: Opportunities for new fields of application and challenges. *Food Research International*, *107*, 423-436.

Lukic, M., Trbovic, D., Karan, D., Petrovic, Z., Jovanovic, J., Milijasevic, J. B., & Nikolic, A. (2021). The nutritional and health value of beef lipids-fatty acid composition in grass-fed and grain-fed beef. In *IOP Conference Series. Earth and Environmental Science* (Vol. 854, No. 1). IOP Publishing.

Lunn, D. (2020). Managing and feeding for marbling and carcass quality in beef cattle : *A report to Nutrifax* . Shur-Gain, Nutreco Canada Inc. http://www.wrightsfeeds.ca/wpcontent/uploads/Managing-and-Feeding-for-Marbling-andCarcass-Quality-in-Beef-Cattle.pdf

Ly, B. C. K., Dyer, E. B., Feig, J. L., Chien, A. L., & Del Bino, S. (2020). Research techniques made simple: Cutaneous colorimetry: A reliable technique for objective skin color measurement. *Journal of Investigative Dermatology*, *140*(1), 3-12.

Matsumoto, H., Haniu, H., & Komori, N. (2019). Determination of Protein Molecular Weights on SDS-PAGE. *Methods in molecular biology (Clifton, NJ)*, *1855*, 101-105.

McNeill, S. H. (2014). Inclusion of red meat in healthful dietary patterns. *Meat science*, *98*(3), 452-460.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Ménard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S., Weitschies, W., & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food & Function*, *5*(6), 1113-1124.

Mwangi, F. W., Charmley, E., Gardiner, C. P., Malau-Aduli, B. S., Kinobe, R. T., & Malau-Aduli, A. E. (2019). Diet and genetics influence beef cattle performance and meat quality characteristics. *Foods*, *8*(12), 648-672.

Nieva-Echevarría, B., Goicoechea, E., & Guillén, M. D. (2020). Food lipid oxidation under gastrointestinal digestion conditions: A review. *Critical reviews in food science and nutrition*, *60*(3), 461-478.

Nosworthy, M. G., & House, J. D. (2017). Factors influencing the quality of dietary proteins: Implications for pulses. *Cereal Chemistry*, *94*(1), 49-57.

Orsavova, J., Misurcova, L., Ambrozova, J. V., Vicha, R., & Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International journal of molecular sciences*, *16*(6), 12871-12890.

Oz, F., Aksu, M., & Turan, M. (2017). The effects of different cooking methods on some quality criteria and mineral composition of beef steaks. *Journal of Food Processing and Preservation*, *41*(4), e13008.

Pathare, P. B., & Roskilly, A. P. (2016). Quality and energy evaluation in meat cooking. *Food Engineering Reviews*, *8*(4), 435-447.

Pennings, B., Groen, B. B., van Dijk, J. W., de Lange, A., Kiskini, A., Kuklinski, M., ... & van Loon, L. J. C. (2013). Minced beef is more rapidly digested and absorbed than beef steak, resulting in greater postprandial protein retention in older men. *American Journal of Clinical Nutrition*, *98*(1), 121-128.

Pereira, P. M. D. C. C., & Vicente, A. F. D. R. B. (2013). Meat nutritional composition and nutritive role in the human diet. *Meat science*, *93*(3), 586-592.

Pighin, D., Pazos, A., Chamorro, V., Paschetta, F., Cunzolo, S., Godoy, F., Messina, V., Pordomingo, A., & Grigioni, G. (2016). A contribution of beef to human health: a review of the role of the animal production systems. *The Scientific World Journal*, *2016*, 1-10.

Pighín, D. G., Davies, P., Pazos, A. A., Ceconi, I., Cunzolo, S. A., Mendez, D., Buffarini, M., & Grigioni, G. (2015). Biochemical profiles and physicochemical parameters of beef from cattle raised under contrasting feeding systems and pre-slaughter management. *Animal Production Science*, *55*(10), 1310-1317.

Ponnampalam, E., Mann, N., & Sinclair, A. (2006). Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and trans fatty acids in Australian beef cuts: potential impact on humnan health. *Asia Pacific Journal of Clinical Nutrition*, *15*(1), 21-29.

Priolo, A., Micol, D., & Agabriel, J. (2001). Effects of grass feeding systems on ruminant meat colour and flavour. A review. *Animal Research*, *50*(3), 185-200.

Promeyrat, A., Gatellier, P., Lebret, B., Kajak-Siemaszko, K., Aubry, L., & Santé-Lhoutellier, V. (2010). Evaluation of protein aggregation in cooked meat. *Food Chemistry*, *121*(2), 412-417.

Purchas, R. W., Wilkinson, B. H., Carruthers, F., & Jackson, F. (2014). A comparison of the nutrient content of uncooked and cooked lean from New Zealand beef and lamb. *Journal of Food Composition and Analysis*, *35*(2), 75-82.

Raes, K., Balcaen, A., Dirinck, P., De Winne, A., Claeys, E., Demeyer, D., & De Smet, S. (2003). Meat quality, fatty acid composition and flavour analysis in Belgian retail beef. *Meat science*, *65*(4), 1237-1246.

Remond, D., Machebeuf, M., Yven, C., Buffière, C., Mioche, L., Mosoni, L., & Patureau-Mirand, P. (2007). Postprandial whole-body protein metabolism after a meat meal is influenced by chewing efficiency in elderly subjects. *American Journal of Clinical Nutrition*, *85*(5), 1286-1292.

Roseland, J. M., Nguyen, Q. V., Williams, J. R., Douglass, L. W., Patterson, K. Y., Howe, J. C., ... & McNeill, S. H. (2015). Protein, fat, moisture and cooking yields from a US study of retail beef cuts. *Journal of Food Composition and Analysis*, *43*, 131-139.

Roseland, J. M., Nguyen, Q. V., Douglass, L. W., Patterson, K. Y., Howe, J. C., Williams, J. R., Thompson, L. D., Brooks, J. C., Woerner, D. R., Engle, T. E., Savell, J. W., Gehring, K. B., Cifelli, A. M., & Mcneill, S. H.. (2018). Fatty acid, cholesterol, vitamin, and mineral content of cooked beef cuts from a national study. *Journal of Food Composition and Analysis*, *66*, 55–64.

Saláková, A. (2012). Instrumental measurement of texture and color of meat and meat products. *Maso International Brno*, *2*(2), 107-114.

Salim, A. P. A., Suman, S. P., Canto, A. C., Costa-Lima, B. R., Viana, F. M., Monteiro, M. L. G., Silva, T. J., & Conte-Junior, C. A. (2019). Muscle-specific color stability in fresh beef from grain-finished Bos indicus cattle. *Asian-Australasian Journal of Animal Sciences*, *32*(7), 1036.

Santé-Lhoutellier, V., Astruc, T., Marinova, P., Greve, E., & Gatellier, P. (2008). Effect of meat cooking on physicochemical state and in vitro digestibility of myofibrillar proteins. *Journal of Agricultural and Food Chemistry*, *56*(4), 1488-1494.

Science of Meat: What is Meat? (2018). Exploratorium: Science of cooking. https://www.exploratorium.edu/cooking/meat/INT-what-is-meat.html.

Schönfeldt, H. C., & Strydom, P. E. (2011). Effect of age and cut on cooking loss, juiciness and flavour of South African beef. *Meat science*, *87*(3), 180-190.

Schwingshackl, L., & Hoffmann, G. (2014). Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. *Lipids in health and disease*, *13*(1), 1-15.

Scollan, N., Hocquette, J.-F., Nuernberg, K., Dannenberger, D., Richardson, I., & Moloney, A. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science*, *74*(1), 17-33.

Scollan, N. D., Dannenberger, D., Nuernberg, K., Richardson, I., MacKintosh, S., Hocquette, J. F., & Moloney, A. P. (2014). Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science*, *97*(3), 384-394.

Scozzafava, G., Corsi, A. M., Casini, L., Contini, C., & Loose, S. M. (2016). Using the animal to the last bit: Consumer preferences for different beef cuts. *Appetite*, *96*, 70-79.

Simopoulos, A. P. (2011). Importance of the omega-6/omega-3 balance in health and disease: evolutionary aspects of diet. In *Healthy agriculture, healthy nutrition, healthy people* (Vol. 102, pp. 10-21). Karger Publishers.

Singh, H., & Gallier, S. (2014). Processing of food structures in the gastrointestinal tract and physiological responses. In *Food structures, digestion and health* (pp. 51-81). Academic Press.

Siriwardhana, N., Kalupahana, N. S., & Moustaid-Moussa, N. (2012). Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid. *Advances in food and nutrition research*, *65*, 211-222.

Sha, L., & Xiong, Y. L.. (2020). Plant protein-based alternatives of reconstructed meat: Science, technology, and challenges. *Trends in Food Science & Technology*, *102*, 51–61.

Shen, Y., Kim, S., Yoon, D., Lee, H., Kang, H., & Seo, K. (2012). Proteome analysis of bovine longissimus dorsi muscle associated with the marbling score. *Asian-Australasian Journal of Animal Sciences*, *25*(8), 1083.

Shokryazdan, P., Rajion, M. A., Meng, G. Y., Boo, L. J., Ebrahimi, M., Royan, M., Sahebi, M., Azizi, P., Abiri, R., & Jahromi, M. F. (2017). Conjugated linoleic acid: A potent fatty acid linked to animal and human health. *Critical Reviews in Food Science and Nutrition*, *57*(13), 2737-2748.

Sjögren, P., Sierra-Johnson, J., Gertow, K., Rosell, M., Vessby, B., De Faire, U., ... & Fisher, R. M. (2008). Fatty acid desaturases in human adipose tissue: relationships between gene expression, desaturation indexes and insulin resistance. *Diabetologia*, *51*(2), 328-335.

Smith, A. M., Harris, K. B., Haneklaus, A. N., & Savell, J. W. (2011). Proximate composition and energy content of beef steaks as influenced by USDA quality grade and degree of doneness. *Meat science*, *89*(2), 228-232.

Smith, M. E., & Morton, D. G. (2011). *The Digestive System: Systems of the Body Series*. Elsevier Health Sciences.

Smith, S. B., & Johnson, B. J. (2016). Marbling: Management of cattle to maximize the deposition of intramuscular adipose tissue. *Journal of Animal Science*, *94*, 382-382.

Smith, S. B., Kawachi, H., Choi, C. B., Choi, C. W., Wu, G., & Sawyer, J. E. (2009). Cellular regulation of bovine intramuscular adipose tissue development and composition. *Journal of Animal Science*, *87*(suppl_14), 72-82.

Smith, S. B., & Smith, D. R. (2014). Adipose tissue. In M. Dikeman & C. Devine (Eds.), *Encyclopedia of meat sciences* (2nd ed., pp. 222-234). Academic Press.

Srigley, C. T., & Mossoba, M. M. (2016). Current Analytical Techniques for Food Lipids. *Food Safety: Innovative Analytical Tools for Safety Assessment*, 33-64.

Strasburg, G. M., & Xiong, Y. L. (2017). Physiology and chemistry of edible muscle tissues. In *Fennema's Food Chemistry* (pp. 955-1015). CRC Press.

Sun, S.-W., Lin, Y.C., Weng, Y.M., & Chen, M.J. (2006). Efficiency improvements on ninhydrin method for amino acid quantification. *Journal of Food Composition and Analysis*, *19*(2-3), 112–117.

Swartz, D. R., Greaser, M. L., & Cantino, M. E. (2009). Muscle structure and function. In M. Du, & R. J. McCormick (Eds.), *Applied muscle biology and meat science*, (pp.1-195). CRC Press.

Terevinto, A., Cabrera, M. C., & Saadoun, A. (2019). Oxidative stability, fatty acid composition and health lipid indices of Longissimus dorsi muscle from Aberdeen Angus steers produced in different feeding systems. *Ciência Rural*, *49*(12).

Thavamani, A., Sferra, T. J., & Sankararaman, S. (2020). Meet the Meat Alternatives: The Value of Alternative Protein Sources. *Current Nutrition Reports*, 1-10.

The Effect of Marbling on Beef Eating Quality. (2018). (Tips & Tools - Meat Standards Australia, Issue.

Toldrá, F. (2017). *Lawrie's Meat Science*. Woodhead Publishing.

Tortora, G. J., & Derrickson, B. H. (2018). *Principles of anatomy and physiology*. John Wiley & Sons.

Trommelen, J., Holwerda, A. M., Pinckaers, P. J., & van Loon, L. J. (2021). Comprehensive assessment of post-prandial protein handling by the application of intrinsically labelled protein in vivo in human subjects. *Proceedings of the Nutrition Society*, 1-9.

Troy, D. J., Tiwari, B. K., & Joo, S. T. (2016). Health Implications of Beef Intramuscular Fat Consumption. *Korean Journal for Food Science of Animal Resources*, *36*(5), 577-582.

Vaclavik, V. A., & Christian, E. W. (2014). *Essentials of Food Science* (4th ed.). Springer.

Vahmani, P., Rolland, D. C., Mapiye, C., Dunne, P. G., Aalhus, J. L., Juárez, M., McAllister, T.A., Prieto, N., & Dugan, M. E. R. (2017). Increasing desirable polyunsaturated fatty acid concentrations in fresh beef intramuscular fat. *CAB Reviews*, *12*(20), 1-17.

Vahmani, P., Ponnampalam, E. N., Kraft, J., Mapiye, C., Bermingham, E. N., Watkins, P. J., Proctor, S. D., & Dugan, M. E. (2020). Bioactivity and health effects of ruminant meat lipids. Invited Review. *Meat Science*, *165*, 108-114.

Van Elswyk, M. E., & McNeill, S. H. (2014). Impact of grass/forage feeding versus grain finishing on beef nutrients and sensory quality: The US experience. *Meat Science*, *96*(1), 535-540.

Vessby, B., Gustafsson, I. B., Tengblad, S., & Berglund, L. (2013). Indices of fatty acid desaturase activity in healthy human subjects: effects of different types of dietary fat. *British journal of nutrition*, *110*(5), 871-879.

Warensjö, E., Rosell, M., Hellenius, M. L., Vessby, B., De Faire, U., & Risérus, U. (2009). Associations between estimated fatty acid desaturase activities in serum lipids and adipose tissue in humans: links to obesity and insulin resistance. *Lipids in Health and Disease*, *8*(1), 1-6.

Warriss, P. D. (2001). *Meat science*. Cabi Publishing.

Watkins, P. J., Frank, D., Singh, T. K., Young, O. A., & Warner, R. D. (2013). Sheepmeat flavor and the effect of different feeding systems: a review. *Journal of Agricultural and Food Chemistry*, *61*(15), 3561-3579.

Wildman, R. E., & Medeiros, D. M. (2019). *Advanced human nutrition*. Boca Raton, FL. CRC press.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R., & Enser, M.. (2004). Effects of fatty acids on meat quality: a review. *Meat Science*, *66*(1), 21–32.

Woolworths New Zealand Limited (2022). https://shop.countdown.co.nz/

Wyness, L. (2016). The role of red meat in the diet: nutrition and health benefits. *Proceedings of the Nutrition Society*, *75*(3), 227-232.

Yu, T. Y., Morton, J. D., Clerens, S., & Dyer, J. M. (2017). Cooking-induced protein modifications in meat. *Comprehensive Reviews in Food Science and Food Safety, 16*(1), 141-159.

Zhou, H., Hu, Y., Tan, Y., Zhang, Z., & McClements, D. J. (2021). Digestibility and Gastrointestinal Fate of Meat versus Plant-Based Meat Analogs: An in Vitro Comparison. *Food Chemistry*, 130439.

Zhu, X., Ye, A., Verrier, T., & Singh, H. (2013). Free fatty acid profiles of emulsified lipids during *in vitro* digestion with pancreatic lipase. *Food Chemistry, 139(1-4),* 398-404.

Appendices

Appendix A - Individual free fatty acids released at 0 min simulated gastro-small intestinal digestion for striploin grain-finished (SLG), striploin pasture-fed (SLP), tenderloin grain-finished (TLG), tenderloin pasture-fed (TLP), topside grain-finished (TSG), topside pasture-fed (TSP), and plant-based meat alternative (BB).

Fatty acids		**Total initial fatty acids (TFA, mg/g cooked meat)**						
		SLG*	**SLP***	**TLG***	**TLP***	**TSG***	**TSG***	**BB^**
Individual SFA								
C8:0	Caprylic acid	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	1.80 ± 0.24 ^{a}
C10:0	Capric acid	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	1.59 ± 0.20 ^{a}
C12:0	Lauric acid	0.04 ± 0.01 ^{b}	0.03 ± 0.01 ^{b}	0.06 ± 0.02 ^{b}	0.04 ± 0.02 ^{b}	0.03 ± 0.01 ^{b}	0.02 ± 0.01 ^{b}	13.56 ± 1.71 ^{a}
C14:0	Myristic acid	1.64 ± 0.34 ^{bc}	1.08 ± 0.25 ^{bcd}	1.85 ± 0.50 ^{b}	1.08 ± 0.50 ^{cd}	1.05 ± 0.22 ^{cd}	0.55 ± 0.08 ^{d}	5.65 ± 0.67 ^{a}
C15:0	Pentadecylic acid	0.31 ± 0.03 ^{ab}	0.23 ± 0.04 ^{b}	0.43 ± 0.15 ^{a}	0.39 ± 0.11 ^{ab}	0.23 ± 0.05 ^{b}	0.17 ± 0.04 ^{b}	0.06 ± 0.00 ^{c}
C16:0	Palmitic acid	15.51 ± 1.60 ^{ab}	13.69 ± 2.28 ^{abc}	19.53 ± 4.67 ^{a}	13.56 ± 4.12 ^{bc}	12.35 ± 2.57 ^{bc}	8.81 ± 1.31 ^{c}	13.55 ± 1.21 ^{abc}
C17:0	Heptadecanoic acid	1.00 ± 0.24 ^{ab}	0.57 ± 0.07 ^{b}	1.29 ± 0.38 ^{a}	0.70 ± 0.16 ^{b}	0.71 ± 0.19 ^{b}	0.37 ± 0.08 ^{c}	0.15 ± 0.01 ^{d}
C18:0	Stearic acid	8.25 ± 1.20 ^{b}	10.05 ± 1.17 ^{ab}	12.75 ± 2.61 ^{a}	12.49 ± 3.20 ^{a}	6.53 ± 1.22 ^{b}	6.48 ± 1.30 ^{b}	7.67 ± 1.03 ^{b}
Individual MUFA								
C14:1	Myristoleic acid	0.42 ± 0.06 ^{a}	0.17 ± 0.05 ^{bc}	0.38 ± 0.14 ^{a}	0.15 ± 0.08 ^{bc}	0.30 ± 0.11 ^{ab}	0.10 ± 0.03 ^{c}	Not detected
C16:1	Palmitoleic acid	1.96 ± 0.24 ^{a}	1.20 ± 0.32 ^{bc}	2.08 ± 0.60 ^{a}	0.99 ± 0.30 ^{c}	1.76 ± 0.53 ^{ab}	0.84 ± 0.10 ^{c}	0.22 ± 0.02 ^{d}
C17:1	cis-10 Heptadecanoic acid	1.00 ± 0.23 ^{a}	0.23 ± 0.05 ^{b}	1.01 ± 0.31 ^{a}	0.32 ± 0.06 ^{b}	0.76 ± 0.23 ^{a}	0.25 ± 0.04 ^{b}	0.12 ± 0.01 ^{c}
C18:1 *c* 9	Oleic acid	28.83 ± 2.73 ^{bc}	19.32 ± 2.74 ^{cd}	32.89 ± 9.14 ^{b}	18.34 ± 4.78 ^{cd}	22.91 ± 6.40 ^{bcd}	13.77 ± 1.87 ^{d}	61.08 ± 5.03 ^{a}
C18:1 *c* 11	cis-Vaccenic acid	1.38 ± 0.12 ^{b}	0.58 ± 0.12 ^{c}	1.70 ± 0.42 ^{b}	0.72 ± 0.17 ^{c}	1.4 ± 0.31 ^{b}	0.58 ± 0.07 ^{c}	2.60 ± 0.20 ^{a}
Individual PUFA								
C18:2 n-6 (LA)	Linoleic acid	1.42 ± 0.29 ^{bc}	0.88 ± 0.12 ^{c}	2.46 ± 0.57 ^{b}	1.65 ± 0.11 ^{bc}	2.13 ± 0.42 ^{b}	1.55 ± 0.09 ^{bc}	23.81 ± 1.43 ^{a}
C18:2 *c* 9, *t* 11 (CLA)	Conjugated linoleic acid	0.12 ± 0.03 ^{c}	0.25 ± 0.05 ^{bc}	0.31 ± 0.18 ^{b}	0.26 ± 0.07 ^{bc}	0.13 ± 0.06 ^{c}	0.14 ± 0.03 ^{c}	0.94 ± 0.06 ^{a}
C18:3 n-3 (ALA)	α-Linolenic acid	0.22 ± 0.04 ^{d}	0.52 ± 0.11 ^{cd}	0.45 ± 0.12 ^{cd}	0.91 ± 0.16 ^{b}	0.30 ± 0.06 ^{d}	0.71 ± 0.04 ^{bc}	6.25 ± 0.45 ^{a}
C20:4 n-6	Arachidonic acid	0.40 ± 0.02 ^{c}	0.36 ± 0.07 ^{c}	0.58 ± 0.03 ^{b}	0.54 ± 0.05 ^{b}	0.69 ± 0.09 ^{a}	0.69 ± 0.03 ^{a}	0.34 ± 0.02 ^{c}
C20:5 n-3 (EPA)	Eicosapentaenoic acid	0.15 ± 0.01 ^{d}	0.20 ± 0.02 ^{c}	0.20 ± 0.02 ^{c}	0.31 ± 0.03 ^{b}	0.30 ± 0.04 ^{b}	0.40 ± 0.03 ^{a}	Not detected
C22:5 n-3 (DPA)	Docosapentaenoic acid	0.26 ± 0.01 ^{d}	0.31 ± 0.03 ^{cd}	0.34 ± 0.03 ^{c}	0.47 ± 0.04 ^{ab}	0.42 ± 0.03 ^{b}	0.53 ± 0.05 ^{a}	Not detected
C22:6 n-3 (DHA)	Docosahexaenoic acid	0.03 ± 0.00 ^{b}	0.03 ± 0.00 ^{b}	0.05 ± 0.01 ^{a}	0.05 ± 0.01 ^{a}	0.06 ± 0.01 ^{a}	0.06 ± 0.01 ^{a}	Not detected

Total intial fatty acid (TFA, mg/g cooked meat)							
mg/g cooked meat	**SLG***	**SLP***	**TLG***	**TLP***	**TSG***	**TLP***	**BB^**
Σ SFA	26.76 ± 3.02 ^{bcd}	25.65 ± 3.53 ^{bcd}	35.91 ± 8.07 ^{ab}	28.28 ± 7.91 ^{abc}	20.90 ± 4.00 ^{cd}	16.40 ± 2.74 ^{d}	44.04 ± 5.06 ^{a}
Σ MUFA	33.55 ± 3.15 ^{bc}	21.50 ± 3.16 ^{cd}	38.06 ± 10.49 ^{b}	20.53 ± 5.36 ^{cd}	27.07 ± 7.45 ^{bc}	15.54 ± 1.99 ^{d}	64.02 ± 5.25 ^{a}
Σ PUFA	2.60 ± 0.35 ^{cd}	2.54 ± 0.30 ^{d}	4.40 ± 0.79 ^{b}	4.18 ± 0.25 ^{b}	4.03 ± 0.64 ^{bc}	4.08 ± 0.15 ^{bc}	31.37 ± 1.95 ^{a}
Σ n-6 PUFA	1.81 ± 0.31 ^{cd}	1.24 ± 0.17 ^{d}	3.04 ± 0.55 ^{b}	2.19 ± 0.12 ^{bcd}	2.83 ± 0.49 ^{bc}	2.24 ± 0.11 ^{bcd}	24.16 ± 1.44 ^{a}
Σ n-3 PUFA	0.66 ± 0.05 ^{d}	1.05 ± 0.13 ^{c}	1.05 ± 0.10 ^{c}	1.74 ± 0.18 ^{b}	1.08 ± 0.12 ^{c}	1.69 ± 0.10 ^{b}	6.28 ± 0.45 ^{a}
Σ EPA,DHA	0.18 ± 0.01 ^{d}	0.23 ± 0.02 ^{cd}	0.25 ± 0.03 ^{c}	0.36 ± 0.03 ^{b}	0.36 ± 0.05 ^{b}	0.48 ± 0.03 ^{a}	Not detected
FA ratios and DI							
MUFA/SFA	1.27 ± 0.04 ^{b}	0.84 ± 0.02 ^{de}	1.06 ± 0.09 ^{c}	0.73 ± 0.04 ^{e}	1.28 ± 0.13 ^{b}	0.95 ± 0.07 ^{cd}	1.58 ± 0.06 ^{a}
(PUFA+MUFA)/SFA	1.38 ± 0.05 ^{b}	0.94 ± 0.01 ^{d}	1.18 ± 0.09 ^{c}	0.89 ± 0.07 ^{d}	1.47 ± 0.12 ^{b}	1.21 ± 0.10 ^{c}	2.35 ± 0.10 ^{a}
n-6/n-3 PUFA	2.72 ± 0.31 ^{b}	1.18 ± 0.10 ^{c}	2.89 ± 0.34 ^{b}	1.27 ± 0.15 ^{c}	2.61 ± 0.24 ^{b}	1.33 ± 0.10 ^{c}	3.85 ± 0.05 ^{a}
DI16**	0.13 ± 0.01 ^{ab}	0.09 ± 0.02 ^{cd}	0.11 ± 0.01 ^{bc}	0.07 ± 0.01 ^{d}	0.14 ± 0.02 ^{a}	0.10 ± 0.01 ^{cd}	0.02 ± 0.00 ^{e}
DI18***	3.52 ± 0.28 ^{b}	1.92 ± 0.13 ^{de}	2.56 ± 0.28 ^{c}	1.48 ± 0.13 ^{e}	3.46 ± 0.50 ^{b}	2.15 ± 0.21 ^{cd}	8.01 ± 0.48 ^{a}

Values of TFA with different superscripts (a-e) within the same row differ significantly ($p < 0.05$).

* N =5, all measurements taken for 5 carcasses in triplicate.

^ N =3, all measurements taken for 1 sample in triplicate.

Appendix B - Copyright permission from John Wiley & Sons, Inc (for Figure 2.2)

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

Aug 22, 2021

This Agreement between Ms. Amrutha Elamurugan ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	5134120103017
License date	Aug 22, 2021
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Wiley Books
Licensed Content Title	Principles of Anatomy and Physiology, 2nd Asia-Pacific Edition
Licensed Content Author	Brendan Burkett Bryan H. Derrickson Danielle Dye Gerard J. Tortora Gregory Peoples Julie Cooke Latika Samalia Mark McKean Rebecca Mellifont Tara Diversi
Licensed Content Date	Oct 1, 2018
Licensed Content Pages	1
Type of use	Dissertation/Thesis
Requestor type	University/Academic

Format	Print and electronic
Portion	Figure/table
Number of figures/tables	2
Will you be translating?	No
Title	Protein and lipid oral-gastro-small intestinal digestibility in vitro of pasture-raised beef, grain-finished beef, and meat substitute: A comparative study.
Institution name	Massey University
Expected presentation date	Jan 2022
Portions	Figure 10.3, Figure 10.4, Table 10.1
Requestor Location	Ms. Amrutha Elamurugan 572 Ruahine Street Hokowhitu Palmerston North, 4410 New Zealand Attn: Massey University
Publisher Tax ID	EU826007151
Total	0.00 USD

Terms and Conditions

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a"Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that

you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.

- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, **and any CONTENT (PDF or image file) purchased as part of your order,** is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.

- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner.**For STM Signatory Publishers clearing permission under the terms of the STM Permissions Guidelines only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts,** You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone basis, or any of the rights granted to you hereunder to any other person.

- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto

- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY,

INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.

- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.
- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.
- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.
- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.
- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.
- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.
- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.
- These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.
- In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions,

these terms and conditions shall prevail.

- WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.
- This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.
- This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

WILEY OPEN ACCESS TERMS AND CONDITIONS

Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

The Creative Commons Attribution License

The Creative Commons Attribution License (CC-BY) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

Creative Commons Attribution Non-Commercial License

The Creative Commons Attribution Non-Commercial (CC-BY-NC)License permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.(see below)

Creative Commons Attribution-Non-Commercial-NoDerivs License

The Creative Commons Attribution Non-Commercial-NoDerivs License (CC-BY-NC-ND) permits use, distribution and reproduction in any medium, provided the original work is properly cited, is not used for commercial purposes and no modifications or adaptations are made. (see below)

Use by commercial "for-profit" organizations

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee.

Further details can be found on Wiley Online Library
http://olabout.wiley.com/WileyCDA/Section/id-410895.html

Other Terms and Conditions:

Appendix C - Copyright permission from Elsevier, Inc. (for Figure 2.3)

ELSEVIER LICENSE
TERMS AND CONDITIONS

Aug 10, 2021

This Agreement between Ms. Amrutha Elamurugan ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	5125690362759
License date	Aug 10, 2021
Licensed Content Publisher	Elsevier
Licensed Content Publication	Journal of Investigative Dermatology
Licensed Content Title	Research Techniques Made Simple: Cutaneous Colorimetry: A Reliable Technique for Objective Skin Color Measurement
Licensed Content Author	Bao Chau K. Ly,Ethan B. Dyer,Jessica L. Feig,Anna L. Chien,Sandra Del Bino
Licensed Content Date	Jan 1, 2020
Licensed Content Volume	140
Licensed Content Issue	1
Licensed Content Pages	11
Start Page	3
End Page	12.e1
Type of Use	reuse in a thesis/dissertation

Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title	Protein and lipid oral-gastro-small intestinal digestibility in vitro of pasture-raised beef, grain-finished beef, and meat substitute: A comparative study.
Institution name	Massey University
Expected presentation date	Jan 2022
Portions	The CIELAB color space diagram
Requestor Location	Ms. Amrutha Elamurugan 572 Ruahine Street Hokowhitu Palmerston North, 4410 New Zealand Attn: Massey University
Publisher Tax ID	GB 494 6272 12
Total	0.00 USD
Terms and Conditions	

INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at http://myaccount.copyright.com).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier's permissions helpdesk here). No modifications can be made to any Lancet figures/tables and they must be reproduced in full.

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation**: This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article.

16. **Posting licensed content on any Website**: The following terms and conditions apply as follows: Licensing material from an Elsevier journal: All content posted to the web site must maintain the copyright information line on the bottom of each image; A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx or the Elsevier homepage for books at http://www.elsevier.com; Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

Licensing material from an Elsevier book: A hyper-text link must be included to the Elsevier homepage at http://www.elsevier.com . All content posted to the web site must maintain the copyright information line on the bottom of each image.

Posting licensed content on Electronic reserve: In addition to the above the following clauses are applicable: The web site must be password-protected and made available only to bona fide students registered on a relevant course. This permission is granted for 1 year only. You may obtain a new license for future website posting.

17. **For journal authors:** the following clauses are applicable in addition to the above:

Preprints:

A preprint is an author's own write-up of research results and analysis, it has not been peer-reviewed, nor has it had any other value added to it by a publisher (such as formatting, copyright, technical enhancement etc.).

Authors can share their preprints anywhere at any time. Preprints should not be added to or enhanced in any way in order to appear more like, or to substitute for, the final versions of articles however authors can update their preprints on arXiv or RePEc with their Accepted Author Manuscript (see below).

If accepted for publication, we encourage authors to link from the preprint to their formal publication via its DOI. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help users to find, access, cite and use the best available version. Please note that Cell Press, The Lancet and some society-owned have different preprint policies. Information on these policies is available on the journal homepage.

Accepted Author Manuscripts: An accepted author manuscript is the manuscript of an article that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and editor-author communications.

Authors can share their accepted author manuscript:

- immediately
  - via their non-commercial person homepage or blog
  - by updating a preprint in arXiv or RePEc with the accepted manuscript
  - via their research institute or institutional repository for internal institutional uses or as part of an invitation-only research collaboration work-group
  - directly by providing copies to their students or to research collaborators for their personal use
  - for private scholarly sharing as part of an invitation-only work group on commercial sites with which Elsevier has an agreement
- After the embargo period
  - via non-commercial hosting platforms such as their institutional repository
  - via commercial sites with which Elsevier has an agreement

In all cases accepted manuscripts should:

- link to the formal publication via its DOI
- bear a CC-BY-NC-ND license - this is easy to do
- if aggregated with other manuscripts, for example in a repository or other site, be shared in alignment with our hosting policy not be added to or enhanced in any way to appear more like, or to substitute for, the published journal article.

Published journal article (JPA): A published journal article (PJA) is the definitive final record of published research that appears or will appear in the journal and embodies all value-adding publishing activities including peer review co-ordination, copy-editing, formatting, (if relevant) pagination and online enrichment.

Policies for sharing publishing journal articles differ for subscription and gold open access articles:

Subscription Articles: If you are an author, please share a link to your article rather than the full-text. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help your users to find, access, cite, and use the best available version.

Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

If you are affiliated with a library that subscribes to ScienceDirect you have additional private sharing rights for others' research accessed under that agreement. This includes use for classroom teaching and internal training at the institution (including use in course packs and courseware programs), and inclusion of the article for grant funding purposes.

Gold Open Access Articles: May be shared according to the author-selected end-user license and should contain a CrossMark logo, the end user license, and a DOI link to the formal publication on ScienceDirect.

Please refer to Elsevier's posting policy for further information.

18. **For book authors** the following clauses are applicable in addition to the above: Authors are permitted to place a brief summary of their work online only. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version. **Posting to a repository:** Authors are permitted to post a summary of their chapter only in their institution's repository.

19. **Thesis/Dissertation**: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for Proquest/UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission. Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

Elsevier Open Access Terms and Conditions

You can publish open access with Elsevier in hundreds of open access journals or in nearly 2000 established subscription journals that support open access publishing. Permitted third party re-use of these open access articles is defined by the author's choice of Creative Commons user license. See our open access license policy for more information.

Terms & Conditions applicable to all Open Access articles published with Elsevier:

Any reuse of the article must not represent the author as endorsing the adaptation of the article nor should the article be modified in such a way as to damage the author's honour or reputation. If any changes have been made, such changes must be clearly indicated.

The author(s) must be appropriately credited and we ask that you include the end user license and a DOI link to the formal publication on ScienceDirect.

If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source it is the responsibility of the user to ensure their reuse complies with the terms and conditions determined by the rights holder.

Additional Terms & Conditions applicable to each Creative Commons user license:

CC BY: The CC-BY license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article and to make commercial use of the Article (including reuse and/or resale of the Article by commercial entities), provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. The full details of the license are available at http://creativecommons.org/licenses/by/4.0.

CC BY NC SA: The CC BY-NC-SA license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article, provided this is not done for commercial purposes, and that the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the

work. Further, any new works must be made available on the same conditions. The full details of the license are available at http://creativecommons.org/licenses/by-nc-sa/4.0.

CC BY NC ND: The CC BY-NC-ND license allows users to copy and distribute the Article, provided this is not done for commercial purposes and further does not permit distribution of the Article if it is changed or edited in any way, and provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, and that the licensor is not represented as endorsing the use made of the work. The full details of the license are available at http://creativecommons.org/licenses/by-nc-nd/4.0. Any commercial reuse of Open Access articles published with a CC BY NC SA or CC BY NC ND license requires permission from Elsevier and will be subject to a fee.

Commercial reuse includes:

- Associating advertising with the full text of the Article
- Charging fees for document delivery or access
- Article aggregation
- Systematic distribution via e-mail lists or share buttons

Posting or linking by commercial companies for use by customers of those companies.

20. **Other Conditions**:

v1.10

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Appendix D - Copyright permission from John Wiley & Sons, Inc. (for Figure 2.5)

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

Aug 10, 2021

This Agreement between Ms. Amrutha Elamurugan ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	5125700531387
License date	Aug 10, 2021
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Journal of Food Science
Licensed Content Title	Does It Look Cooked? A Review of Factors That Influence Cooked Meat Color
Licensed Content Author	Rosemary Whyte, Nicola J. King (née Turner)
Licensed Content Date	Jun 5, 2006
Licensed Content Volume	71
Licensed Content Issue	4
Licensed Content Pages	10

Type of use	Dissertation/Thesis
Requestor type	University/Academic
Format	Print and electronic
Portion	Figure/table
Number of figures/tables	1
Will you be translating?	No
Title	Protein and lipid oral-gastro-small intestinal digestibility in vitro of pasture-raised beef, grain-finished beef, and meat substitute: A comparative study.
Institution name	Massey University
Expected presentation date	Jan 2022
Portions	Characteristics of the myoglobin pigments in meat, their dynamic relationships, and the denatured products formed during cooking
Requestor Location	Ms. Amrutha Elamurugan 572 Ruahine Street Hokowhitu Palmerston North, 4410 New Zealand Attn: Massey University
Publisher Tax ID	EU826007151
Total	0.00 USD

Terms and Conditions

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a"Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.

- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, **and any CONTENT (PDF or image file) purchased as part of your order,** is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.

- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner.**For STM Signatory Publishers clearing permission under the terms of the STM Permissions Guidelines only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts,** You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone basis, or any of the rights granted to you hereunder to any other person.

- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto

- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.

- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.

- These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement