Nutritional characterization of different cuts in goat kid meat

Nan Jiang

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Approved by

Supervisors:

Dr. Michelle Yoo:

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List of abbreviations

ALA Alanine

AA Amino Acids

AQC 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate

ANOVA Analysis of Variance

ASN Asparagine

ASP Aspartic acid

BW Body weight

CIE Commission Internationale de l'Eclairage

CLA Conjugated linoleic acid

DFA Desirable fatty acids

Dabsyl-Cl 4-dimethylaminoazobenzenesulfonylchloride

EAAs Essential free amino acids

FMOC-Cl 9-fluoroenylmethyl chloroformate

FAO Food and Agriculture Organization

FAAs Free amino acids

GC Gas Chromatography

GI Gastrointestinal

GLU Glutamic acid

GLN Glutamine

GLY Glycine

GTB Green tea by-products

HHP High pressure process

HIS Histidine

HILIC Hydrophilic interaction liquid chromatography

HO-PRO Hydroxyproline

HCl Hydrochloric acid

ILE Isoleucine

LEU Leucine

LC-MS Liquid chromatography–mass spectrometry

LD *Longissimus dorsi*

LYS Lysine

MET Methionine

NZ New Zealand

N/A Not Available

NI Not Indicated

OPA O-phthaldehyde

PHE Phenylalanine

PITC Phenylisothiocyanate

PUFA Polyunsaturated fatty acids

PRO Proline

PDCAAS Protein digestibility-corrected amino acid score

PER Protein efficiency rate

QF *Quadratus Femoris*

RPLC Reversed-phase liquid chromatography

SFA Saturated fatty acids

SM *Semimembranosus*

SER Serine

SGF Simulated gastric fluids

SIF Simulated intestinal fluids

SSF Simulated salivary fluids

SDS–PAGE Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SED Standard error of difference

THR Threonine

TAAs Total free amino acids

TRP Tryptophan

TYR Tyrosine

UFA Unsaturated fatty acids

VAL Valine

WBS Warner–Bratzler Shear force

WHC Water-holding capacity

Attestation of Authorship

I hereby declare that this submission is my own work and that, to be the best of my knowledge and belief, 'Nutritional characterization of different cuts in goat kid meat', contains no material previously published or written by another person (except where explicitly defined in the acknowledgements) nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Name: ……Nan Jiang …….

Signed: …….

Date: ……06/10/2017…….

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Abstract

Compared to the other frequently-consumed red meat like beef and lamb, goat meat occupies a narrow market and is cheap in price due to the historical prejudice towards tough and stringy goat meat. However, nutritionally, goat meat has been proven not to be inferior to beef and lamb, and it is regarded as a lean meat with low fat content. Perceptually, goat kid meat can satisfy consumers' organoleptic requirements of a tender and juicy meat. Hence the under-utilized goat kid meat can make a great contribution to the growing need of meat worldwide. Within goat kid carcasses, the nutritional value differs among various goat cuts. Therefore, the aim of the current study is to characterize the differences of nutritional composition in goat cuts to provide a thorough information for consumers when purchasing goat meat.

The proximate composition in terms of moisture, crude fat, crude protein and ash content of 14 cuts (*longissimus dorsi*, tenderloin, flap, knuckle, rump, outside round, hind shank, inside, cube roll, neck, fore shank, blade roll, cross cut and bolar) from milk-fed Saanen male goat kids (aged 31 days with a living weight of approximate 8.2 kg) were determined using the Official Methods of Analysis (AOAC). The *in vitro* gastrointestinal digestion was simulated to compare the protein digestibility of the 14 cuts. The free amino acids (FAAs) were measured quantitatively by LC-MS in three phases (prior to digestion, at the end of the gastric digestion phase and the intestinal digestion phase). Protein and peptides were determined qualitatively by SDS-PAGE in these three phases as well.

The flap was found to have a significantly low moisture content ($P < 0.001$). The ventral trunk (flap and bolar) was found to have a relatively high fat content ($P < 0.001$). All of the other cuts did not show statistical differences in moisture and fat content ($P > 0.05$), varying around 75% and 2.5% of the raw meat, respectively. The protein content was around 20% for all of the 14 cuts with small variance. But statistically, the *longissimus dorsi* (LD) and the tenderloin possessed a higher protein content than that of the ventral trunk (flap and bolar), knuckle, cube roll, fore shank and blade roll ($P < 0.001$). Ash content was around 1% and did not show significant difference in all of the cuts. After the simulation of *in vitro* gastrointestinal digestion, the fore shank had the highest digestibility in respect of the release of the total free amino acids (TAAs) at the end of intestinal digestion, which was not significantly different with the digestibility of the cross cut, blade roll, tenderloin and knuckle, but higher than that of all of the remaining

cuts ($P < 0.001$). As for SDS-PAGE profile, the proteins and large peptides showed sparser and lighter bands in molecular weights over 14 kDa with the progression of *in vitro* digestion, indicating the degradation and disintegration of meat proteins by digestive enzymes.

The result of the current study indicates the less preferred goat kid cuts like the fore shank and the cross cut do not have an inferior nutritional attributes than those highly preferred cuts like the LD, especially in terms of the protein digestibility. Further investigations on sensory attributes of these cuts could be applied to better understand the differences in cuts so that consumers can make a better choice when purchasing goat kid meat cuts, or even the red meat cuts.

Chapter 1 Introduction

Due to the rapid growth of world population and fast development of industrialization and urbanization, the demand for meat as a good source of animal protein is also rising. According to Food and Agriculture Organization (FAO), the world total meat production has incredibly increased from 84 million (84 M) tonnes in 1965 to 317 million (317 M) tonnes in 2014 as shown in Figure 1 (FAOSTAT, 2014). During those 49 years, poultry which only accounted for 13.1% of the world meat production (11 M out of 84 M) in 1965 increased to 35.6% (113 M out of 317M) in 2014. Goat meat showed a slow increase from 1.19% to 1.74% and pig meat maintained a steady trend around 36%. The proportion of cattle meat declined considerably from 38.1% in 1965 to 20.35% in 2014, and similar tendency was also found in sheep meat, dropping from 5.95% to 2.84% (FAOSTAT, 2014).

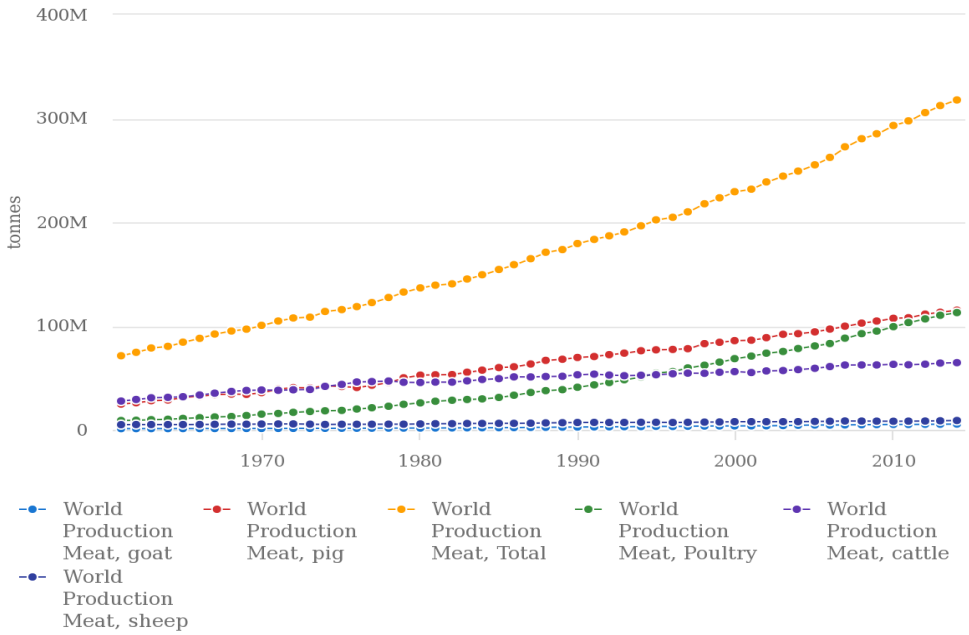

Figure 1. World meat production (FAOSTAT, 2014).

The decreasing proportion of beef and mutton over the last few decades indicates the reduced demand and consumption of these two species, which is mainly caused by their high prices and associated health and food safety concerns such as bovine spongiform encephalopathy from cattle (Cawthorn & Hoffman, 2014). In contrast to cattle and sheep, the unconventional livestock goat, which is also one of human's most enduring sources of high-quality meat and milk, gets exploitation, and is stepping into people's dietary and gradually becoming a crucial livestock species (Sheridan, Hoffman, & Ferreira, 2003). Over the past few decades, the world goat population had a rapid growing of more than 100%, which was much higher than that of the sheep (3%) and the cattle (19%) (FAOSTAT, 2014). The population of goat has reached over 996 million around the world while more than 90% are concentrated in developing countries such as India and the tropical areas of Africa and Asia (Cawthorn & Hoffman, 2014; Webb, 2014).

Goats are bred for meat, fibre, milk and skin (Dubeuf, Morand-Fehr, & Rubino, 2004). Goat fibre mainly contains two products: Cashmere hair which mostly is produced and exported from China, and Angora Mohair of which 50% is produced in South Africa. New Zealand and Australia also provide small quantities of quality fibre. Goat milk production accounts for a small portion of the world milk production, about 2 ~ 2.5%, and the same as goat meat, only occupies less than 2% of the total world meat production (Cawthorn & Hoffman, 2014; Morand-Fehr et al., 2004). India, China, Iran and Nigeria are among the top six countries which contribute to 67% of the world total goat meat production (Dubeuf et al., 2004).

Goats have a great adaptive capacity to different natural conditions, especially to harsh environments because they have a better ability to browse cell wall-rich plant resources like shrubs (Bas, Dahbi, Aich, Morand-Fehr, & Araba, 2005). They are disease-tolerant and have an efficient feed utilization ability, and goat meat is cheap in price compared with beef and mutton. Hence goat meat is widely-accepted by many poor farmers, households and rural communities, and provide a healthy and inexpensive alternative protein source which can partially solve the animal protein shortage in developing countries (Babiker, El Khider, & Shafie, 1990; Cawthorn & Hoffman, 2014). For example, goat meat is more preferred than sheep meat in India and tropics due to the

abovementioned reasons (Madruga, Dantas, Queiroz, Brasil, & Ishihara, 2013; Sen, Santra, & Karim, 2004). In some traditional Mediterranean dishes like curry goat stew and Kleftiko, goat meat accounts as one of the main ingredients (Nediani et al., 2017). The meat of goat kids which are usually less than 3 ~ 7 months old with a carcass weight of 6 ~ 12 kg is particularly very much appreciated in Latin America, most Mediterranean countries (e.g. Spain, Portugal, Italy and Greece) and the western part of India (Boyazoglu & Morand-Fehr, 2001; Madruga & Bressan, 2011).

Western consumers have not favoured goat meat in the past, partly attributed to the social stigma that goat meat is an inferior meat and it is related to poverty and lower-income classes (Cawthorn & Hoffman, 2014). But this perspective is changing. Currently, consumers are aware that excessive consumption of high-fat animals will cause overabundant intake of calories leading to obesity, diabetes, cardiovascular heart disease, stroke and hypertension (Sheridan et al., 2003). Because the lean goat meat with low fat and cholesterol contents is health-beneficial, the economic value and global consumption of goat meat has gradually increased over the past two decades, leading to the double price of goat meat in 2005 than that in 1990 (Gillingham, 2008; Webb, 2014). Although the production and consumption of goat meat is lower than that of the lamb and beef, its rapid increase is inevitable and foreseen along with human's enhanced consciousness and demand about eating healthy meat (Tshabalala, Strydom, Webb, & Kock, 2003).

Based on the studies by Babiker et al. (1990), Niedziółka et al. (2006), Rhee et al. (1999), Sen et al. (2004), Shija et al. (2013) and Turner et al. (2014), it can be concluded that the biological and nutritional value of goat meat is not inferior to lamb or beef. But due to a series of inherent factors like high tissue fibre, low fat content and high muscle collagen (the major protein of connective tissue) with low solubility, the organoleptic quality of adult goat meat is not as good as lamb and beef, especially in terms of tenderness, resulting in the prejudice towards goat meat (Tshabalala et al., 2003). However, this problem can be alleviated by castrating goats or using goat kid meat as fresh resale meat. Goat kids have a pale pink colour, pleasant milky odour and desirable tenderness, and they also hold a high nutrition and readily digested ability (Longobardi et al., 2012). The old goat meat can be used for processing because those unfavourable properties can be overcome during food processing by interacting with

other ingredients and are hardly reflected in the final products (Rhee et al., 1999; Webb, Casey, & Simela, 2005).

According to Statistics New Zealand's agricultural production, by 30 June 2012, the estimated sheep and goat quantities in New Zealand (NZ) were 31.3 million and 90 thousands, respectively (Statistics NZ, 2017). Hogg et al. (1992) reported that New Zealand used to focus on goat fibre production rather than meat production, while Mohammed (2011) found that in the past few years, chevon exportation from New Zealand and Australia to USA increased a lot due to the growing requirement of goat meat in USA. The total meat production in NZ increased from 807,147 tonnes in 1965 to 1,375,408 tonnes in 2014, and the goat meat production also presents an obvious growth from 600 tonnes (1965) to 1299 tonnes (2014) as Figure 2 shows (FAOSTAT, 2014). In 2014, sheep and goat contributed to about 35.51% of the total animal meat production in NZ while goat only provided 0.27% meat production (FAOSTAT, 2014), thus there still has a large development space for goat meat in NZ market.

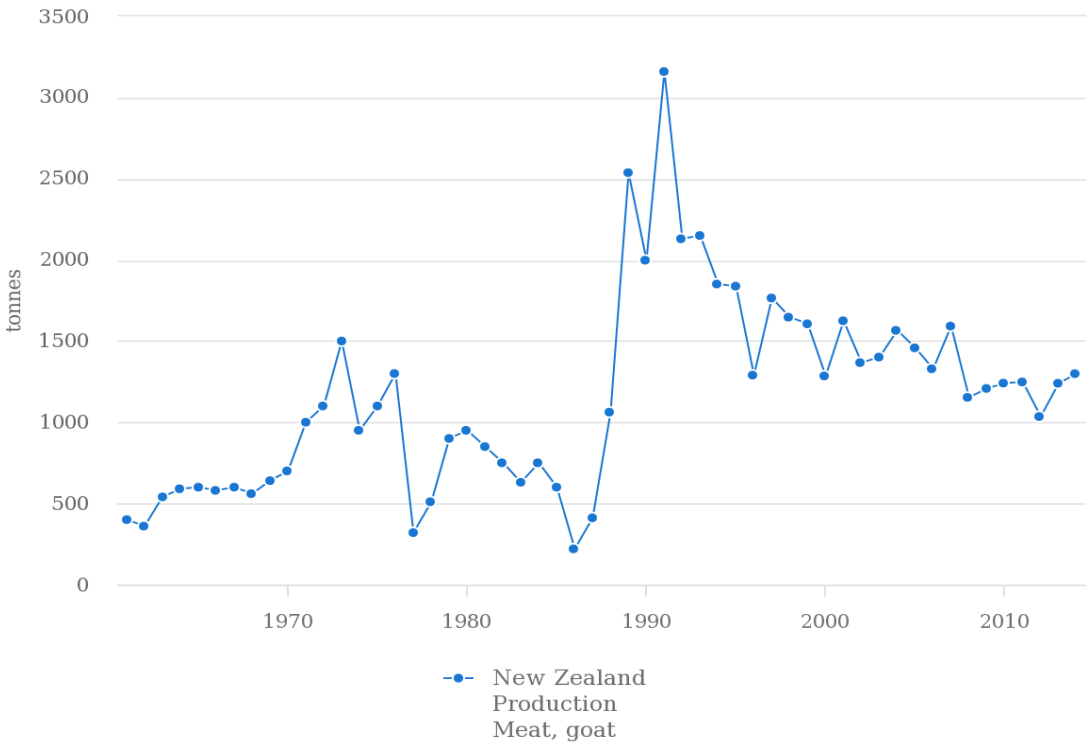

Figure 2. New Zealand goat meat production (FAOSTAT, 2014).

Compared to cattle and sheep, knowledge of goat meat quality, especially kid meat, is extremely limited. Some literatures have compared the different carcass traits and

composition of sheep and goat meat (Babiker et al., 1990; Niedziółka & Pieniak-Lendzion, 2006; Rhee et al., 1999; Sen et al., 2004; Sheridan et al., 2003; Shija et al., 2013; Turner et al., 2014). However, only a few studies have compared the differences among goat kid cuts to date (Hogg et al., 1992; Sheridan et al., 2003; Tshabalala et al., 2003).

Nowadays, the primal resale meat cuts which can be found in market are neck, shoulder, fore shank, loin, breast, rump and leg. Different countries have different preferences among various cuts. Asian and African countries usually prefer breast cuts while in Western countries, hind limb and loin cuts are more popular (Webb, 2014). To my knowledge, there is not a specific and comprehensive study carried out to compare the distribution of nutrients among cuts. In consideration of the fact that the potential of goat kid meat is not fully exploited and the possibility that it could make a great contribution to the growing need for meat worldwide, the aim of the current study was to analyze the nutritional profiles, particularly the protein digestibility of different cuts. Protein is known to provide humans energy and essential amino acids. A high protein digestibility represents a high protein utilization efficiency and more energy is provided. The Deficiency intake of protein can cause mental degradation, stunted growth and more seriously losing skeletal muscle mass and strength (sarcopenia) (Veronique Santé-Lhoutellier, Astruc, Marinova, Greve, & Gatellier, 2008). In the current study, the proximate composition of nutrients in different goat kid cuts was determined. The protein digestibility among the goat cuts was compared after *in vitro* simulated gastrointestinal digestion, in terms of the release of free amino acids and peptides. Such information on the proximate content and digestibility in goat kid cuts would help consumers make an informed decision when purchasing meat cut. More details can be found in section 2.9.

Chapter 2 Literature review

2.1 Overview of meat from animals

In living animals, skeletal muscle, which consists of muscle tissue, nerve fibers, blood vessels and connective tissue coverings, is structured by connective tissues. Epimysium, the toughest connective tissue, bundles muscle fascicles together and covers the whole skeletal muscle. Muscle fascicle that comprises a group of muscle fibers is surrounded by perimysium. Muscle fiber, which is also known as muscle cell, is wrapped by a thin connective tissue covering called the endomysium (Brantley, 2013). Muscle fiber consists of organelles like nuclei and mitochondria, sarcolemma namely cell membrane, sarcoplasm namely cytoplasm, and myofibrils which are composed of thin filaments (actin) and thick filaments (myosin) (Brantley, 2013; Pearce, Rosenvold, Andersen, & Hopkins, 2011). Apart from these two main proteins, there are also other regulatory proteins surrounded such as tropomyosin and troponin (Pearce et al., 2011).

After animal is slaughtered, oxygen supply ceases. To resynthesize ATP, glycogen starts anaerobic respiration during which pH drops. When ATP is almost fully depleted, muscle starts to shorten and myosin head will bind to actin to form the actomyosin complex leading to inextensible muscle (Pearce et al., 2011). This process converts muscle to meat, which is also known as rigor mortis. But nowadays, meat is often considered unambiguously synonymous with muscle (Robert, 2012).

Officially, meat is defined as meat flesh which includes skeletal muscle and any attached fat or connective tissue (Williams, 2007). Meat consists of water, protein, fat, vitamins, minerals and a small amount of carbohydrates. Meat coming from goat, sheep or cattle is referred to as red meat, also known as ruminant meat, which is an ideal dietary source of some health-benefiting nutrients like conjugated linoleic acid isomers, omega-3 polyunsaturated fat, long chain polyunsaturated fatty acids, protein, vitamin B6 and B12, niacin, minerals like zinc, selenium, phosphorous and iron and bioactive molecules such as creatine, taurine and carnitine (Ebrahimi, Rajion, & Goh, 2014; Pereira & Vicente, 2013; Williams, 2007).

As shown in Figure 3, meat quality is normally evaluated by researchers from three aspects: meat physical attributes such as colour, water-holding capacity (WHC), shear force and pH; meat nutritive values and meat organoleptic properties. The nutritive value of meat can be evaluated either by the physical dissected tissues i.e. separated lean meat, bones and fat (mainly subcutaneous and intermuscular fat), or chemically analysed constituents such as moisture, protein, fat, ash, minerals, cholesterol, vitamins, microelements and the bioavailability of these nutrients like digestibility and utilization (Tomović et al., 2016). The approximate determination of four dominating analytes in meat (moisture, protein, fat and ash) is referred to as proximate analysis, the most basic and common method to assess the proximate nutrition in meat (Karakok, Ozogul, Saler, & Ozogul, 2010). In terms of the most important two analytes: protein and fat, further analyses such as the composition of amino acids and fatty acids can also be determined to evaluate meat nutritive values. Sensory evaluation of cooked meat mainly refers to colour, tenderness, juiciness, flavour, odour and overall palatability, in which tenderness and flavour are the most important meat quality attributes that consumer will consider and evaluate (Madruga et al., 2013; Ouali et al., 2006).

Internally, these meat attributes are mutually interactive to each other rather than being independent, and externally, they are affected by factors including but not limited to species, age, diet, breed, gender, cuts and so on (Brzostowski, Niżnikowski, & Tański, 2008). In the following sections, the physical attributes of goat meat (section 2.2), the nutritive values of goat meat (section 2.3) in terms of proximate composition (section 2.3.1), fatty acids (section 2.3.2) and amino acids (section 2.3.3), and sensory attributes of goat meat (section 2.4) will be discussed in detail. The influences of species, age and diet are discussed along with each attribute, and the effects of breed and gender as well as cuts are briefly summarized in section 2.5 and 2.6.

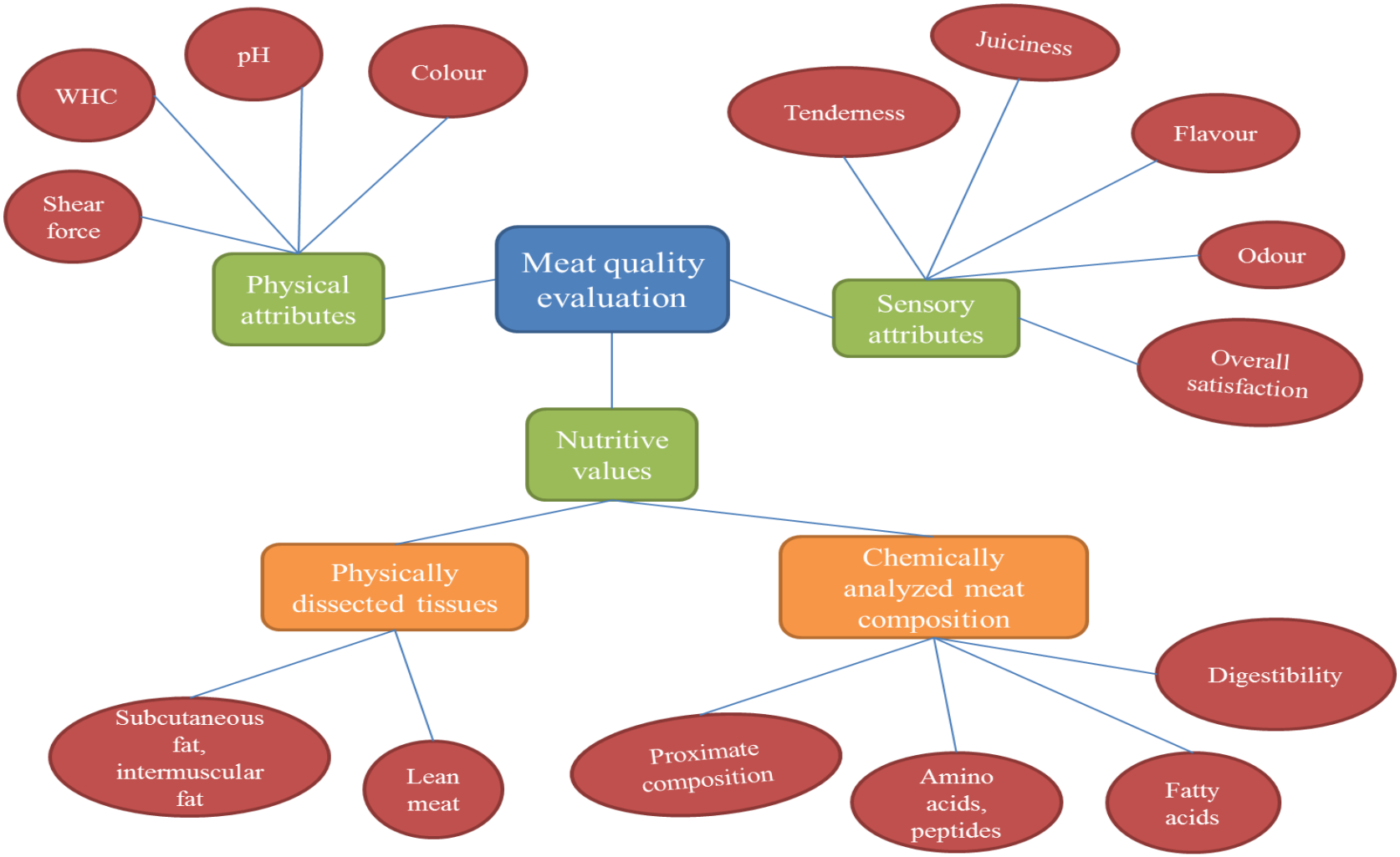

Figure 3. Meat quality evaluation.

2.2 Physical attributes

2.2.1 Water-holding capacity

Water content accounts for about 70 ~ 77% of raw meat (Young, Frost, & Agnew, 2012), and plays an important role in affecting other meat properties such as tenderness and shelf life. Thus many processed meat products will lower moisture content to inhibit the growth of microorganisms and improve the preservation life (Milica, Snezana, & Zorica, 2015; Young et al., 2012).

Water-holding capacity (WHC), just as its name implies, describes the ability of post-mortem muscle (meat) to remain inherent water under some exterior pressures like gravity or heat (Huff-Lonergan & Sosnicki, 2002). WHC can be evaluated from drip loss or cooking loss. Drip loss normally means a passive exudation process of water lost from fresh meat (Huff-Lonergan & Sosnicki, 2002). Cooking loss contains the expelled fluid during cooking and the volatile loss which includes the evaporation of water and other volatile molecules (Meischke, Van Laack, & Smulders, 1997). Generally, the higher the WHC is, the lower the dripping loss or cooking loss.

As shown in Table 1, most authors found there was no significant difference in WHC content, as well as cooking loss, between comparable goat and sheep meat (Lee, Kannan, Eega, Kouakou, & Getz, 2008; Sen et al., 2004; Shija et al., 2013). However, Babiker et al. (1990) found that *Semimembranosus* muscle of desert goat had a significantly higher WHC and lower cooking loss (34.2%) compared to lamb (36.6%). Diet does not have a big influence on WHC, but this ability decreases with age. Dhanda et al. (1999) found young capretto had a lower cooking loss ($P < 0.05$) than the mature chevon.

2.2.2 Colour

When consumers purchase meat, colour is the first evaluative criterion which will be taken into account (Ouali et al., 2006). The colour change of meat is mainly caused by

the oxygenation of water-soluble heme protein pigment myoglobin. When meat is exposed to oxygen, the original purplish-red colour will turn into appealing bright red because myoglobin is oxygenated to oxymyoglobin (Mancini & Hunt, 2005). Normally, metmyoglobin which gives meat a brown colour can be found in the subsurface area between the superficial oxymyoglobin and interior myoglobin where only low concentration oxygen exists (around 0.5%). During this discoloration process, myoglobin and oxymyoglobin will be oxidized, and ferrous ion (Fe^{2+}) will be converted into ferric ion (Fe^{3+}). When the brown metmyoglobin reaches meat surface, the meat is considered as spoilage with a bad quality (Ouali et al., 2006). Thus to reduce the influence of colour degradation attributed to oxygen and light, colour should be determined immediately after cutting samples (Nediani et al., 2017).

The International Commission on Illumination, also known as CIE (Commission Internationale de l'Eclairage), recommends one uniform colour scale: CIELAB (L*a*b*), where L* represents lightness, a* (redness) expresses the red/green value and b* (yellowness) denotes yellow/blue value. This CIELAB system is frequently-used when performing instrumental color analysis nowadays (Nediani et al., 2017). Compared to sheep meat, goat meat always has a darker brown colour (higher a* and lower L*) due to the lower intramuscular fat content. For example, Babiker et al. (1990) found goat meat had a significantly higher a* (13.1) value than lamb raised under same conditions (11.96), and lower b* (4.9 versus 5.7) and L* (34.8 versus 36.2) values as seen in Table 1. But Lee et al. (2008) reported a different result with no significance in L* and a lower a* value in goat meat compared to lamb.

Diet can influence meat colour in a certain extent. Basically, a low energy diet will give meat a lower lightness value, namely, a darker colour than a high energy diet (Priolo, Micol, Agabriel, Prache, & Dransfield, 2002). As Table 1 shows, Priolo et al. (2002) found lambs finished on low energy pasture had a darker colour compared to concentrate finished lambs. However, this is not always the case, Kouakou et al. (2008) reported a lower L* and b* values of loin muscle from goats fed with high energy concentrated diet ($P < 0.05$) than those fed with hay diet.

Goat kid meat is paler than old meat because its muscle pigment concentration is 2 ~ 3 times lower than that of adult goats (Bañón, Vila, Price, Ferrandini, & Garrido, 2006). For example, Dhanda et al. (1999) found the concentration of total pigment in

longissimus muscle in Feral capretto was 1.9 mg/g, which was significantly lower compared to 3.7 mg/g in Feral chevon. Besides, with age, b* value will increase because the colour of fat will become more yellow.

2.2.3 Shear force

Literally, shear force means the force required to shear muscle fibres (Shija et al., 2013). Shear force can be measured through the widely-accepted "Warner-Bratzler shear force test" where the kilograms of force to shear 1 cubic centimeter muscle sample are recorded, or through a sensory panel test to access the tenderness of meat (Savell et al., 1994). There is a close correlation between Warner-Bratzler shear method and sensory panel scores (Shija et al., 2013). Usually the higher shear force value, the tougher meat is and the lower tenderness and liking scores customers will give. For Australian and New Zealand consumers, the acceptable limit of lamb tenderness is < 5 kgf (namely < 3 kg Warner–Bratzler shear force). If tenderness is over 11 kgf (6 kg WBS), lamb is rated as unacceptably tough (Webb et al., 2005).

As revealed in Table 1, except one finding from Babiker et al. (1990) showing goat and lamb have similar value of shear force, most studies found there is a significantly higher shear force value in goat meat compared to mutton (Lee et al., 2008; Sen et al., 2004; Shija et al., 2013). For example, Shija et al. (2013) reported a higher shear force value (34.07 N) in goat meat ($P < 0.05$) than that of sheep meat (29.83 N). The high shear force value is mainly because goat meat has relatively thicker myofibrils, larger fibre bundles as well as more fibrous tissue residues compared to sheep meat, resulting in a coarser and less tender goat meat. Young goat kids are always reported to have a lower shear force value compared to the older ones (Dhanda et al., 1999; Schönfeldt, Naude, et al., 1993), which indicates goat kid meat is more tender and desirable.

2.2.4 pH

The ultimate pH (pHu) plays an important role in meat quality control. Normally, after slaughter, during the first 24h post-mortem period, the meat pH will fall from 7.2 to around 5.8 (Brewer, Zhu, Bidner, Meisinger, & McKeith, 2001). The decline of pH is due to the anaerobic glycolysis converting glycogen to lactate, which will release and

accumulate hydrogen ions (H^+) during this ATP re-synthesis process (Laack, 2000; Meischke et al., 1997; Vetharaniam & Daly, 2000). pHu is influenced by a lot of factors such as species, the concentration of muscle glycogen, lactate, ATP and creatine phosphate at slaughter, the reaction to pre-slaughter stress and so forth (Shija et al., 2013; Vetharaniam & Daly, 2000). Stronger firmness, darker colour, lower cooking loss and higher water-holding capacity are always correlated with a higher pHu (Laack, 2000). Higher tenderness value and lower shear forces value are usually related to a lower pHu (Webb, 2014).

As indicated in Table 1, the pHu of goat carcass normally varies between 5.8 and 6.2, and expresses a higher value than sheep (Shija et al., 2013; Webb, 2014). For example, Shija et al. (2013) found the decline of pH recorded for goat was slower than that of sheep, and a higher pHu at 24 h post-mortem was observed in goat than in sheep (5.88 vs 5.74). While Sen et al. (2004) reported a similar pHu between goat and sheep meat. Age and diet have a small influence on pH as Table 1 exhibits.

Table 1. Comparison of meat physical attributes.

Classification	Sample	Cut	Meat physical attributes							References
			Colour			Water-holding capacity	Shear force	Cooking loss	pH	
			L	a	b					
Species	Tanzania sheep and goats: n=17 Live BW: 22.59±0.50 kg Age: 1.5-2 yrs	LD	N/A			N/A	Goat (34.07 N) > sheep (29.83 N)	Goat (18.79%) ns sheep (20.31%)	Goat (5.88) > sheep (5.74)	(Shija et al., 2013)
	Lambs: n=16; 38.9 kg; ♂ Boar × Spanish goats: n=16; 31.5 kg; ♂	LD	Goat (36.18) ns lamb (36.65)	Goat (12.21) < lamb (14.24)	Goat (10.38) < lamb (11.25)	N/A	Goat (2.12 kg) > lamb (1.29 kg)	Goat (16.95%) ns lamb (16.69%)	N/A	(Lee et al., 2008)
	Yearling sheep and goats: n=12 Age: 1 yr Gender: ♀	LD	N/A			Goat (57.03%) ns sheep (59.50%)	Goat (7.42 kg/cm2) > sheep (3.74 kg/cm2)	Goat (22.67%) ns sheep (20.74%)	Goat (5.48) ns sheep (5.46)	(Sen et al., 2004)
	Angora and Boer goats and sheep: n=27	LD	N/A			N/A	Boer (62.94 N) > Angora (45.76 N) > sheep (32.05 N)	N/A	N/A	(Schönfeldt et al., 1993)
	Desert lambs and goats: n=10 Live BW: 35 kg	SM	Goat (34.8) < lamb (36.2)	Goat (13.1) > lamb (11.96)	Goat (4.9) < lamb (5.7)	Goat (2.84) > lamb (2.14)	Goat (4 kg/cm2) ns lamb (3.6 kg/cm2)	Goat (34.2%) < lamb (36.6%)	N/A	(Babiker et al., 1990)

Diet	Crossbred Boer × Indigenous goats: n=40 Live BW: 15.6 kg Age: 5 months Gender: ♂ Diet: Soybean meal substituted with peanut cake at 0%, 34%, 67% and 100% level (presented as S0, S34, S67 and S100)	LD	S0 (33.6) ns S34 (33.1) ns S67 (32.9) ns S100 (33.2)	S0 (11.5) ns S34 (11.8) ns S67 (12.1) ns S100 (11.5)	S0 (6.99) ns S34 (7.08) ns S67 (6.88) ns S100 (7.01)	N/A	S0 (21.48 N/cm2) ns S34 (26.87 N/cm2) ns S67 (24.30 N/cm2) ns S100 (25.60 N/cm2)	S0 (21%) ns S34 (19.8%) ns S67 (21.7%) ns S100 (24.4%)	N/A	(Silva et al., 2016)
	Boer × Spanish goats: n=36 Live BW: 18 kg Age: 4 months Gender: ♂ Diet: Hay diet (H); 18% CP concenrtrate diet (C)	LD	H (43.57) > C (39.81)	H (9.34) ns C (9.91)	H (12.45) > C (11.09)	N/A	H (3.79 kg) ns C (3.73 kg)	H (22.66%) ns C (28.83%)	N/A	(Kouakou et al., 2008)
	Ile-de-France lambs: n=32 Live BW: 15.3 kg Age: 37 days Gender: ♂ Diet: Pasture (P); concentrate-based diet (C)	LD	P (46.1) < C (49.23)	P (7.60) ns C (7.35)	P (9.79) ns C (10.71)	N/A	N/A	N/A	P (5.62) ns C (5.57)	(Priolo et al., 2002)
Age	Feral capretto and chevon: n=25	LD	Capretto (41.1) > chevon (37.1)	Capretto (11.5) < chevon (14.4)	Capretto (4.1) ns chevon (2.0)	N/A	Capretto (3.6 kg/cm2) < chevon (4.3 kg/cm2)	Capretto (39%) < chevon (44.6%)	Capretto (5.7) ns chevon (5.7)	(Dhanda et al. 1999)

	Angora and Boer goat kids: n=27 A age group: with no permanent incisors; B age group: 1-6 permanent incisors; C age group: 7-8 permanent incisors.	LD	N/A	N/A	A (45.86 N) < B (46.61 N) < C (47.65 N)	N/A	N/A	(Schönfeldt et al., 1993)
	Criollo goats: n=10 Gender: Castrated ♂ Live BW: 12, 16, 20 and 24 kg	LD	N/A	N/A	12 BW (7.86 kg/cm2) ns 16 BW (6.31 kg/cm2) ns 20 BW (6.59 kg/cm2) ns 24 BW (7.79 kg/cm2)	N/A	12 BW (6.09) ns 16 BW (5.98) ns 20 BW (6.11) ns 24 BW (5.77)	(Nuñez Gonzalez et al., 1983)
Breed	French Alpine (FA) goat kids and French Alpine × Boer crossbreds (FAB) goat kids: n=12 Age: 50 days Gender: ♂	QF	N/A	FA (7.02 cm2) < FAB (8.09 cm2)	N/A	N/A	FA (5.78) > FAB (5.70)	(Brzostowski et al., 2008)

n = No. of observations BW: Body Weight SM: M. *semimembranosus* LD: M. *longissimus dorsi* QF: M. *quadratus femoris* N/A: Not Available

>: Significantly higher <: Significantly lower ns: Not significant (P > 0.05).

2.3 Nutritive values

2.3.1 Proximate composition

Due to the traditionally lower economic contribution of goats compared to other domesticated ruminants like cattle and sheep, the knowledge of goat carcass yield and meat quality is limited and not many of scientific studies have been devoted on goat meat (Tshabalala et al., 2003). However, based on the present publications (Dhanda et al., 1999; Ivanovic, Nesic, Pisinov, & Pavlovic, 2016; Johnson, Eastridge, Neubauer, & McGowan, 1995; Turner et al., 2014), it can be concluded that, in terms of proximate composition as shown in Table 2, goat carcass is comparable and not inferior to other red meat species under similar ages.

Fat deposits in different parts of animal body, which can be divided into visceral fat (surrounding the organs) and carcass fats including subcutaneous fat (under the skin), intermuscular fat (between muscles) and intramuscular fat (marbling). A trimmed lean meat usually means the removal of external fat that surrounds the muscle, namely the subcutaneous fat and intermuscular fat (Williams, 2007). The fat content measured in the proximate analysis of the fresh muscle, if not specified, defaults to the intramuscular fat.

As shown in Table 2, except Sheridan et al. (2003) found a higher fat content and a lower protein content in goat meat than lamb, almost all of the other scientists discovered goat meat had a higher moisture proportion, a lower fat percentage and no significant difference of protein present in comparable goat and sheep meat. For example, Shija et al. (2013) found a significantly higher moisture content (76.5%), a lower intramuscular fat content (2.49%) in goat meat than that from sheep (66.96% and 5.82% respectively), slaughtered at the similar age (1.5 ~ 2 yrs) with an average living body weight (BW) of 22.59 ± 0.50 kg, whereas there was no significant difference in protein and ash contents between the two species. Similar trend had also been reported by Babiker et al. in 1990. Niedziółka and Pieniak-Lendzion (2006) found goat kid meat possesses a larger total protein content in comparison to lamb meat. Elgasim and Alkanhal (1992) compared the chemical composition of four red meat species (camel,

steer, lamb and goat). They did not apply statistical analysis so significance cannot be found here. But the results showed goat meat had a numerically higher moisture value (74.5%) than that of beef (73.4%) and lamb (72.2%), a lower fat content (3.3%) than that of lamb (6.2%) and beef (4.7%), and a slightly lower protein content (19.8%) than that of beef (20.4%) and lamb (20.1%). In 1999, a lower fat content (2.48%) was confirmed in ground lean goat meat than that of beef (8.74%) and lamb (7.56%) by Rhee, et al. (1999).

The reason of a lean goat meat may be during the feeding period, goat synthesizes more water and slightly more protein while sheep deposits more intramuscular fat, therefore goat meat is healthier and more advantageous to people with restricted or deficient protein intake (Sheridan et al., 2003). Ash content which occupies a small and similar proportion in both species varies among different lab works, but the value is around 1% of fresh meat basis.

The diet composition and supplemental feedstuffs can influence the livestock carcass traits. An appropriate supplementation can be given to livestock when farming to increase the meat economic value. Either overnutrition or malnutrition cannot achieve a desirable carcass composition. For example, Titi et al. (2000) found the goat kids fed with 16% crude protein ration had significantly higher body weaning weight and growth rate compared to those fed with 12, 14 or 18% ration, while those values of goat kids were proportionally lower than that of lamb fed under same conditions. Atti et al. (2004) reported that the *longissimus dorsi* muscle of goat kids fed with medium protein level (130 g/kg of dry matter) had significantly less fat, more protein and moisture than the kids given lower (100 g/kg of dry matter) or higher (160 g/kg of dry matter) protein level. Ahmed et al. (2015) studied castrated male goats fed with 0%, 0.5%, 1.0% and 2.0% green tea by-products (GTB) which provides a valuable crude protein source around 22 ~ 35%. They found the protein content of goat meat from *gluteus medius* increased from 20.7% to 21.4% by lifting the level of GTB from 0% to 2.0%, while the crude fat and cholesterol content decreased from 1.02% to 0.92% and from 59.1mg/100 g to 55.9mg/100 g of meat, respectively. The meat protein content supplemented with 1.0% GTB was 22.8%, higher than that fed with 0.5% GTB (22.0%) and 2% GTB (21.4%).

Younger animals have a higher protein synthesis and degradation rate compared to the older animals, but their protein level stays relatively constant with aging, and their moisture and fat contents display an inverse relationship. As indicated in Table 2, younger animals, either steers or goats, present a higher moisture level and a lower fat level than that of the older animals (Nuñez Gonzalez, Owen, & Arias Cereceres, 1983; Watanabe, Ueda, & Higuchi, 2004).

Table 2. Comparison of meat proximate composition.

Classification	Sample	Cut	Proximate composition (% of fresh muscle weight)				References	Note
			Moisture	Fat	Protein	Ash		
Species	Katahdin lambs, Boer × Kiko goats: n=24 Age: 1 yr Gender: Castrated ♂	LD	N/A	Goat (1.8) < lamb (3.9)	Goat (21.5) ns lamb (21.0)	Goat (4.7) > lamb (4.3)	(Turner et al., 2014)	N/A
	Tanzania sheep and goats: n=17 Live BW: 22.59±0.50 kg Age: 1.5-2 yrs	LD	Goat (76.5) > sheep (66.96)	Goat (2.49) < sheep (5.82)	Goat (23.45) ns sheep (22.49)	Goat (4.4) ns sheep (3.9)	(Shija et al., 2013)	N/A
	Lambs: n=16; 38.9 kg; ♂ Boar × Spanish goats: n=16; 31.5 kg; ♂	LD	Goat (68.32) ns lamb (68.96)	Goat (4.97) ns lamb (4.56)	Goat (23.41) ns lamb (23.39)	Goat (1.73) > lamb (1.17)	(Lee et al., 2008)	N/A
	Yearling sheep and goats: n=12 Age: 1 yr Gender: ♀	LD	Goat (74.23) > sheep (68.85)	Goat (3.16) < sheep (8.47)	Goat (20.38) ns sheep (21.02)	N/A	(Sen et al., 2004)	N/A
	South African Merino lambs: n=32; 32.72 kg; castrated ♂ Boar goats: n=32; 26.23 kg; castrated ♂	8-9-10-rib cut	Goat (65.14) > lambs (54.70)	Goat (13.47) < lamb (24.47)	Goat (17.68) > lambs (15.26)	Goat (3.39) ns lamb (2.99)	(Sheridan et al., 2003)	Meat and fat were minced together.

	Colesberg Boer goats and Damara sheep: n=12 Gender: Castrated ♂	NI	Goat (69.4) > sheep (60.15)	Goat (10.45) < sheep (20.37)	Goat (22.76) ns sheep (22.49)	Goat (0.95) > sheep (0.83)	(Tshabalala et al., 2003)	Muscle and subcutaneous fat were minced together.
	Angora and Boer goats and sheep: n=27	LD	Goat (65.37) > sheep (64.74)	Goat (4.71) < sheep (7.00)	Goat (27.24) ns sheep (26.64)	Goat (0.99) ns sheep (1.07)	(Schönfeldt et al., 1993)	N/A
	Camel: n=6; 248 kg; 2 yrs Steer: n=5; 162 kg; 7 months Lamb: n=5; 41 kg; 6 months Goat: n=3; 25 kg; 5 months	Leg and loin	Camel (77.2) > goat (74.5) > beef (73.4) > lamb (72.2)	Camel (2.6) < goat (3.3) < beef (4.7) < lamb (6.2)	Beef (20.4) > lamb (20.1) > goat (19.8) > camel (19.3)	Camel (0.9) < goat (1.4) < beef (1.5) = lamb (1.5)	(Elgasim & Alkanhal, 1992)	Average data of leg and loin was taken.
	Desert lambs and goats: n=10 Live BW: 35 kg	SM	Goat (75.04) > lamb (74.12)	Goat (2.8) < lamb (3.5)	Goat (20.8) ns lamb (21.2)	Goat (1.23) ns lamb (1.24)	(Babiker et al., 1990)	N/A
Diet	Goats: n=48, Live BW: 30.25 kg Age: 7 months Gender: castrated ♂ Diet: Basal diet supplemented with 0%, 0.5%, 1.0% and 2.0% green tea by-products (GTB)	*gluteus medius*	0% GTB (76.2) ns 0.5% GTB (75.7) ns 1.0% GTB (76.3) ns 2.0% GTB (75.6)	0.5% GTB (0.54) < 1.0% GTB (0.71) < 2.0% GTB (0.92) < 0% GTB (1.02)	0% GTB (20.7) < 2.0% GTB (21.4) < 0.5% GTB (22.0) < 1.0% GTB (22.8)	0% GTB (1.07) ns 0.5% GTB (0.98) ns 1.0% GTB (1.03) ns 2.0% GTB (1.02)	Ahmed et al. (2015)	N/A

	Boer × Spanish goats: n=36 Live BW: 18 kg Age: 4 months Gender: ♂ Diet: Hay diet (H); 18% CP concenrtrate diet (C)	LD	H (77.09) > C (74.70)	H (1.32) < C (2.67)	H (20.78) ns C (21.30)	H (1.32) ns C (1.38)	(Kouakou et al., 2008)	N/A
	Tunisia goat kids: n=15, Live BW: 23.3 kg Age: 5 months Gender: castrated ♂ Diet: Concentrated diet containing low (L), medium (M) and high (H) crude protein levels (100, 130, 160 g/kg of dry matter)	LD	M (74.3) > L (73.0) > H (72.1)	M (7.6) < L (11.2) ns H (16.0)	M (87.8) ns L (85.2) > H (80.3)	M (4.6) ns L (3.6) ns H (3.7)	Titi et al. (2000)	Fat, protein and ash were based on % of dry matter.
Age	Steers: n=25 Age: 15, 25 and 35 months old (recorded as 15M, 25M and 35M) Gender: ♂	LD	15M (74.07%) > 25M (70.31%) > 35M (64.98%)	35M (11.28%) > 25M (6.9%) > 15M (3.12%)	15M (19.23%) ns 25M (18.80%) ns 35M (18.49%)	N/A	Watanabe rt al. (2004).	N/A
	Criollo goats: n=10 Gender: Castrated ♂ Live BW: 12, 16, 20 and 24 kg	Dissected carcass lean	12 BW (78.66) > 16 BW (77.93) > 20 BW (76.86) > 24 BW (76.38)	24 BW (16.12) > 16 BW (11.88) ns 20 BW (11.52) > 12 BW (7.9)	20 BW (23.98) > 16 BW (22.25) ns 12 BW (22.21) > 24 BW (19.84)	20 BW (1.03) ns 12 BW (1.00) ns 16 BW (0.97) ns 24 BW (0.97)	(Nuñez Gonzalez et al., 1983)	N/A

Gender	Saanen × Angora goats: n=36 18 ♀: 15.1 kg 18 castrated ♂: 16.5 kg	Loin	♂ (71.43) ns ♀ (70.32)	♂ (6.79) < ♀ (8.01)	♂ (20.71) ns ♀ (20.68)	♂ (0.98) ns ♀ (0.96)	(Hogg et al., 1992)	N/A
Breed	French Alpine (FA) goat kids and French Alpine × Boer crossbreds (FAB) goat kids: n=12 Age: 50 days Gender: ♂	QF	FA (77.79) ns FAB (76.79)	FA (1.67) < FAB (1.96)	FA (19.44) ns FAB (19.74)	FA (1.10) ns FAB (1.13)	(Brzostowski et al., 2008)	N/A
	Japanese Wagyu (B), Japanese Shorthorn (S), Holsteins (D) steers: n=22 Gender: ♂	LD	D (72.19%) ns S (71.04%) > B (64.35%)	B (11.86%) > S (5.81%) ns D (5.63%)	S (19.46%) ns D (18.54%) ns B (17.98%)	N/A	Watanabe rt al. (2004).	N/A

n = No. of observations BW: Body Weight SM: *M. semimembranosus* LD: *M. longissimus dorsi* QF: M. *quadratus femoris* N/A: Not Available NI: Not Indicated

>: Significantly higher <: Significantly lower ns: Not significant (P > 0.05)

2.3.2 Fatty acids profile

2.3.2.1 Fat and fatty acids

Fat content which is a matter of concern in terms of consuming meat is affected by a host of factors such as origin, breed, diet, sex, age, cuts, physiological state, environmental condition and genetic ability (Pereira & Vicente, 2013; Webb et al., 2005). Compared with sheep, goat meat is leaner due to its more visceral deposits rather than carcass deposits, especially less subcutaneous and intramuscular fat depots (Babiker et al., 1990; Madruga & Bressan, 2011; Webb et al., 2005). Within goat carcass, more intermuscular fat is developed than subcutaneous fat (Casey, 2000). Besides that, the deposition rate of subcutaneous fat is also slower (Turner et al., 2014). This leads to a less fat covering in goat carcass compared to the contemporary sheep carcass at same age and sex. Therefore, when post-mortem chilling is undertaken rapidly under the same conditions, goat meat becomes tougher than mutton because cold shortening causes muscle to contract more quickly and irreversibly with a little exterior fat cover. The electrical stimulation, however, can ease this cold shortening phenomenon to some extent (Webb, 2014). A less fat coverage can also lead to a rapid temperature decline in post-mortem carcass due to a quick dissipation of heat so Shija et al. (2013) found that a lower temperature presented in goat carcass compared to sheep carcass after 12 hours and 24 hours post-mortem.

Desirable fatty acids (DFA) are stearic acid C18:0 and all the polyunsaturated fatty acids (PUFA), which have beneficial implications such as decreasing plasma cholesterol amount or reducing the risk of cardiovascular diseases (Brzostowski et al., 2008; Karakok et al., 2010). The ratio between PUFA and saturated fatty acids (SFA), namely PUFA/SFA, which differs in different species resulting in various fat quality (Niedziółka & Pieniak-Lendzion, 2006), ought to be high in meat, ideally 0.45 (Webb et al., 2005). Goat meat owns a higher PUFA/SFA ratio (with a median of 0.32) than mutton (0.19) and beef (0.25) (Banskalieva, Sahlu, & Goetsch, 2000; Webb et al., 2005). Within PUFA, omega-3 fatty acids whose precursors are linolenic (C18:3n−3) acids should have a high percentage. While omega-6 PUFA derivatives whose precursors are linoleic (C18:2n−6) acids are considered unideal because they may cause coronary diseases and thrombosis by yielding more eicosanoids than omega-3 PUFA

derivatives. The adequate intake of omega-3 fatty acids per day is 160 mg for men and 90 mg for women, which is recommended by the National Health and Medical Research Council of Australia and New Zealand (Intakes, 2005). In addition, conjugated linoleic acid (CLA) isomers should also account for a large proportion in PUFA because they act preventively and help to maintain blood sugar level by preventing the formation of obesity and improve human health through anti-atherosclerosis and anti-carcinogenesis (Ivanovic et al., 2016).

2.3.2.2 Fatty acids in meat

Meat usually has less than 50% SFA, but up to 70% unsaturated fatty acids (UFA) (Jiménez-Colmenero, Carballo, & Cofrades, 2001). The intramuscular fat of goat kid meat contains a higher amount of UFA and PUFA, a lower proportion of SFA and a lesser total fat content compared to lambs fed under the same conditions (Banskalieva et al., 2000; Brzostowski et al., 2008; Madruga et al., 2013; Niedziółka & Pieniak-Lendzion, 2006). Banskalieva et al. (2000) summarized the available literatures to compare the fatty acid composition between goats and other animals. The data derived from different experiments were pooled together and they found the mean concentration of SFA in goat muscle lipids was 41.58%, which was not significantly different from that in lamb (41.6%) and beef (40.4%). The PUFA occupied 13.45% in goat muscle lipids, which was higher compared with lamb (7.46%) and beef (9.30%) (Banskalieva et al., 2000).

Goat muscle contains four main fatty acids which are stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and palmitic acid (C16:0) in which the amount of C18:2 in goat meat is almost twice as much as that in the mutton (Webb et al., 2005). Bañón et al. (2006) reported C4 ~ C8 and other short chain fatty acids were all metabolized by goat kids and hardly accumulated in their adipose tissue, and the ratio of SFA/UFA in goat meat varies between 1 ~ 2.

The diet composition can considerably influence the fat deposition and fatty acids profile of goat meat. And, in turn, the fatty acids composition of goat muscle or adipose tissue can, to some extent, reflect the fatty acids composition of ingested diet as well (Bas et al., 2005). For example, increase in omega-3 and decrease in omega-6 fatty

acids will lead to a desirable n−6/n−3 ratio in animal tissues so goats can be supplemented with diets rich in omega-3 sources such as fish oil, flaxseed oil, forages and linseed oil to elevate long chain omega-3 intake (Webb et al., 2005). Ebrahimi et al. (2014) found the n−6/n−3 ratio was 11.67 when goats were fed diets supplemented with sunflower oil that is the main source of linoleic acid (C18:2*n*-6) and this ratio decreased to 3.07 when replaced with flaxseed oil which contains approx. 50-70% α-linolenic acid (C18:3n−3). Goats fed on pasture-based diets which were rich in 18:3 (n-3) also had more Omega-3 fatty acids in their meat and leaner carcasses compared to those finished with high grain diets (Turner et al., 2014). Suckling goat kids fed with milk or milk replacer are highly valued in Spain (Bañón et al., 2006). The *Longissimus dorsi-lumborum* muscle of goat kids fed with milk replacer rich in coconut fat had a significant higher UFA content (48.32%) than the kids fed with goat milk (38.83%). Ahmed et al. (2015) found the proportions of monounsaturated fatty acid (MUFA) and PUFA increased linearly, and the ratio of PUFA/SFA raised from 0.25 to 0.51 as the concentration of green tea by-products (GTB) increased in diets.

The fatty acids (FA) composition of goats will change with age due to rumen bacteria activity. Older goats will have a higher level of trans FA, odd chain-length FA, branched-chain FA, conjugated linoleic acid (CLA) isomers and total saturated FA contents (Bas et al., 2005). Banskalieva, et al. (2000) also clarified when increasing goat kids age, in most fat depots, the SFA level increased while the proportion of MUFA and stearic acid (C18:0) that is counted as desirable fatty acids (DFA) decreased.

2.3.3 Amino acids profile

2.3.3.1 Protein and amino acids

There are 190 amino acids (AAs) present while only 20 compose meat proteins (Pereira & Vicente, 2013). Within these 20 amino acids, 9 amino acids must be supplied from food because human body cannot synthesize them. These 9 essential amino acids (EAAs) are valine (VAL), threonine (THR), phenylalanine (PHE), leucine (LEU), isoleucine (ILE), methionine (MET), histidine (HIS), lysine (LYS) and tryptophan (TRP) (Toldra & Aristoy, 2008b). Another 6 amino acids, namely proline (PRO), glycine (GLY), arginine (ARG), tyrosine (TYR), glutamine (GLN) and cysteine (CYS),

are conditionally essential due to the limited synthesis under individual health status and special conditions. For example, ARG is nutritionally essential for infants due to the inefficient synthesis while adults do not need to supplement it from food (Toldra & Aristoy, 2008b). The remaining 5 amino acids can be synthesized in human body so they are defined as nonessential amino acids (NEAAs) which are glutamic acid (GLU), asparagine (ASN), alanine (ALA), serine (SER) and aspartic acid (ASP) (Toldra & Aristoy, 2008a).

There are some free amino acids present in meat while the majority is in the form of protein which can be hydrolyzed into amino acids or peptides by enzymes after ingested by the humans. The original free amino acids plus those hydrolyzed amino acids are called total amino acids that can be absorbed into body when human beings digest meat (Toldra & Aristoy, 2008a). The protein efficiency rate (PER) is one of an efficient method to evaluate protein quality. A PER value below 1.5, between 1.5 ~ 2.0 or above 2.0 refers to a low, medium and good protein quality respectively (Toldra & Aristoy, 2008b). The protein quality of a muscle meat is quite high because its PER value is over 2.7 and it contains a large amount of all EAAs which are indispensable for physical well-being and body growth (Pereira & Vicente, 2013; Toldra & Aristoy, 2008a). As for other diets like legumes are poor in MET and cereals such as wheat have low amounts of LYS, therefore, legumes and cereals need to be ingested together to get all EAAs for vegetarians (Pereira & Vicente, 2013). Additionally, the amino acid profiles of connective tissue whose proteins are mainly elastin and collagen are also not very balanced because they are rich in GLY and PRO, but poor in TRP (Toldra & Aristoy, 2008b). Apart from PER, the digestibility of protein which produces free amino acids (FAAs) and peptides, is also an alternative way to access protein quality and availability. The digestibility of meat protein is further mentioned in section 2.7, and the determination of FAAs and peptides is described in section 2.8.

2.3.3.2 Amino acids in meat

Webb, Casey and Simela (2005) exhibited the amino acid profile of goat meat which shows that goats contain all 9 EAAs. Sheridan, Hoffman and Ferreira (2003) found goat meat had higher total contents of EAAs (8.89 g/100 g rib cut) than that from the sheep (7.12 g/100 g rib cut) but the TRP level was significantly higher in sheep compared to

goat (0.31 versus 0.22 g/100 g). The dominant amino acids present in goat and sheep meat were GLY, ASP, GLU, LEU and LYS, and no significant differences were found between these two species in terms of HIS, ALA, SER and GLY (Sheridan et al., 2003). Elgasim and Alkanhal (1992) found that the EAAs in goat meat accounted for 47.39 % of the total AAs content, which was higher than that of the beef (46.26%) and the lamb (45.42%). Amongst all of the individual EAAs in goat meat, LYS occupied a superior proportion of about 21.4% out of the total EAAs. Moya et al. (2001) studied the content change of FAAs and peptides during the post-mortem aging process of pork meat. They found almost all of the amino acids increased their concentration during this aging process, and THR, MET, PHE, ILE, SER, GLU and TYR showed a remarkable increase at the end of 24 h post-mortem aging, but the two dipeptides, anserine and carnosine, did not have a significant change.

Watanabe et al. (2004) observed a significant difference in the concentration of almost all the FAAs and dipeptides between animal age groups. They found 35-month-old steers had lower FAAs, anserine and carnosine compared to younger steers of 25 months of age.

Diet, undoubtedly, has a significant effect on meat amino acid composition. Koutsidis et al. (2008) analyzed the FAAs in *longissimus lumborum* muscle derived from steers aged 24 months, fed with either low energy grass silage diet or high energy concentrate diet. Their results revealed silage-fed animals had significantly higher amount of all EAAs compared to the concentrate-fed animals. Especially the LEU had shown a 25% higher level when animals were fed with silage. Besides, MET and LYS are limiting amino acids in grain-based diet (Titgemeyer, Merchen, Berger, & Deetz, 1988).

2.4 Organoleptic attributes

2.4.1 Tenderness and juiciness

When animals are slaughtered, the conversion of muscle to meat can be summarized to three steps: pre-rigor phase, rigor mortis and tenderising phase (aging). Meat toughness will raise during rigor mortis but its tenderization will improve progressively with post-mortem aging because during this period, proteolysis and oxidation will involve in

improving the tenderness. For example, endogenous peptidases will soften the structure of myofibrillar protein (Ouali et al., 2006; Shija et al., 2013). Apart from post-mortem aging, electrical stimulation can also improve the meat tenderness (Hogg et al., 1992).

Meat juiciness is associated with the water-holding capacity (WHC) so the efflux of intracellular water to extracellular space will decrease the intensity of juiciness. Because WHC is also impacted by pH, the bound water will be released when the pH declines to the isoelectric point of myofibrillar protein, resulting in the loss of juiciness (Ouali et al., 2006).

Tenderness and juiciness are undoubtedly both closely related with meat intramuscular fat. When cooking, the melted fat can act as a lubricant to improve meat tenderness and juiciness (Schönfeldt, Naudé, et al., 1993). Generally, the more fat the meat has, the more tender the meat, and the more quickly juices will be released when chewing and the less residue will remain in the mouth after chewing (Tshabalala et al., 2003).

Apart from fat, connective tissue is one of the decisive factors for meat tenderness. Collagen is the major component of connective tissue, which has a rigid and tough structure and can form cross-linking to enhance toughness. The total collagen content and solubility can cause variation in tenderness. Goat normally has a higher insoluble collagen content than that of the sheep. Schönfeldt et al. (1993) reported a significantly higher collagen content in *Longissimus dorsi* muscle of Boer goat than that of the same muscle from sheep (3.74 vs 3.18), and a significantly lower collagen solubility (14.62% vs 17.09%). Thus a higher collegan content with lower solubility, a less intramuscular fat content and a larger and thicker myofibril bundles result in a tougher goat meat when compared to sheep meat, which has been proved by a lot of publications as summarized in Table 3 (Schonfeldt, 1989; Sen et al., 2004; Swan, Esguerra, & Farouk, 1998).

The young goat kids are tender and less stringier than old goats, not only because the total collagen content increases with age. But also because collagen can be broken down and denatured to form a gelatin-like component which can make meat tender when cooking, while this gelatinization ability decreases with age (Webb et al., 2005). For example, Schönfeldt et al. (1993) found goat kids with no permanent incisors had a lower collagen content (3.69) but higher collagen solubility (20.55%) compared to the

goats with 7-8 permanent incisors (3.78 and 10.05% respectively), resulting in a more tender kid meat.

As shown in Table 3, though not statistically significant, juiciness of goat meat was found to be lesser when compared with the lamb (Babiker et al., 1990; Sen et al., 2004; Smith, Pike, & Carpenter, 1974; Swan et al., 1998). However, meat juiciness from young animals was always rated to be higher than that of the old animals. This is in accordance with the result that young kids have a higher water-holding capacity (Dhanda et al., 1999), resulting in a juicier meat.

2.4.2 Flavor and odour

In cooked meat, flavour and odour have a very complicated development process in which different components react to produce intermediate or final flavour and aromatic compounds (Bañón et al., 2006). The flavour and odour of cooked meat mainly come from Maillard reaction and thermal degradation of fat (Mottram, 1998). Maillard reaction between amino acids and reducing sugars results in roast and boiled flavours from the formation of heterocyclic compounds. The unsaturated lipids are very reactive to heat and can generate more aromatic substances such as furanthiols, furan sulfides and disulfides (Bañón et al., 2006; Mottram, 1998).

According to Madruga et al. (2013), grilled lamb and goat have 133 identified headspace volatiles, which consist of 46 Maillard-derived substances and 87 lipid-derived compounds. Volatile products like alcohols, aldehydes, ketones, carboxylic acids and aliphatic hydrocarbons are regarded as main contributors to meat flavour profile. In cooked lamb and goat, quantitatively abundant volatiles are alcohols, aldehydes and ketones, during which the most dominant volatiles are heptanal, 2-butanone, hexanal, octanal and nonanal (Madruga et al., 2013).

The composition of fatty acids affects the volatile composition. For example, large amounts of PUFA can increase the lipid oxidation concentration, especially unsaturated and saturated aliphatic aldehydes. The higher concentration of linolenic acid in goat also gives a higher level of oxidation product derived from linolenic acid, such as 2-ethylfuran and 2-ethylbenzaldehyde. Similarly, lamb always contains more linoleic acid

hence the oxidation products of linoleic acid (hexanal and 1-pentanol) are detected in larger amounts in lamb (Madruga et al., 2013). The strong smell of goat is mainly attributed to 4-ethylocatanoic fatty acid (Ivanovic et al., 2016).

Schönfeldt, et al. (1993) reported sheep meat possessed a significantly stronger species-related flavour than goat meat and they also found no matter goat or sheep, the species-related flavour was less typical in the meat from younger animals. Smith, et al. (1974) found when samples of comparable maturity were compared, goat meat was significantly less tender than beef, pork and lamb with a lower palatability rating. However, when goat kids were compared with other animals, the overall satisfaction of kid meat was rated higher than that of beef and lamb.

In conclusion, compared to sheep meat under the same gender and comparable age, goat meat has a coarser texture and a darker red color that gives a better visual appeal. It has a characteristic flavor which is typically different from mutton or lamb but less strong without obvious off-odour. Their differences in odour and juiciness are not notably obvious but the tenderness of goat meat is usually rated to be lower than the sheep meat. Thus the overall palatability varies among various experiments, while most scientists found a similar rating in overall liking (Babiker et al., 1990; Rhee et al., 1999; Sen et al., 2004). However, meat from young animals proves to be juicier and tender with a less species-related flavor and odor compared to yearling or old animals. Thus the younger kid meat is fit for being sold as fresh meat to get rid of the sensory defects brought by the old goats, and the yearling or older adult goat meat is suitable for the other processed food production (Kirton, 1970; Webb et al., 2005).

Table 3. Comparison of meat organoleptic attributes.

Classification	Sample	Cut	Sensory Evaluation of cooked meat					References
			Tenderness	Juiciness	Odour/aroma	Flavour desirability	Overall acceptability	
Species	Yearling sheep and goats: n=12 Age: 1 yr Gender: ♀	LD	Goat (3.37) < sheep (4.25)	Goat (3.87) ns sheep (3.87)	Goat (4.00) ns sheep (3.87)	N/A	Goat (3.87) ns sheep (3.75)	(Sen et al., 2004)
	Boer, Cashmere, Boer×Cashmere crossbred goats and lambs: Age: 1 yr Gender: ♀	LD	Crossbred (29) ns Boer (34) < Cashmere (43) < lamb (74)	Boer (19) ns Crossbred (20) ns Cashmere (27) < lamb (39)	Cashmere (43) ns Boer (48) < Crossbred (56) ns lamb (56)	Boer (40) ns Cashmere (40) ns Crossbred (43) < lamb (62)	N/A	(Swan et al., 1998)
	Angora and Boer goats and sheep: n=27	LD	Boer goat (2.42) < Angora goat (3.07) < sheep (4.75)	N/A	Boer goat (4.33) ns Angora goat (4.32) < sheep (4.59)	Boer goat (4.16) ns Angora goat (4.27) < sheep (4.49)	N/A	(Schönfeldt et al., 1993)
	Desert lambs and goats: n=10 Live BW: 35 kg	LD	Goat (2.8) ns lamb (3.1)	Goat (2.3) ns lamb (2.6)	N/A	Goat (2.5) < lamb (2.8)	Goat (3.2) ns lamb (3.4)	(Babiker et al., 1990)

	109 goats, 71 lambs, 34 cattles, 30 pigs	Rib or loin samples	Goat (4.2) < beef (5.9) < pork (6.6) < lamb (7.9)	Goat (5.2) ns beef (5.9) ns pork (5.2) ns lamb (5.7)	N/A	Goat (5.3) < beef (6.1) ns pork (6.1) < lamb (6.8)	Goat (4.9) < beef (6.0) ns pork (6.6) < lamb (7.0)	(Smith et al., 1974)
Age	Angora and Boer goat kids: n=27 A age group: with no permanent incisors; B age group: 1-6 permanent incisors; C age group: 7-8 permanent incisors.	LD	A (3.87) > B (3.22) > C (3.16)	N/A	A (4.48) ns B (4.40) ns C (4.35)	A (4.33) ns B (4.28) ns C (4.32)	N/A	(Schönfeldt et al., 1993)
	Feral capretto and chevon: n=25	LD	Capretto (5.7) > chevon (5.4)	Capretto (5.7) > chevon (5.5)	N/A	Capretto (5.6) > chevon (6.2)	Capretto (5.6) ns chevon (5.7)	(Dhanda et al. 1999)
	9 goats: goat kids, yearling goats, old goats with 6 or more permanent incisors	LD	Young (6.41) > yearling (5.07) > old goats (4.81)	Young (5.84) > yearling (4.68) > old goats (4.52)	N/A	N/A	Young (6.54) > yearling (5.49) > old goats (5.27)	(Kirton, 1970)
Diet	Crossbred Boer × Indigenous goats: n=40 Live BW: 15.6 kg Age: 5 months Gender: ♂ Diet: Soybean meal substituted with peanut cake at 0%, 34%, 67% and 100% level (presented as S0, S34, S67 and S100)	LD	S0 (6.80) ns S34 (6.49) ns S67 (7.13) ns S100 (6.31)	S0 (5.14) ns S34 (5.02) ns S67 (5.00) ns S100 (4.64)	S0 (1.95) ns S34 (2.19) ns S67 (2.16) ns S100 (2.39)	S0 (2.08) ns S34 (2.36) ns S67 (2.54) ns S100 (2.47)	S0 (6.94) ns S34 (6.87) ns S67 (6.66) ns S100 (6.35)	(Silva et al., 2016)

Gender	Sheep and 36 feral goats: 18 ♀, 18 ♂	LD	Sheep (6.17) > goat ♀ (5.50) > goat ♂ (4.62)	Sheep (4.83) ns goat ♀ (5.30) ns goat ♂ (4.91)	N/A	N/A	Sheep (6.30) > goat ♀ (5.68) > goat ♂ (5.32)	(Kirton, 1970)
Breed	French Alpine (FA) goat kids and French Alpine × Boer crossbreds (FAB) goat kids: n=12 Age: 50 days Gender: ♂	QF	FA (4.25) < FAB (4.46)	FA (4.58) < FAB (4.75)	FA (4.83) ns FAB (4.92)	FA (4.67) ns FAB (4.75)	FA (4.65) ns FAB (4.76)	(Brzostowski et al., 2008)

n = No. of observations BW: Body Weight SM: *M. semimembranosus* LD: *M. longissimus dorsi* QF: M. *quadratus femoris* N/A: Not Available

>: Significantly higher <: Significantly lower ns: Not significant (P > 0.05)

2.5 The influence of breed and gender

Goats have about 1156 different breeds including South African Boer goats, fibre-producing Cashmere goats, Boer × Cashmere crossbreed goats and other breeds (Swan, Esguerra, & Farouk, 1998). In Korea, 80% of the entire goat population is occupied by Korean indigenous black goat (*Capra hircus coreanae*, KNG) (Ahmed et al., 2015). New Zealand also has a wide range of goat breeds, such as Saanen, Sable, Anglo Bubian, British Alpine and Kiko (Batten, 1987). Widespread Saanen goat kids which originate from Saanen valley in Switzerland have been studied by Madruga et al. (2010).

As for breeds, it's very difficult to conclude a similar trend of the breed effects on carcass traits because there are too many breeds in one species and even the same breed in different region will give a various nutritional value. In general, breeds have a small influence on carcass compositions (McGowan, Nurse, & Anous, 1995). Johnson et al. (1995) reported that many of the carcass characteristics were not influenced by breeds. Florida native goats, Florida native × Nubian goats and Florida native × Spanish goats did not have significant differences in soft tissue composition, lean texture, firmness, marbling and skeletal maturity. Tshabalala et al. (2003) reported that Indigenous Victoria West goats had proportionally larger heads, feet, livers and spleens than the Boer goats, while the skin yield and total subcutaneous fat of Boer goats were higher than that of the Indigenous goats. Growth rate varies in different breeds. A rapid growth potential usually means more meat production. Saanen and Saanen×Angora crossbred kids were found to have a higher growth rate of 128 ~ 209 g per day than that of the native Tunisian goat kids (84 ~ 105 g per day), but their carcass fat content was also higher compared to that of the Tunisian goat kids (19 ~ 24% versus 12%) (Atti et al., 2004).

Compared to breed factor, gender has a more obvious effect on carcass properties. In Table 2, it can be seen that female goat carcass was always reported to have more fat, less water, protein and muscular tissue than the male (Hogg et al., 1992; Kirton, 1970; McGowan et al., 1995). Male goats were found to have heavier heads, skins and stomach contents than those from females, when comparing of the same live weight. Their visceral organs, like the spleens, livers, lungs, stomachs, in turn, accounted for a

lower proportion (Kirton, 1970). Hogg et al. (1992) reported that castrated males had significantly higher lean meat in shank and breast but females contained significantly more fat in rack and loin, and the fat distribution of castrated males was more favorable than that of females. As shown in Table 3, meat from female goats turned out to be tender than the males while they do not have a significant difference in juiciness (Kirton, 1970). Johnson et al. (1995) also found that female muscles were significantly more tender and desirable than the muscles from either intact male muscles or castrates.

2.6 The influence of different cuts

Meat from different species or breeds possesses different nutrient profile. *Longissimus dorsi* is the most widely studied meat cut to date. Other cuts, especially the shank, breast and loin cuts of goat meat, possess higher proportion of lean meat compared to that of the sheep. Within the same breed, individual cuts also have variances in their nutritional composition. For example, Lombardi-Boccia et al. (2005) reported beef fillet was the richest in trace elements like copper, zinc and iron than the other four beef cuts (roast beef, topside, thick flank and sirloin). Liméa et al. (2010) reported heavier forequarters in the Creole goat carcasses than the hindquarters. Oliveira et al. (2015) found the weights and yields of retail cuts from Boer goats were not affected by the different supplementation levels of sunflower cake, but leg presented the highest carcass weight yield proportion of 30.8% and loin exhibited the lowest yield of 2.84%. Shoulder, neck and ribs all had similar yields, around 22%.

The daily cholesterol intake for human being recommended by The American Heart Association is less than 300mg. Although meat provides a small portion, less than 75 mg/100 g, customers are advised to choose lean meat cuts and remove visible fat before cooking (Jiménez-Colmenero et al., 2001; Keeton, 1994). Fat content varies significantly in different retail meat cuts. For example, the leanest portion in poultry meat is breast but in pork and beef, the leanest part is loin (Pereira & Vicente, 2013). Sen et al. (2004) found that the highest proportion of lean meat in yearling female goats was in leg cut (76.77%) and the lowest was in loin cut (62.36%). The percentage of subcutaneous and intermuscular fat was highest in loin (26.83%), followed by breast and fore shank (18.02%), rack (14.93%), neck and shoulder (12.71%), and leg (6.93%). Tshabalala et al. (2003) also described a relatively higher subcutaneous fat proportion in

dorsal (3.84%) and ventral (4.39%) trunk of castrated Boer goats, compared to fore shank (3.19%) and hind shank (1.76%). Hogg et al. (1992) discovered that the loin and breast from female Saanen × Angora goats always showed a higher fat content and a lower lean meat compared to males slaughtered at similar live weight. Franco et al. (2010) studied the proximate composition of six different muscles from Blonde Galician male veal calves. These muscles were *semitendinosus* (ST), *biceps femoris* (BF), *semimembranosus* (SM), *longisimus dorsi* (LD), Masseter (MS) and cardiac muscle (CM). They found a quite similar proximate composition in all 6 muscles with about 76% moisture, 20% protein, 2.5% intramuscular fat and 1% ash. As for amino acid content, Franco et al. (2010) found in LD muscle, leucine and lysine were the major essential amino acids (EAAs). In the non-essential fraction, LD had a high proportion of glutamic acid and aspartic acid.

Generally, mid-line cuts like shoulder, rack, loin and leg no matter from sheep or goat, have a relatively higher commercial value than other cuts because they account for 78.5% ~ 79.5% of animal carcass weight. Shoulder and leg deposit more lean muscle, while loin and rib have less connective tissue with desirable tenderness (Kirton, Mercer, Duganzich, Clarke, & Woods, 1999; Oliveira et al., 2015). In New Zealand market, take Countdown supermarket for example, the butchery leg boneless of lamb from Silver Fern Farms is $3.88/100 g while the lamb rump from the same industry is $3.75/100 g. And the butchery lamb knuckle from Gourmet is only $1.7/100 g. Studies on species, breeds, gender and diet have been around for years, but differentiation coming from cuts has not been studied well to date. In New Zealand, unlike other meat sources, goat meat cuts sold in the market are restricted. Hence more insights into goat cuts are required to better understand and fully utilize goat kid meat. In the current study, the protein digestibility among different goat cuts was determined to provide more nutritional information in broadening the goat meat market.

2.7 Digestibility

2.7.1 Human digestive system

The nutritional attributes of foods are evaluated not only by their fatty acids and amino acids compositions, but also by digestibility and bioavailability. The human digestive

system is literally to break down the ingested foods into small pieces, digest food, absorb and assimilate nutrients into the bloodstream. This system roughly works in three stages: mouth, stomach and intestine digestion. It comprises the gastrointestinal (GI) tract and other accessory organs, including tongue, liver, pancreas and gallbladder.

When a solid food is ingested, it is lubricated by saliva and chewed in oral cavity to form a cohesive bolus. Human saliva has 99.5% water, 0.3% proteins which is mainly composed of α-amylase, lactoferrin and mucins and some electrolytes such as phosphate, magnesium and calcium (Minekus et al., 2014). The α-amylase hydrolyzes starch to monosaccharides or oligosaccharides, and because of the short retention time, protein and fat almost rarely get digested in mouth (Hur, Lim, Decker, & McClements, 2011; Minekus et al., 2014).

The bolus is then swallowed and passed down to esophagus which contracts and relaxes rhythmically to move the bolus down to the stomach. Gastric juice in the stomach primarily consists of sodium chloride, hydrochloric acid and enzymes. Peristalsis by the muscular wall of the stomach can further mix bolus with enzymes. Pepsin with optimum pH range from 2 to 4 is the only proteolytic enzyme in the stomach, and a pH above 6 will deactivate it irreversibly (Egger et al., 2016). Gastric lipase with optimal pH activity value between 4 and 6 accounts for a low proportion and it is not as efficient as pancreatic lipase. It can only break down fat to a small extent due to the low concentration and the pH of the stomach being outside its optimal range, to pH 2 in the fasted state (Paeschke & Aimutis, 2010).

Gastric digestion lasts for one or two hour, forming a semi-liquid chyme. When the pyloric valve that separates the stomach from the duodenum opens, the chyme enters the small intestine which is composed of three sections: duodenum, jejunum and ileum. In duodenum, acidic chyme is neutralized with bile from gallbladder and bicarbonate from pancreatic duct and duodenal gland (Paeschke & Aimutis, 2010). Here it meets and mixes with a number of digestive enzymes produced by the pancreas. Pancreatic amylase hydrolyses the residual carbohydrates. Pancreatic lipase transforms triacylglycerols to monoacylglycerols or free fatty acids forming chylomicrons which are small emulsified fat particles. The rate of lipolysis is improved markedly by the existence of bile salts, calcium and co-lipase which can adhere to lipase to facilitate the lipase to bind the substrate (Geissler & Powers, 2005; Paeschke & Aimutis, 2010).

Trypsin and chymotrypsin are the major intestine proteolytic enzymes that decompose proteins into smaller peptides or free amino acids (Geissler & Powers, 2005). The midsection, jejunum, mainly absorbs nutrients, including digested sugars, fatty acids and amino acids into the bloodstream. Bile acids, vitamins and any remaining nutrients are assimilated in ileum. Segmentation contraction of small intestine aids to achieve 95% absorption of nutrients in foods.

The semi-solid residue passes through the cecum into large intestine where some minerals and water are reabsorbed back into the bloodstream. The colonic microflora biotransforms large polyphenols into absorbable simple phenolic compounds (Sadeghi Ekbatan et al., 2016). In addition, the flora in human gut ferments the digestible matter producing waste, known as feces, which is propelled forward to reach the rectum and exist from the anus via defecation.

2.7.2 Sous-vide cooking

In most cases, meat is eaten cooked. Different cooking methods, for example roasting, stewing, grilling, smoking, boiling, steaming, frying or dry-curing can affect meat nutritional values, particularly the protein bioavailability and digestibility in the gastrointestinal tract because of the protein coagulation, aggregation, oxidation and proteolysis during cooking (Li, Liu, Zhou, Xu, & Li, 2017; Silva, Ferreira, Madruga, & Estévez, 2016). If protein cannot be digested, small intestine then cannot absorb them, thus some will move to colon and cecum, which will influence the gut microbiota environment (Li, Liu, Zhou, et al., 2017). However, meat digestion can be accelerated by cooking at a moderate temperature (around 70^{o}C). Protein cleavage sites are exposed to enzymes under this mild denaturation. A prolonged high temperature over 100^{o}C is known to decrease the enzymatic proteolysis by formation of aggregates and intermolecular cross-links, hence influencing amino acid release and digestibility (Kaur, Maudens, Haisman, Boland, & Singh, 2014; Li, Liu, Zhou, et al., 2017; Li, Liu, Zou, et al., 2017).

Sous-vide cooking, also known as vacuum cooking, is a method to cook vacuum-packed food in water bath with a moderate temperature (usually around 55 ~ 60^{o}C) at a relatively longer cooking time (normally 2 hours) (Baldwin, 2011). The aim of this

method is to maximize the nutrition retention and retain moisture in the premise of cooking the whole item (both inside and outside) thoroughly.

Research shows sous-vide could not only enhance the food succulence by minimizing the water loss, and also inhibit the loss of flavour volatiles and the appearance of some off-flavours (Borrisser-Pairó et al., 2017). Compared to the food cooked under conventional methods, sous-vide cooking can prevent oxidative rancidity and retard the growth of microorganism so the shelf-life will be prolonged (Borrisser-Pairó et al., 2017; Naveena et al., 2017). Hence in this study, sous-vide cooking was used to cook goat kid cuts (section 3.3.1).

2.7.3 *In vitro* digestion model

Recently, there has been an increase in interest to study how food or pharmaceutical components are released and delivered in the human body to maximize the benefit to the human health (Hur et al., 2011). As *in vivo* human trial is costly and ethically debatable, *in vitro* delivery systems which mimic the human GI tract have been used to simulate the digestive process (Minekus et al., 2014). The *in vitro* system can be dynamic with real-time transporting, changed pH and enzyme concentration over time, or static with invariable concentration of salts and enzymes in each step (Minekus et al., 2014). Hitherto, simulated static *in vitro* assay is widely used to evaluate the bioaccessibility and digestibility of foods, macronutrients, micronutrients and metabolites (Denis et al., 2016; Minekus et al., 2014). Although *in vitro* assay is less expensive, faster and controllable than *in vivo* method, it's very hard to simulate the whole system accurately due to the complex physicochemical and physiological environments in human GI tract. *In vitro*, food matrices are released all at once rather than gradually, and some digestive products that may inhibit enzymes cannot be removed. Thus static models cannot fully simulate the *in vivo* kinetic behavior (Egger et al., 2016; Minekus et al., 2014), and the results of *in vitro* digestion model are different to the results of the *in vivo* model (Hur et al., 2011; Rodrigues, Mariutti, & Mercadante, 2016). *In vitro* has a tendency to provide a lower protein digestibility compared to *in vivo* assay (Yi, Van Boekel, Boeren, & Lakemond, 2016).

Typically, *in vitro* simulated model contains three consecutive phases: oral, gastric and intestinal. Usually large intestine is not taken into account because the main absorption occurs in small intestine (Hur et al., 2011). Several aspects need to be considered when conducting *in vitro* digestion experiments, such as the composition of digestive fluid in each phase (e.g. enzymes); the environmental condition for each step like temperature (37^oC), pH and salinity (Hur et al., 2011). In *in vitro* assay, α-amylase, pepsin, trypsin, pancreatin, lipase and bile salt are widely used. Depending on the experimental objective and digestive phases, different types of enzymes are chosen and added sequentially. Amylases digest starch and they are secreted in oral phase. Proteases like pepsin are added to stomach to digest protein. Pancreatin including trypsin and lipase, simulates the digestion of protein and fat in small intestine (Hur et al., 2011). Although this study aims to identify the free amino acids released from the meat protein, bile salts and other digestive enzymes (i.e. lipase, amylase) were also used because the hydrolysis and presence of lipids and carbohydrates have been reported to influence the protein digestion (Bordoni et al., 2014).

Minekus et al. presented a consolidated *in vitro* digestion method in 2014 as a result of the European Cooperation in Science and Technology (COST) action named INFOGEST to standardize the individual parameters to minimize the research variability and decrease the replicated numbers so that different research groups from worldwide can compare their results under the consistent parameters (Rodrigues et al., 2016). The digestion part of this research (section 3.3) was adapted from the INFOGEST method to compare the *in vitro* digestibility of different cuts of goat meat because this INFOGEST method is proven to be consistent and comparable during the inter-laboratory *in vitro* digestion study (Egger et al., 2016).

2.7.4 Application of *in vitro* digestion

Based on both, the type of food and individual variation such as different habits, physical situation, age, chewing times and so on, even in the human body the enzyme substrate ratio, the gastrointestinal transition time, digestive fluid secretion, as well as gastric emptying time vary widely. When applying to *in vitro* static methods, different authors have made slight changes to adapt for their experimental goals as well as their samples, as summarized in Table 4.

In *in vivo* setting, meat protein is highly digestible whose digestibility is estimated to reach 95% (Sayd, Chambon, & Santé-Lhoutellier, 2016). Gastrointestinal digestion breaks down muscle proteins into free amino acids (FAAs) and peptides. The released FAAs can be absorbed directly but the absorption of peptides is usually in the form of small di- and tri- peptides, which is regarded as a more efficient way of amino acid intake. Amino acid composition is an important criterion to assess meat nutritive value. Some of the biologically active peptides, especially low molecular weight peptides that are either initially present in food or generated during food digestion, also have various health-benefiting biological activities like antimicrobial, antihypertensive, immunomodulatory and antithrombotic (Bauchart et al., 2006). Escudero et al. (2010) observed an extensive proteolysis of pork muscle proteins after *in vitro* pepsin and pancreatin digestion. 51 different peptides with fragment size ranged from 6 to 16 amino acids were found in pork meat digests. Although actin has a smaller molecular weight of 42 kDa than that of the myosin (250 kDa), it gave a relative higher amount of hydrolyzed peptides after *in vitro* digestion (Sayd et al. 2016).

Santé-Lhoutellier et al. (2008) indicated that pasture or concentrate diet did not influence the myofibrillar protein digestibility of lamb meat. However, storage time had no significant effect on gastric protein digestion but significantly increased the intestinal protein digestibility. In terms of cooking temperature, Bax et al. (2012) proposed a mechanism that proteins would partially unfold to make the cleavage site more accessible to digestive protease under moderate cooking temperature. However, a high temperature would induce protein oxidation as well as aggregation. The intermolecular cross-links and aggregates can cause the stacking of protein and masking of cleaving sites, slowing the enzymatic proteolysis and reducing digestibility (Kaur et al., 2014). Bax et al. (2012) found a low gastric digestibility of pig meat when cooked at 100 °C, but the overall protein digestibility improved (Bax et al., 2012). Liu and Xiong (2000) reported, compared to the unoxidized myosin, pepsin-digested oxidized myosin showed a decreased band intensity of higher molecular masses and new peptide bands with lower molecular masses ranging from 97 to 200 kDa appeared. They clarified that oxidation can cause the degradation and fragmentation of myosin because oxidative attack can lead to the scission of polypeptide backbone and many amino acid residue side chains.

Table 4. Comparison of *In vitro* digestion model.

Sample	***In vitro* digestion model**						Key findings	References
	Gastric phase			**Intestinal phase**				
	Enzymes	**pH**	**Time**	**Enzymes**	**pH**	**Time**		
Dry-cured ham, smoked ham, emulsion-type sausage and dry-cured sausage	Porcine gastric pepsin	2	2 h	Porcine trypsin	7.5	2 h	*Dry-cured ham had the highest *in vitro* digestibility, while smoked ham showed the lowest. *In gastric phase, emulsion sausage revealed higher digestibility than dry-cured sausage, but the result was reverse in intestinal phase.	(Li et al., 2017)
Pork *longissimus dorsi* muscles (cooked pork, emulsion-type sausage, dry-cured pork and stewed pork)	Porcine gastric pepsin	2	2 h	Porcine trypsin	7.5	2 h	*Emulsion-type sausage had highest digestibility but stewed pork had lowest. *Cooked pork showed higher digestibility than dry-cured pork in intestinal phase while no difference was observed in gastric phase.	(Li et al., 2017)
Bovine *longissimus dorsi* muscles	Pepsin	1.9	1 h	Pancreatin	8	2 h	*Meat gastric digestibility was improved by high pressure processing. *The effect of high pressure and cooking are not comparable.	(Kaur et al., 2016)
Cooked *semimembranosus* muscles from bull	Pepsin	2	2 h	Trypsin; chymotrypsin	7	2 h	Muscle contraction and structure proteins were preferentially enzymatically hydrolyzed in the small intestine.	(Sayd et al., 2016)

Cooked and uncooked eye of round of beef	Pepsin	1.9	1 h	Pancreatin	8	2 h	After pepsin hydrolysis, cooked meat had more peptides with intermediate molecular weight (15-30 kDa), while raw meat had more large peptides (74-91 kDa).	(Kaur et al., 2014)
Cooked pig *longissimus dorsi* muscles	Porcine gastric pepsin	1.8	90 min	Trypsin and α-chymotrypsin	8	60 min	Pepsin digestibility was increased at 70°C but decreased when temperature was over 100°C.	(Bax et al., 2012)
Raw pork *longissimus dorsi* muscles	Porcine gastric pepsin	2	2 h	Porcine pancreatin	7.2	3 h	51 different peptides were identified.	(Escudero et al., 2010)
Longissimus dorsi muscles from castrated lamb	Porcine gastric pepsin	1.8	1 h	Porcine trypsin and α-chymotrypsin	8	30 min	*Diet didn't influence the myofibrillar protein digestibility. *Storage time had no significant effect on gastric protein digestion but significantly increased the intestinal protein digestibility.	(Santé-Lhoutellier et al., 2008)
Chicken pectoralis muscle	Porcine gastric pepsin	1.8	1 h	Porcine trypsin and α-chymotrypsin	8	1 h	Oxidation decreased the myosin digestibility under nonreducing conditions.	(Liu & Xiong, 2000)

N/A: Not Available

Oral phase was missing in all of the reported references in this table.

2.8 Determination of free amino acids and peptides

2.8.1 Determination of free amino acids

Amino acids can be detected through the traditional post-column derivatization such as cation-exchange chromatography using ninhydrin, pre-column derivatization followed by either reversed-phase liquid chromatography (RPLC) or gas chromatography (GC), and the hydrophilic interaction liquid chromatography (HILIC) without derivatization (Chou et al., 2007). The ion-exchange used to be the most common strategy but it is time-consuming. HILIC is a good alternative approach because it can separate the underivatized polar amino acids based on the interaction with its polar stationary phase (Buszewski & Noga, 2012; Prinsen et al., 2016), while compared to RPLC, it has poor separation efficiency and long equilibration times (Castellanos, Van Eendenburg, Gubern, & Sanchez, 2016). The pre-column derivatization using RPLC is rapid and sensitive in detection limits with a range varying from picomoles to femtomoles (Cohen & Michaud, 1993), but it has poor reproducibility and instability of derivatives (Prinsen et al., 2016).

The pre-column derivatization technique can be achieved through various derivatizing reagents, for example with phenylisothiocyanate (PITC), 4-dimethylaminoazobenzenesulfonylchloride (Dabsyl-Cl), 9-fluoroenylmethyl chloroformate (FMOC-Cl) and o-phthaldehyde (OPA). However, every reagent has some drawbacks. For example, sample preparation is complicated for PITC and the derivatives are unstable. Multiple derivatives are yielded by Dabsyl-Cl and FMOC-Cl. Secondary amino acids cannot react with OPA and the reaction between cysteine and OPA is weak, and it also produces some unstable derivatives (Castellanos et al., 2016; Cohen & Michaud, 1993; Yi et al., 2016).

6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC reagent or Accutag reagent) was introduced by Cohen and Michaud in 1993 because this reagent could overcome most abovementioned shortcomings. The primary and secondary amino-containing compounds can react with AQC, forming stable derivatives which can be detected by a highly reproducible and sensitive reverse phase high performance liquid chromatograph combined with a mass spectrometer (LC-MS) (Gray et al., 2017). This AQC reagent can

optimize the chromatographic separation and minimize the reagent interference because of the special fluorescence property of AQC derivatives (Cohen & Michaud, 1993). Besides, it is insensitive to salts, common buffers and detergents so AQC derivatization technique is one of the most commonly used method to analyze amino acids (Azilawati, Dzulkifly, Jamilah, Shuhaimi, & Amin, 2016).

The derivative will ionize after the AQC reagent reacts with the amine group in amino acids. Multiple reaction monitoring (MRM) is used to quantify each individual derivatized amino acid. The mass-to-charge ratio (m/z) of the common fragment ion is 171 so in MRM quantification, this common fragment ion and the specific parent ion with a fixed m/z ratio are selected to detect different amino acids. If amino acids have more than one amine site like lysine and cystine, then more AQC units will be involved in this reaction. The most abundant m/z parent ion values of lysine and cystine are 244 and 291 respectively, which actually are doubly derivatized and doubly charged ($[M+2xAcq+2H]^{2+}$). While small amount of singly derivatized and singly charged parent ions ($[M+1xAcq+H]^{+}$) as well as doubly derivatized and singly charged ions ($[M+2xAcq+H]^{+}$) also present in both amino acids whose m/z values are 317 and 487 for lysine and 411 and 581 for cystine (Gray et al., 2017). In room temperature, these derivatives are stable for up to one week. If stored in $4^{o}C$, they are stable for at least 15 days (Castellanos et al., 2016). RPLC has a non-polar stationary phase which normally is conventional C18 silica column. The mobile phase is composed of different gradients which start with highly aqueous solvent to elute the polar compounds first (Castellanos et al., 2016).

AQC derivatization technique has been applied to analyse amino acids by many researchers (Armenta et al., 2009; Diana, Rafecas, & Quílez, 2014; Lu, Lv, Gao, Shi, & Yu, 2015; Nakamura et al., 2015; G. Sharma et al., 2014). Nakamura et al. (2015) confirmed using liquid chromatography–tandem quadrupole mass spectrometry (LC–MS/MS) to detect AQC–amino acids was able to identify the nitrogen metabolism in living organisms. Sharma et al. (2014) achieved a good reproducibility and repeatability when using this AQC reagent to separate 26 amino acids in human plasma. Lu et al. (2015) used AQC derivatization kit to quantify 16 amino acids in milk powders, and they found the amino acids concentrations were in line with the previous work. Here in this study, the pre-column derivatization using AQC reagent in combination with LC-

MS was applied to goat meat samples to compare the free amino acids released before and after the *in vitro* digestion simulation.

2.8.2 Determination of peptides

Laemmli (1970) reported that using polyacrylamide gel electrophoresis in the presence of the anionic detergent sodium dodecyl sulfate, known as SDS–PAGE, protein and peptides based on their molecular weights can be separated. When dissolved, sodium dodecyl sulfate (SDS) can denature the secondary and tertiary structures of protein by binding to the hydrophobic side of protein molecule giving it a net negative charge and facilitating protein moving towards the anode in an electric field (Brunelle & Green, 2014).

In Laemmli SDS-PAGE system, the discontinuous polyacrylamide gel consists of two layers, where the lower layer is separating gel with a pH of 8.8 aiming to separate proteins and peptides by molecular weight, and the upper layer containing sample wells is stacking gel whose pH is 6.8 aiming to compress proteins into a tight band before entering the resolving gel (Brunelle & Green, 2014). Stacking gel has a low polyacrylamide concentration and a large pore size which allows protein to move freely, while resolving gel has a higher percentage of polyacrylamide and small pores so protein with high molecular weight moves slower than low molecular weight protein (Sharma & Rajput, 2015).

SDS-PAGE has two buffer systems: glycine and tricine. Laemmli SDS-PAGE is also regarded as glycine-SDS-PAGE (Sharma & Rajput, 2015). When electrophoresis starts, glycinate ions which are trailing ions from running buffer have lower mobility than protein-SDS complexes, which in turn, have a lower migration rate than Cl- ions which are leading ions from stacking gel, therefore protein moves between leading ion and trailing ion and stacks in a very thin band. Once the glycinate arrives at the separating gel, high pH makes it fully negatively-charged thus its migration rate increases leaving protein-SDS complexes behind to migrate at their own rate (Sharma & Rajput, 2015). Glycine is usually used for big protein separation with a molecular weight over 20 kDa, while tricine who moves much faster than glycine in stacking gel has a superior resolution of low molecular weight protein (< 20 kDa) (Schagger, 2006; Schägger &

von Jagow, 1987). Claeys et al. (2004) found Tricine-SDS–PAGE offers a better separation to quantify peptides in fresh meat in the molecular weight range of 3 ~ 17 kDa. However, if the size of hydrolysed peptides is under 5 kDa, it is almost undetectable using SDS-PAGE assay (Bordoni et al., 2014).

Kaur et al. (2016) investigated the effects of high pressure processing (HPP) on protein digestibility of bovine steaks using Tricine-SDS-PAGE. The main protein bands identified in bovine muscle digests were myosin heavy chain MHC (>220 kDa); β-actinin (132 kDa); actin (42 kDa); tropomyosin- β-chain (37 kDa); troponin T (35 kDa); tropomyosin- α-chain (33 kDa), MLC1 (22 kDa), MLC2 (16 kDa) and troponin C (19 kDa). They observed a greater and faster *in vitro* gastric digestion of polypeptides when applying to HPP treatment. Kaur et al. (2014) also found after pepsin hydrolysis of raw beef, some large peptides (74 ~ 91 kDa) which may come from the myosin heavy chain appeared, while cooked beef had more peptides with intermediate molecular weight (15 ~ 30 kDa). Thus they concluded meat cooking could lead to a greater and faster digestion of protein and polypeptides with a molecular weight over 25 kDa. During post-mortem storage, muscle pHu plays an important role in myofibrillar degradation. Beef structural proteins such as titin, filamin and nebulin had the rapidest degradation rate when the pHu was highest (G. Wu, Farouk, Clerens, & Rosenvold, 2014). In the current study, glycine-SDS-PAGE was applied to goat meat digests to compare the peptides released before and after *in vitro* gastric and intestinal digestion.

2.9 Overview of this study

From the above literatures, it is evident that goat meat is not nutritionally inferior to other frequently-consumed red meat like beef and lamb. The demand of lean goat meat is increasing worldwide. Considering adult goat is a bit tough and stringy, goat kid meat which has a better organoleptic quality than adult goat meat, in terms of low fatness, high meatiness etc, is able to meet both the nutritional and sensory requirements for the consumers.

The knowledge of the nutritional value of different cuts in goat kid meat is rarely present in the literature. Therefore, in this study, the proximate composition of 14 various cuts in goat kids was determined and protein digestibility was investigated by

comparing the free amino acids and peptides released before and after simulated *in vitro* digestion to provide in depth nutritional information of goat kid cuts.

Chapter 3 Materials and methods

3.1 Design of the study

Figure 4 shows the design of my study. After power calculation, 9 goats were chosen to apply proximate analysis whose specific procedures could be seen in section 3.2. Based on the results of proximate analysis, 3 goats were selected to accomplish further *in vitro* digestibility study (section 3.3). After sous-vide cooking (section 3.3.1), samples were prepared and collected in three phases: before (section 3.3.2), gastric (section 3.3.3.4) and intestinal (section 3.3.3.5). Two types of analyses were conducted on all the samples: key analysis on free amino acids (FAAs) using LC-MS (section 3.3.4) and subsidiary peptides analysis which did not apply statistics using SDS-PAGE (section 3.3.5).

In Tomović et al. (2016)'s publication on proximate composition of four muscles from Saanen goat male kids, the average standard deviation (SD) value was 0.425 g/100 g of muscle calculated based on the maximum SD 0.81 g/100 g and minimum SD 0.04 g/100 g. The averaged detectable difference was 0.8475 g/100 g. Using this detectable difference and SD we found that 9 samples (goats) should give our study more than 80% power at the 5% level of significance. Therefore 9 dressed carcasses of milk-fed Saanen male goat kids were supplied from AgResearch Ltd (Hamilton, New Zealand) after removing skin, viscera, head, fore feet and hind feet. They were slaughtered at an age of 31 days and had an average living weight of approximate 8.2 kg. Each carcass was deboned, trimmed of external fat, and cut into 14 parts: *Longissimus dorsi*, tenderloin, flap, knuckle, rump, outside round, hind shank, inside, cube roll, neck, fore shank, blade roll (chuck tender), cross cut and bolar as showed in Figure 5. All the cuts were vacuum-packed and stored in freezer until analysis.

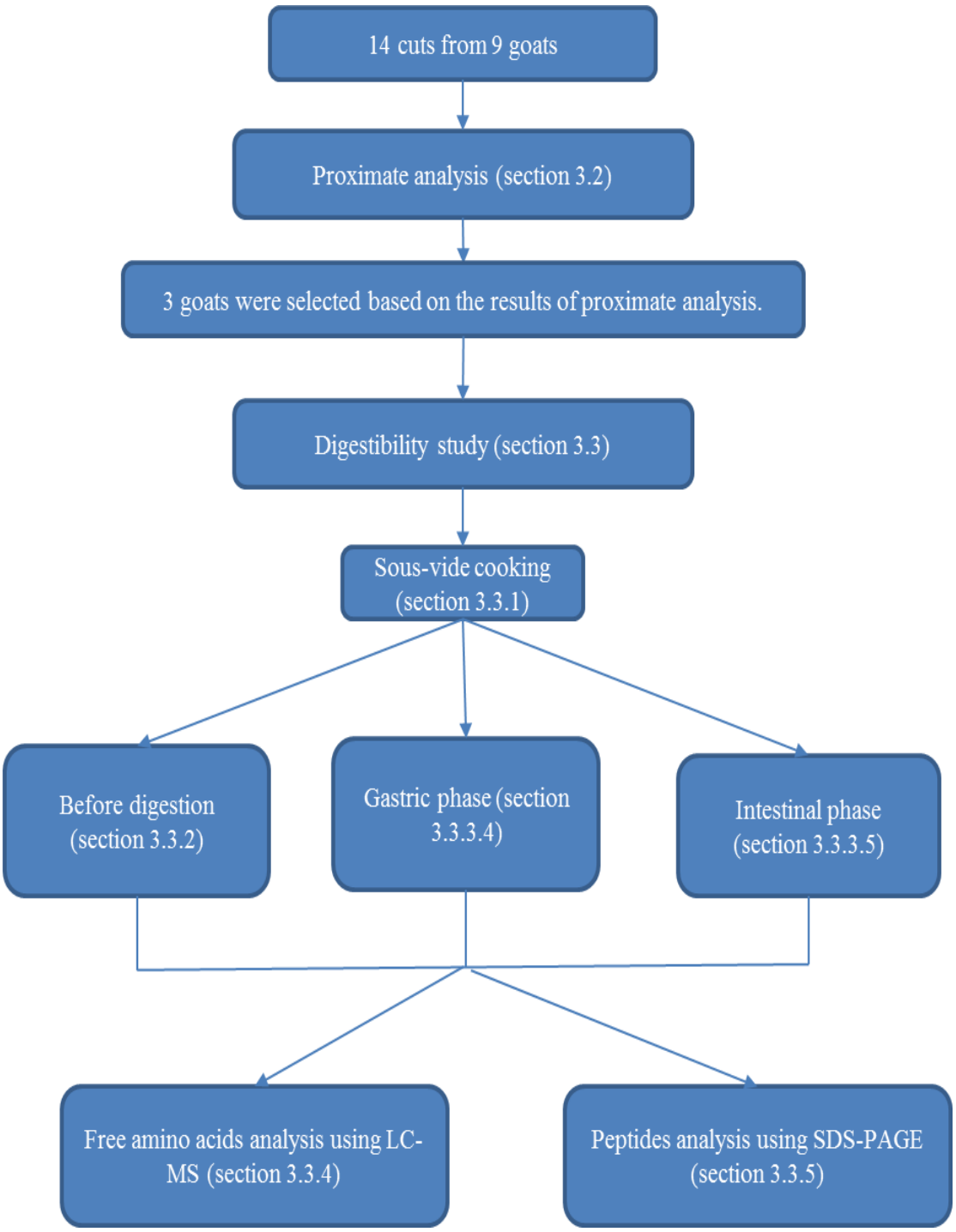

Figure 4. Overview of this study.

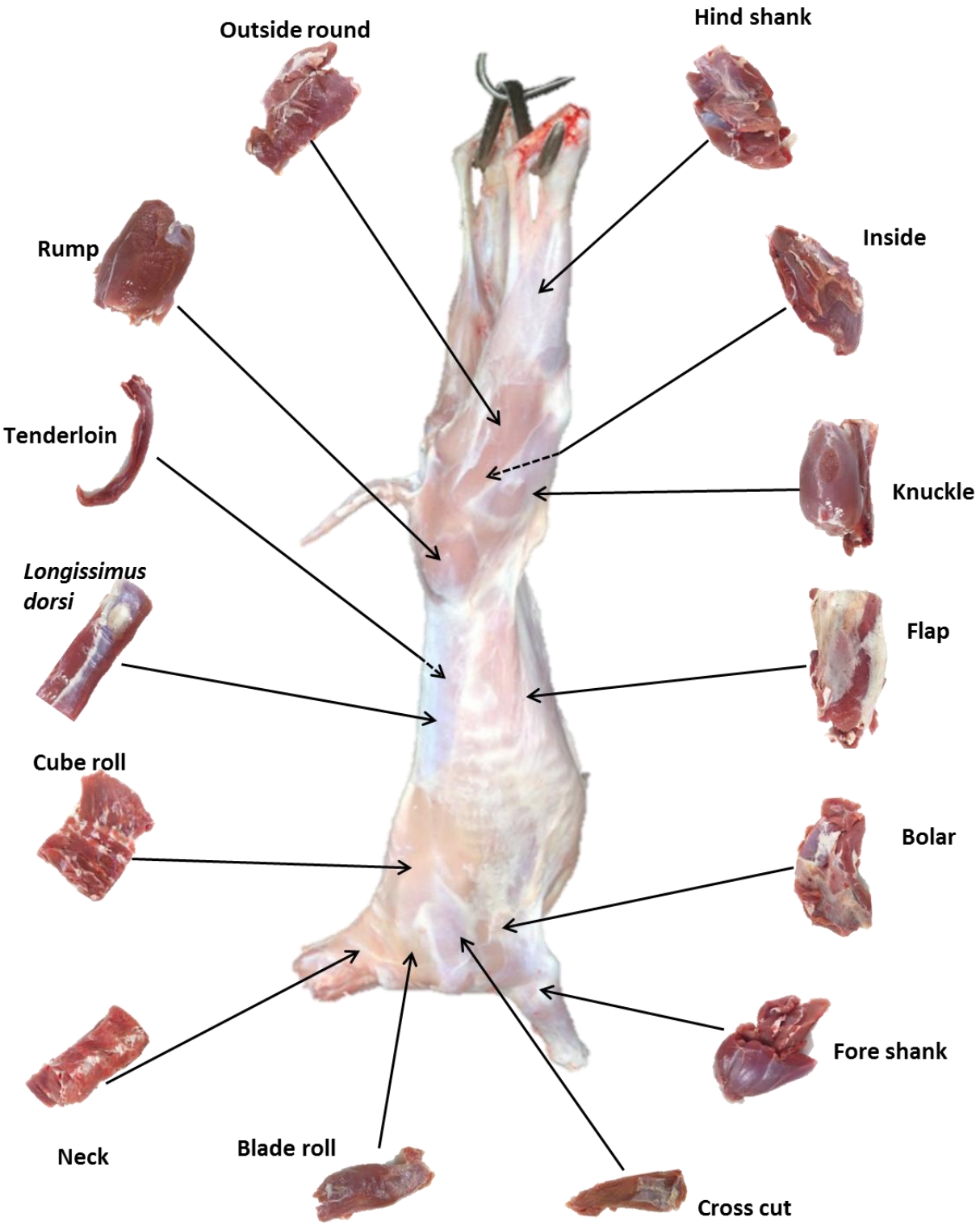

Figure 5. Anatomy of 14 goat cuts.

3.2 Proximate analysis

Proximate analysis refers to the determination of five constituents: moisture, protein, fat, ash and carbohydrates. The front four components can be measured via chemical experiments, while the determination of carbohydrates is based on the difference calculation between 100 and the sum of four others. With the development of food industry, a statistically refined and verified method has been developed to isolate and measure these constituents, which is known as the Official Methods of Analysis of AOAC international (Young et al., 2012). AOAC number is referenced to all individual proximate determinations. Adequate sampling and tissue mixing is a big concern when using AOAC method, thus samples should be finely minced and ground to make sure sampling is representative and even (Robert, 2012).

Before analysis, all 14 cuts of these 9 goat kid carcasses were defrosted overnight at 4^{o}C in a refrigerator and then divided into half. One half was completely minced into meat paste using a coffee blender (BCG200, Breville) and was used for proximate analysis. The other half was stored in high density polyethylene vacuum-packed bags (ZeroPak, New Zealand) in a freezer (-20^{o}C) for other analysis. All samples (14 cuts × 9 goats = 126 samples) were analysed in duplicates for proximate analysis.

3.2.1 Moisture determination

Moisture content is normally measured by traditional oven drying due to the volatility of water when temperature is above 100°C. This standard method has a low cost and is easy to handle. It can analyse a large number of samples simultaneously with minimal manpower and no chemical consumption (Milica et al., 2015). But this method is quite time-consuming (Young et al., 2012). Except oven drying, moisture can also be determined by ultraviolet light drying, infrared drying, microwave drying and some other methods (Young et al., 2012).

In this study, moisture content was determined using oven drying method according to AOAC 950.46B. Convection oven (SANYO Electric Biomedical Co. Ltd, Japan) was

preheated to 101°C in advance. Empty crucibles were dried in the oven for 3 h and transferred to desiccator to cool until reaching the constant weight. The weight (W_1) was recorded. About 5 g of ground goat meat was weighed (W_2) into crucibles and spread out. The crucibles with meat were placed in the oven and and dried for 16 ~ 18 hours. After drying, crucibles were transferred to the desiccator to cool down to room temperature preventing the moisture absorption in the ambient environment. Dried samples and crucibles were reweighed (W_3) until constant weight was achieved. The moisture content was calculated as follows:

$$\text{Moisture (g/100g)} = \frac{W_2 - (W_3 - W_1)}{W_2} \times 100$$

W_1 = Weight of empty crucibles (g)
W_2 = Weight of initial sample before drying (g)
W_3 = Weight of crucibles and dried samples (g)

3.2.2 Ash determination

The most conventional methodology for measuring ash content is dry ashing which incinerates samples in a muffle furnace at a high temperature around 500 ~ 600°C until the fluffy light gray ash occurs. In this ashing process, organic matters are oxidized to carbon dioxide and nitrogen oxides. Minerals like potassium, phosphorous, calcium, sodium or magnesium will change to oxides, phosphates, sulfates, carbonates or silicates (Milica et al., 2015; Young et al., 2012). This dry ashing method does not need chemicals so it has a low cost as well as the low safety risks, but it's also time-consuming (Milica et al., 2015).

In this study, ash content was measured according to AOAC 920.153 (AOAC, 2012). After moisture determination, crucibles with dried samples were heated over low Bunsen flame until fumes were no longer produced to burn away the organic materials in the samples. The crucibles were placed in a muffle furnace (Model 200, McGregor Kiln Furnace) and heated to 550°C for 6 hours. Upon the completion of ashing, the crucibles were left in the furnace to cool down. The door of furnace was opened carefully to avoid any loss of ash. Crucibles were then transferred to a desiccator to cool

down to room temperature. Gray samples and crucibles were weighed (W_4). The calculation followed the formula below:

$$\text{Ash (g/100g)} = \frac{W_4 - W_1}{W_2} \times 100$$

W_1 = Weight of empty crucibles (g)
W_2 = Weight of initial sample before drying and ashing (g)
W_4 = Weight of crucibles and incinerated samples (g)

3.2.3 Crude protein determination

Standard method for crude protein determination in AOAC is the extensively-accepted Kjeldahl method which was invented by the Danish chemist Johan Kjeldahl in 1993. Protein consists of amino acids and all amino acids contain nitrogen so protein content can be estimated by measuring the quantity of nitrogen if a fixed relationship presents between nitrogen mass and protein mass. Although individual amino acid is different in nitrogen percentage by weight, the primary myofibrillar protein actin, myosin and tropomyosin in meat all contain about 16% nitrogen (Gutheil & Bailey, 1993; Young et al., 2012). Thus the nitrogen-to-protein conversion factor 6.25 is normally used for meat (Young et al., 2012).

The whole Kjeldahl process can be classified into five steps: digestion, neutralization, distillation, condensation and titration (Young et al., 2012). First, sample is decomposed and digested in concentrated sulfuric acid with catalysts which are mainly potassium sulphate and copper sulphate at high temperature. This boiling procedure converts carbonaceous, hydrogenous and nitrogenous components to carbon dioxide (CO_2), water (H_2O) and ammonium sulphate ($(NH_4)_2SO_4$) respectively. The chemical equation is listed below: $Sample + H_2SO_4 \rightarrow (NH_4)_2SO_4 + CO_2 + H_2O + SO_2 +$ *other sample matrix by-products.* After digestion and cooling down, excess alkali, mostly sodium hydroxide (NaOH), is added into that transparent and clear solution, resulting in the conversion of ammonium sulphate to ammonia. This neutralized reaction is conducted as follows: $2NaOH + (NH_4)_2SO_4 \rightarrow Na_2SO_4 + 2\,NH_3 + 2H_2O$. Then the free ammonia

is distilled and carried by steam passing along a condensation coil. Ammonia is condensed after passing over it and collected in a trapping solution which can either be a weak boric acid (H_3BO_3) or a standardized hydrochloric acid (HCl) (Labconco, 1998; Young et al., 2012). If it is received in boric acid, an ammonium-borate complex forms and the pH increases (Labconco, 1998). Then a known concentration hydrochloric acid should be added to neutralize the ammonium-borate complex and drop the pH to the original pH. As the formula shows: $NH_3 + H_3BO_3 \rightarrow NH_4H_2BO_3$. $NH_4H_2BO_3 + HCl \rightarrow NH_4Cl + H_3BO_3$. If it is received in hydrochloric acid, the excess acid will be back titrated to neutrality with a standardized sodium hydroxide. Chemical reactions are as follows: $NH_3 + HCl \rightarrow NH_4Cl$. $NH_4Cl + HCl + NaOH \rightarrow NH_4Cl + NaCl + H_2O$. Except digestion, the other four steps can be finished in a semiautomatic machine to simplify and fasten the whole process.

In this study, the Kjeldahl assay conducted to determine the crude protein was according to AOAC 928.08 (AOAC, 2012). About 1 g of minced meat cuts was accurately weighed and placed at the base of the 250 ml Kjeldahl's digestion tube. 7 g potassium sulphate (K_2SO_4) (BSPPL453.500, LabServ, Thermo Fisher Scientific New Zealand Ltd) and 0.5 g copper sulphate ($CuSO_4$) (CO0096, Scharlau Chemie S.A., Spain) were also weighed and added into the digestion tube as catalysts. The digestion tubes were transferred to fume hood where 12 ml concentrated sulphuric acid (H_2SO_4) (98% Sulphuric acid, AJA534, ThermoFisher Scientific Australia Pty Ltd, Australia) was carefully added to the tube and mixed with other contents prior to digestion. Blank tube contained all the chemicals except the meat sample. Then all the digestion tubes were put on a heating block (Velp DK 20 digester, Italy) and covered with suction cap as shown in Figure 6A. The whole digestion setup comprised of a digester, suction cap, a fume neutralizer and a water jet pump, was turned on. The digester was heated to 420°C. When the temperature went up, a large amount of corrosive and toxic acid fumes including sulfur dioxide (SO_2) and sulfur trioxide (SO_3) evolved. The JP recirculating water pump (VELP Scientifica srl, Italy) containing a plastic water tank in which an impeller is driven by an electric motor was used for fume aspiration by producing a vacuum air flow. The gases produced during digestion were sucked out of the suction cap through Venturi ejectors. The ejected acid fumes were then condensed, neutralized and absorbed by fume neutralizer Scrubber SMS (VELP Scientifica srl, Italy) without releasing any corrosive acid vapors to the environment. After about one and half hour when digestion was completed, the solution in the digestion tube became

clear and transparent with no undigested matter adhering to the walls of the tube. The digester was shut down to let tubes cool down. After digestion, the sample was distilled in an automated VAPODEST 450 distillation unit (Gerhardt GmbH & Co. KG, Germany) as shown in Figure 6B.

Figure 6. The Kjeldahl digestion apparatus.

6A Velp DK 20 digester 6B VAPODEST 450 distillation unit

50 ml of distilled water, 80 ml of 35% NaOH and 80 ml of 4% boric acid were added to the digestion tube. 5 minutes of distillation and titration time were allowed. The tube containing the digested meat sample was placed in the apparatus and the automatic distillation process was started. The sample was made strongly alkaline and the pH of the mixture was increased after the addition of 35% NaOH solution. Under the alkaline condition, the ammonium ions (NH_4^+) were fully converted into ammonia (NH_3). The released ammonia was steam-distilled into a collector filled with 20 ml of 4% boric acid (H_3BO_3) that had a pH of about 3.9 after passing over a condensation coil. As the ammonia collects, the pH of the acid solution increased. When distillation was finished, back titration with 0.1 M HCl started to neutralize the ammonium borate complex until reaching the initial pH of the boric acid. At this point all the ammonia had been titrated. The pH was determined by a pH meter and the consumed volume of 0.1 M HCl was showed on the titrator panel.

The crude protein content in goat meat cut was calculated according to formula below:

$$\text{Protein (g/100g)} = \frac{V(HCl_{sample} - HCl_{blank}) \times C_{HCl} \times Mr(N) \times \text{Conversion factor}}{W_{sample}} \times 100$$

$V(HCl_{sample})$ = Volume of HCl used in sample titration (ml)

$V(HCl_{blank})$ = Volume of HCl used in blank titration (ml)

C_{HCl} (Concentration of HCl) = 0.1 M

Mr(N) (Molar mass of nitrogen) = 14.007 g/mol

Nitrogen-protein conversion factor for meat = 6.25

W_{sample} = Weight of meat samples (g)

3.2.4 Crude fat determination

In crude fat determination, samples should be dried using vacuum drying or low temperature to avoid the co-extraction of water-soluble substances like lactic acid, urea or carbohydrates. Non-toxic and non-flammable solvents with a low boiling point are preferred to use. Ether is a kind of frequently-used organic solvent which mostly extracts triglycerides which occupies 95% of the total lipids, so crude fat is also defined as diethyl ether or petroleum ether-extractable lipid content (Petracci & BaÉZa, 2011). If the organic solvent is the mixture of methanol and chloroform, which is famous as Folch method, polar lipid compounds can be extracted along with crude fat, such as phospholipids, so the polarity of organic solvent is one of the important factors that can influence extraction (Young et al., 2012). Besides that, extraction time, temperature, moisture content, carbohydrates and hydrolysis can also affect extraction.

Traditional method to measure the crude fat is to assemble soxhlet apparatus manually using paper thimble, condenser, extraction tube, bottle and heating mantle, which is quite time-consuming and inconvenient (Petracci & BaÉZa, 2011). The automatic extraction equipment can accelerate this process. After several continuous solvent reflux, crude fat will be extracted and then these fat residues can be dried to a constant weight by evaporating the remaining solvent (Young et al., 2012). The percentage of fat content is calculated based on the initial wet sample weight.

In this study, the determination of crude fat in goat cuts was slightly modified from AOAC 991.36 (AOAC, 2012) using assembled Soxhlet apparatus. Thermotec 2000 oven (Contherm Scientific Ltd, New Zealand) was preheated to 101°C in advance. Empty aluminum dishes (Cole-Parmer 43 mm Aluminum Crimpled-Walled Weighing Dishes with Tab, 20 ml, 1000/C, EW-01017-51, United States) and degreasing cottons were dried in the oven for 3 h and transferred to desiccator to cool until reaching the constant weight. Approx. 5 g of ground meat sample was weighed precisely (W_1) into the dish and completely wrapped by the dish and cotton as shown in Figure 7A. After wrapping, they were put in the oven at 101^{o}C for 18 hours to evaporate all the moisture in the meat samples. The weight of dried meat with dish and cotton was recorded (W_2). The Soxhlet apparatus was assembled with a condenser, an extraction tube, a boiling flask and a heating mantle as shown in Figure 7B.

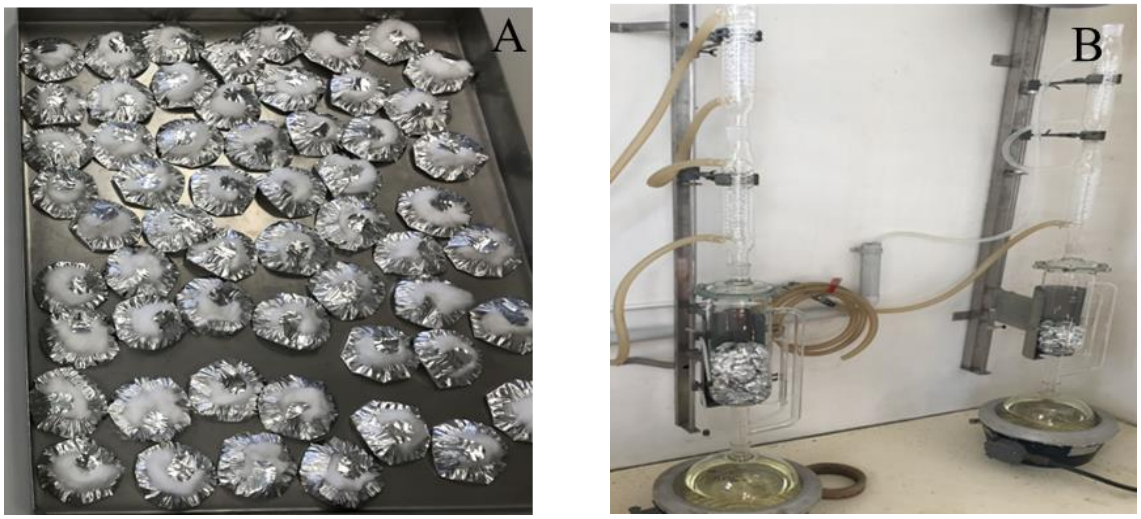

Figure 7. The Soxhlet apparatus.

7A Meat samples wrapped by the dish and cotton 7B Soxhlet apparatus

All of the dried samples were put into extraction tubes to expedite the extraction process. 2.5 L of petroleum ether (ACS reagent, boiling range 40 ~ 60°C, ACROS Organics™, Fisher Scientific, UK) was poured into each boiling flask which then was connected with the other two accessories. The water supply and heating mantle was turned on to start the fat extraction. The sample was extracted for 48 hours at a heating rate of 150 condensed drops per minute. When all the fat had been extracted and almost all the petroleum ether came back to the boiling flask, extraction tube was released and

the samples inside was taken out of the extractor into a fume hood for 2 hours to let the remaining petroleum ether evaporate. When the cotton was dried without any obvious kerosene-like odor, samples were transferred to the oven at 101°C for 2 hours to make sure all the solvents had evaporated. After that, the samples were taken to a desiccator to cool until reaching constant weight. The weight of dried and defatted samples with dish and cotton were recorded again (W_3). The calculation of crude fat in goat meat followed the equation below:

$$\text{Fat (g/100g)} = \frac{W_2 - W_3}{W_1} \times 100$$

W_1 = Weight of initial sample before drying and extraction (g)

W_2 = Weight of dried meat with dish and cotton (g)

W_3 = Weight of dried and defatted meat with dish and cotton (g)

3.3 Digestibility study

Based on the proximate analysis results and the free amino acids content in cured Boar goats published by Paleari et al. in 2003, using the maximum standard error (SE) 59.78 mg/100 g of meat, there would be a 80% chance of detecting a significant difference more than 287.25 mg/100 g of meat in the treatments if 3 animals were used. Thus 3 goats were randomly chosen to accomplish digestibility study. First of all, all of the goat cuts were cooked using sous-vide method (refer to section 3.3.1) to minimize the water loss and maintain nutrients maximally, and then minced for 1 minute using a coffee blender (BCG200, Breville). Before digestion started, free amino acids (FAAs) from cooked and minced samples were extracted (refer to section 3.3.2.1). Minced meat samples were homogenized with HCl to prepare further analysis for SDS-PAGE (section 3.3.2.2). *In vitro* digestion simulation can be found in section 3.3.3. The preparatory work included the preparation of digestion fluids (section 3.3.3.1) and the preparation of enzymes (section 3.3.3.2). This simulated assay contained three phases: oral phase (section 3.3.3.3), gastric phase (section 3.3.3.4) and intestinal phase (section 3.3.3.5). During digestion, samples were collected from the endpoint of stomach and intestine phases, respectively. The FAAs contents (section 3.3.4) and SDS-PAGE analysis (section 3.3.5) were conducted on all the samples taken out from before digestion, gastric and intestinal phases.

3.3.1 Sous-vide cooking

Vacuum-packed goat cuts were defrosted at 4^{o}C overnight in a refrigerator and put into a water bath (Model 360, Conthern, New Zealand) which was preheated to 60^{o}C and then cooked for 2 hours (Baldwin, 2011). All the cooked cuts were finely homogenized for 1 minute using a coffee blender (BCG200, Breville). Liquid coming out of the meat during cooking was poured back into the blender and mixed with meat granules evenly to prevent water loss. The samples were cooked and ground the day before conducting pre- or post- digestion experiment, vacuum-packed and stored overnight in the refrigerator until use.

3.3.2 Prior to digestion

3.3.2.1 FAAs extraction prior to digestion

The extraction of FAAs in cooked goat meat before digestion was adapted from Mustafa et al. (2007). Approx. 1 g homogenized meat sample was weighed into a 50 mL polypropylene centrifuge tube (LBSCN8CT50, LabServ) and mixed with 5.4 g glass beads of 0.2 cm diameter and 7.5 ml of methanol (≥ 99.9% for HPLC, Sigma-Aldrich). The mixture was shaken at a highest speed of 10 (range from 0 to 10) using a vortex-genie (Vortex-Genie 2, Scientific Industries, Inc.) for 30 minutes. Then the tubes were centrifuged at 2000 rpm (1580 R, Gyrozen) for 10 minutes. The supernatant was transferred to a 1.5 ml micro-centrifuge tube and further centrifuged at 10000 rpm (Z 216 MK, Hermle Labortechnik GmbH, Wehingen) for another 10 minutes. After removing all of the meat micelles, the clear supernatant containing FAAs (S_1) was stored in a freezer (-20^{o}C) for future analysis.

3.3.2.2 SDS-PAGE sample preparation prior to digestion

For each cut sampling, approx. 1 g meat from the same cut of those three animals was weighed and three batches samples were put together in a 50 ml polypropylene centrifuge tube. After adding 20 ml of 0.1 M HCl, mixed samples were homogenized

thoroughly (Ultra-Turrax, T25 basic, IKA Labortechnik) at 16000 rpm for 15s to obtain a representative pooled sample (S_2).

3.3.3 Simulated static *in vitro* digestion

The simulated static *in vitro* digestion assay used to study the digestibility of different goat kid cuts was based on a standardized method proposed by Minekus et al. (2014) with slight modifications.

Three consecutive stages: oral, gastric and small intestinal phases were mimicked throughout the digestion. In each phase, corresponding simulated fluids, enzymes and Milli-Q water from a water purification machine (Puripac PP8, Part No. L991543, SUEZ Water Purification Systems Ltd, UK) were added. Cooked meat samples were weighed into screw-capped Schott bottles. A shaking water bath (Gyrotory Water Bath Shaker, Model G76, New Brunswick Scientific Co., INC, Edison, N. J. U.S.A.) which was pre-warmed to 37ºC shaking at a speed of 5 rpm was used to mimic the peristalsis process happening in human GI tract. The pH was monitored every 1 minute by a pH meter (HI 4221, HANA Instruments) and adjusted to 7, 3 and 7 for those 3 consecutive phases using 1 M and 6 M HCl and NaOH (prepared from 37% HCl and NaOH pellets). Then the Schott bottles were put into the shaking water bath and heated to 37ºC followed by adding the relevant enzymes. The incubation time was 5 min for oral phase, 120 min for gastric phase and 180 min for small intestinal phase. Samples taken out from gastric and small intestinal phases were immediately snap-frozen in liquid nitrogen to inactivate enzymes and stored in freezer for further FAAs and SDS-PAGE analysis.

3.3.3.1 Preparation of simulated digestion fluids

Based on the human *in vivo* data, different concentrations of electrolytes in final simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were recommended by Minekus et al. (2014). Various individual electrolyte stock solutions were prepared in accordance to Table 5. Specifically, 37.3 g/L potassium chloride (KCl, L 484, May & Baker Australia Pty Ltd), 68 g/L monopotassium phosphate (KH_2PO_4, Ajax Chemicals, Sydney, Australia), 84 g/L sodium bicarbonate ($NaHCO_3$, SO01310500, reagent grade, Scharlau Chemie S.A.,

Spain), 117 g/L sodium chloride (NaCl, S/3160/60, analytical reagent grade, Fisher Scientific UK), 30.5 g/L magnesium chloride hexahydrate ($MgCl_2(H_2O)_6$, 101494V, BDH, England), 48 g/L ammonium carbonate ($(NH_4)_2CO_3$, 100153W, BDH, England) and 44.1 g/L calcium chloride dihydrate ($CaCl_2(H_2O)_2$, C3306, Sigma-Aldrich) were prepared using Milli-Q water and to achieve the required stock concentration of these constituents. The final digestion fluids SSF, SGF and SIF actually are mixtures containing these individual electrolytes solutions, enzymes, water etc. Thus to get a volume of 500 ml final simulated fluids, 1.25 × concentrates referred to as SSF, SGF and SIF electrolyte stock solutions which contain almost all of these individual electrolyte stock solutions except $CaCl_2(H_2O)_2$ due to the possible precipitation caused by $CaCl_2(H_2O)_2$ were made up to 400 ml first. Then the corresponding volume of enzymes, Ca^{2+} solution, bile salts (Bile extract porcine, B8631, Sigma-Aldrich) and water were added in sequence leading to the required human electrolyte concentration in each final 500 ml digestion mixture.

Take the preparation of SSF electrolyte stock solution as example, 15.1 ml of KCl, 3.7 ml of KH_2PO_4, 6.8 ml of $NaHCO_3$, 0.5 ml of $MgCl_2(H_2O)_6$ and 0.06 ml of $(NH_4)_2CO_3$ were pipetted into the 100 ml volumetric flask and the mixture was diluted to the mark with Milli-Q water. This 100 ml solution was transferred to a large 500 ml Schott bottle and 300 ml Milli-Q water was added to make up a 400 ml SSF electrolyte stock solution. Similar processes were conducted for the other two SGF and SIF stock solutions according to Table 5. These stock solutions were stored in refrigerator for digestion experiments.

3.3.3.2 Preparation of enzymes

Before digestion started, SSF, SGF and SIF electrolyte stock solutions were put into an oven to be incubated to 37ºC. The shaking water bath was set to 37ºC temperature and 5 rpm speed. 1 M and 6 M HCl were made up from concentrated HCl (37% HCl, Sigma-Aldrich). 1 M and 6 M NaOH were prepared from anhydrous NaOH pellets (≥ 98%, Sigma-Aldrich). Enzymes were made freshly on the day before digestion started. 0.5 g α-amylase from the *Aspergillus oryzae* (≥ 150 units/mg protein, A9857, Sigma-Aldrich) was weighed accurately and dissolved in 50 ml SSF electrolyte stock solution to achieve 1500 U/mL. 5 g pepsin from porcine gastric mucosa (P7000, ≥ 250 units/mg solid,

Sigma-Aldrich), 1.07 g pancreatin from porcine pancreas (Pancreatin 3X U.S.P., MP Biomedicals, LLC) and 1.92 g bile (Bile extract porcine, B8631, Sigma-Aldrich) were dissolved by 50 ml SGF, 100 ml SIF electrolyte stock solutions and 50 ml Milli-Q water, respectively, to reach 25000U/mL, 800 U/mL based on trypsin activity in the corresponding electrolyte solution and 160 mM in water as mentioned by Minekus et al. (2014).

Table 5. Preparation of individual electrolyte stock solution and SSF, SGF and SIF electrolyte stock solution (Minekus et al., 2014).

Constituent	Individual stock conc. g/L	SSF electrolyte stock solution pH=7 Vol. of individual stocks (mL)	SGF electrolyte stock solution pH=3 Vol. of individual stocks (mL)	SIF electrolyte stock solution pH=7 Vol. of individual stocks (mL)
KCl	37.3	15.1	6.9	6.8
KH_2PO_4	68	3.7	0.9	0.8
$NaHCO_3$	84	6.8	12.5	42.5
NaCl	117	0	11.8	9.6
$MgCl_2(H_2O)_6$	30.5	0.5	0.4	1.1
$(NH_4)_2CO_3$	48	0.06	0.5	0
$CaCl_2(H_2O)_2$	44.1	-	-	-

3.3.3.3 Oral phase

Mastication process in oral cavity was mimicked by mincing the cooked goat kid meat using coffee blender. 5 g homogenized goat kid meat was weighed into a 50 ml Schott bottle and mixed with 3.5 ml SSF electrolyte stock solution, 25 µl $CaCl_2$ and 975 µl Milli-Q water. The pH of the mixture was adjusted to 7 using 1 M or 6 M NaOH. Schott bottles were then put into the shaking water bath to be heated to 37^{o}C. 0.5 ml amylase

(1500 U/mL, A9857, Sigma-Aldrich) was added into mixture to acquire the final concentration of 75 U/mL and a 50 : 50 (w/v) ratio of meat to SSF. The paste-like meat bolus was shaken at 37°C for 5 minutes. No sample was taken at this step.

3.3.3.4 Gastric phase

In simulated gastric phase, the final ratio of 10 ml of meat bolus to SGF was adjusted to 50 : 50 (v/v) by adding 7.5 ml of SGF electrolyte stock solution, 5 µl of $CaCl_2$, 695 µl of Milli-Q water, 0.2 ml of 1 M HCl and 1.6 ml of 25000U/mL pepsin (P7000, Sigma-Aldrich) after the pH was regulated to 3 using 1 M or 6 M HCl and the solution temperature reached 37°C. The digestion reactor was further incubated for 2 hours at 37°C. A 2 ml sample (S_3) was collected into a 15 ml polypropylene centrifuge tube (LBSCN8CT15, LabServ) at the end of gastric phase and snap-frozen using liquid nitrogen to inactivate pepsin and stop gastric digestion, and was stored in a freezer (-20°C) until further analyses.

3.3.3.5 Intestinal phase

The final ratio between the meat chyme coming from gastric phase and SIF was adjusted to 50 : 50 (v/v). 11 ml of SIF electrolyte stock solution, 40 µl of $CaCl_2$, 1.31 ml of Milli-Q water and 0.15 ml of 1 M NaOH were added first to the chyme. After the pH of the mixture was adjusted to 7 and the temperature reached 37°C, 5 ml pancreatin solution (800 U/mL based on trypsin activity, Pancreatin 3X U.S.P., MP Biomedicals, LLC), 2.5 ml of 160 mM bile (B8631, Sigma-Aldrich) and 0.8 g lipase (originating from *Thermomyces lanuginosus*, Lipozyme® TL 100 L, 100 KLU/g, Novozymes) were added to achieve 100 U/mL based on trypsin activity, 10 mM, 2000U/mL in the final mixture (approx. 40 ml) according to Minekus et al. (2014). A 5 ml sample (S_4) was collected into a 15 ml polypropylene centrifuge tube after 3 hours of incubation time and immediately frozen with liquid nitrogen to avoid further digestion. The sample was stored in a freezer (-20°C) until further analyses.

3.3.4 Free amino acids (FAAs) analysis

3.3.4.1 Amino acids derivatization

Samples taken out after gastric and intestinal phases were defrosted at room temperature and centrifuged at 2000 rpm (Centrifuge 5810 R, Eppendorf) for 10 minutes. The supernatant was transferred to a 1.5 mL micro-centrifuge tube and further centrifuged at 10000 rpm for another 10 minutes to remove the small precipitates. Then for each goat cut, FAAs of three clear supernatants (S_1, S_3 and S_4) were derivatized using a modified method from Armenta et al. (2009).

Prior to derivatization, three reagents and standard samples were prepared. 1 ml formic acid (101156G, 98-100%, BDH, England) and 99 ml ultrapure water were added in a Nalgene bottle to make neutralizing solution. 7.63 g of sodium tetraborate decahydrate (N1025633-1, Pure Science, New Zealand) was weighed into a Nalgene bottle. 90ml ultrapure water and 10ml acetonitrile were added as well, and the pH was adjusted to 8.8 using 1 M HCl. The 200 mM sodium tetraborate buffer with pH 8.8 was called borate buffer. 2.8g/L 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Accutag reagent or AQC reagent, BIB6284, Apollo Scientific Limited, Manchester, UK) was made up in dry acetonitrile. Borate buffer and neutralizing solution were stored in a fridge at 4^{o}C. Accutag reagent was stored in a dessicator in the fridge.

20 µl of 500 µM standard mix (A9906, Sigma-Aldrich) was pipetted to a micro-centrifuge tube. 26.5 µl of 50 mg/L asparagine stock and 29 µl of 50 mg/L glutamine stock were added into the same tube additionally because they were unstable and did not exist in the standard mix. 18.5 µl of 50 mg/L d4-alanine (d4-ALA) stock was added as an internal standard. Then the remaining volume was made up to 100 µl using neutralising solution. This vial was vortexed and labelled with “STD A”. Standard A (100 µM) was serially diluted using neutralising solution to produce concentrations of 50, 25, 12.5, 6.25, 3.125, 1.5625 µM, etc. These standards, samples and a solvent blank (neutralizing solution) were derivatized according to the protocol below.

All the samples, standards and blanks were centrifuged at 10,000 g for 5 minutes at 4°C before derivatization to get rid of all the particulates. For every 20 samples, 15 µl 50 mg/L d4-ALA stock was added to 1485 µl borate buffer in a micro-centrifuge tube to

make a new borate buffer spiked with internal standard. For each sample, 70 µl spiked borate buffer, 10 µl sample and 10 µl Accutag reagent were added in an Eppendorf tube, and the mixture was vortexed immediately. The standards and blanks were derivatized the same way but with unspiked borate buffer. The Eppendorf tubes were incubated at 55°C for 15 minutes. After incubation, the tubes were vortexed again and centrifuged at 10,000 g for 2 minutes at 4°C. 450 µl neutralizing solution and 50 µl supernatant from the reaction mixture were pipetted into a clean, amber, 1.8 ml autosampler vial (THC1109520, ThermoFisher). The autosampler vials were capped, vortexed and stored at 4°C until analyzed by LC-MS.

3.3.4.2 LC-MS condition

The FAAs analysis of derivatized samples were carried out by Agilent 1260 Infinity HPLC system equipped with Agilent 6420 Triple Quadrupole LC/MS system (Agilent Technologies New Zealand Limited, New Zealand) operated a positive electrospray ionization mode with MRM detection. The 100 × 2.1 mm with a 1.7 µm particle size C18 core shell Kinetex LC column (00D-4726-AN, Kinetex®) was used to separate different FAAs. The mobile phase was a binary gradient system consisting of gradient A which was acetonitrile with 0.1% formic acid, and gradient B which was Milli-Q water with 0.6% formic acid. FAAs were eluted with a linear gradient of 1.5% to 80% A and 98.5% to 20% B in 26 minutes followed by a 1.5 min re-equilibration phase to initial conditions. The flow rate value was 0.3 ml/min and the injection volume was 2 µl. The retention time of each amino acid was identified by comparing with individual known amino acid standard. The data was acquired and analysed quantitatively by MassHunter QQQ Quantitative Acquisition software (B.07.00) and qualitatively by MassHunter Qualitative Analysis software (B.06.00). The concentrations of FAAs were corrected by their relative response factors using the value of internal standard d4-ALA and expressed as mg/100 g of cooked meat samples.

3.3.5 SDS-PAGE analysis

3.3.5.1 Buffer preparation and sample dilution

20X NuPAGE® MES SDS running buffer (NP0002, Life Technologies New Zealand Limited) was purchased. 100 ml of 20 × running buffer was diluted with 1900 ml Milli-Q water to get a 1 × MES running buffer. 10 ml of 1 M Tris-HCl (JT Baker® 4109-02) with a pH of 6.8, 20 ml of glycerol (Fisher Chemical G/0650/17), 4 g of SDS (Fisher Chemical, BP166-500), 10 ml 2-mercaptoethanol (BDH Laboratory Supplies, England) and 4 mg of bromophenol blue were mixed together, and the remaining volume was made up to 100 ml with Milli-Q water to acquire 100 ml of 2X SDS sample loading buffer. 1 × sample loading buffer was obtained by diluting 50 ml of 2X sample loading buffer with 50 ml Milli-Q water so the final concentration of 1 × sample loading buffer was 50mM Tris-HCl, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol and 0.002% bromophenol blue. Gel staining dye Coomassie blue G-250 was composed of 34% methanol (Fisher Chemical A452-4), 17% ammonium sulphate (BSPAL941.500, Thermo Fisher Scientific New Zealand Ltd), 2% phosphoric acid (345245, Sigma-Aldrich) and 0.04% Coomassie blue G-250 (161-0406, Bio-Rad).

Aliquots of three batches of samples (S_2, S_3, S_4) were collected into micro-centrifuge tubes and diluted 1 : 1 with 2X SDS sample loading buffer first to achieve a consistent final concentration same with 1 × sample loading buffer, and then diluted with the corresponding times using 1 × sample loading buffer to obtain a similar final protein concentration (about 4 mg/mL) before loading on the gel.

3.3.5.2 Electrophoresis

Proteins and peptides of goat kid meat before and after digestion were quantified by 1D SDS-PAGE using NuPAGE™ 10% Bis-Tris Midi Protein Gels (WG1202BOX, Life Technologies New Zealand Limited). Micro-centrifuge tubes containing samples were heated for 5 minutes at 100°C, well-mixed and centrifuged at 10,000 rpm for 5 minutes. Gel cassette was taken out of packages and Novex® Midi gel adapter (WA0999, Life Technologies New Zealand Limited) was adhered to the cassette. After removing the comb and the white tape at the bottom, gel cassette was inserted the electrophoresis chamber (Criterion™ Vertical Electrophoresis Cell, Bio-Rad) which was filled with 1× MES running buffer. The Novex™ Sharp Pre-stained Protein Standard (3.5 ~ 260 kDa) (LC 5800, Life Technologies New Zealand Limited) was applied as a reference. 8 µl protein standard and approx. 10 µl supernatants from samples were loaded on the gel to

achieve 40 µg protein per well. The chamber was covered by the lid with the red electrode attached to the red wire and the black electrode to the black wire. The electrophoresis was conducted at a constant voltage of 150 V for 1 hour. After finishing the electrophoresis, the gel taken out from the cassette was placed on a tray filled with Milli-Q water and washed for 3 minutes on a shaker. The gel then was stained using Coomassie blue G-250 for 24 hours after removing water, and it was washed with Milli-Q water trice when completing staining. A calibrated densitometer (GS-900™, Bio-Rad) was used to scan the gel and the Image Lab™ software (version 5.2.1, Bio-Rad) was used to analyse the gel.

3.4 Statistical analysis

The collected data were analyzed descriptively and statistically by R 3.3.2 (R core team, 2016) with the different cuts and amino acids as the main effects. Proximate analysis data were analyzed by one way analysis of variance (ANOVA), performed at 5% significance level using basic packages. Linear mixed effect model was performed by installing "lmerTest" package. Multiple comparisons of group means were achieved using "least-squares means lsmeans" package. "Generalized least-squares" model was built to minimize the variance heterogeneity and non-normality when analyzing FAAs data. Differences of $p \leq 0.05$ were considered to be significant. The results were presented in the tables as means and standard error of difference (SED) to compare the difference between means.

Chapter 4 Results and Discussion

4.1 Proximate analysis

The proximate analyses of 14 uncooked cuts from Saanen goat kids were conducted according to the standard procedures of AOAC (2012). The results were given as the mean value of 9 goats and the standard error of difference (SED) to compare the variability of means among different cuts. As shown in Table 6, the proximate composition of these 14 cuts differed from each other in terms of moisture, fat and protein ($P < 0.001$). But as for ash content, there was no significant difference among various cuts ($P > 0.05$).

Table 6. Proximate composition of goat kid cuts (% of raw meat).

		Moisture	Fat	Protein	Ash
Hindquarter	*Longissimus dorsi*	74.77^{b}	2.55^{ab}	22.77^{d}	1.08^{a}
	Tenderloin	74.95^{b}	2.12^{a}	22.71^{d}	1.05^{a}
	Flap	66.71^{a}	12.81^{c}	18.94^{a}	0.97^{a}
	Knuckle	76.27^{b}	2.75^{ab}	20.76^{bc}	1.06^{a}
	Rump	75.50^{b}	2.50^{ab}	21.57^{cd}	1.01^{a}
	Outside round	75.71^{b}	2.34^{a}	21.89^{cd}	1.05^{a}
	Hind shank	76.25^{b}	2.08^{a}	21.58^{cd}	1.07^{a}
	Inside	75.64^{b}	2.07^{a}	22.23^{cd}	1.09^{a}
Forequarter	Cube roll	75.39^{b}	3.09^{ab}	21.09^{c}	0.98^{a}
	Neck	75.49^{b}	2.71^{ab}	21.79^{cd}	0.99^{a}
	Fore shank	76.12^{b}	2.45^{a}	21.03^{bc}	1.02^{a}
	Blade roll	76.93^{b}	1.99^{a}	20.87^{bc}	1.01^{a}
	Cross cut	76.99^{b}	1.76^{a}	21.20^{cd}	0.98^{a}
	Bolar	74.98^{b}	4.63^{b}	19.50^{ab}	1.00^{a}
SED		0.85	$0.19^{\#}$	0.46	0.047
P-value		***	***	***	ns
#: Mean and SED for comparisons of fat analysis are based on log scale.					

Results are presented as Mean and Standard Error of Difference (SED). Values with different superscripts (a,b,c,d) in the same column differ significantly across the cuts. $P > 0.05$ presented as no significance (ns). $P < 0.001$ presented as $***$ for level of significance.

4.1.1 Moisture

Moisture has a remarkable effect in meat processing potential, shelf-life and sensory characteristics. Normally, customers prefer “juicy” rather than “dry” mouth-feeling meats (Atti et al., 2004). Figure 8 shows the moisture content of 14 goat cuts. Mean values of 9 goat kid animals are plotted with error bars representing standard deviations within the 9 observations of each cut. Flap had a significantly lower moisture percentage which was 66.71% compared to other cuts that did not show significant differences in their moisture proportions, all around 75%. Previous work, as shown in Table 2, has reported approx. 65 ~ 75% moisture content is present in goat meat, varying in age, breed and so on. The result in my study was in a reasonable range reported by earlier literatures (section 2.3.1).

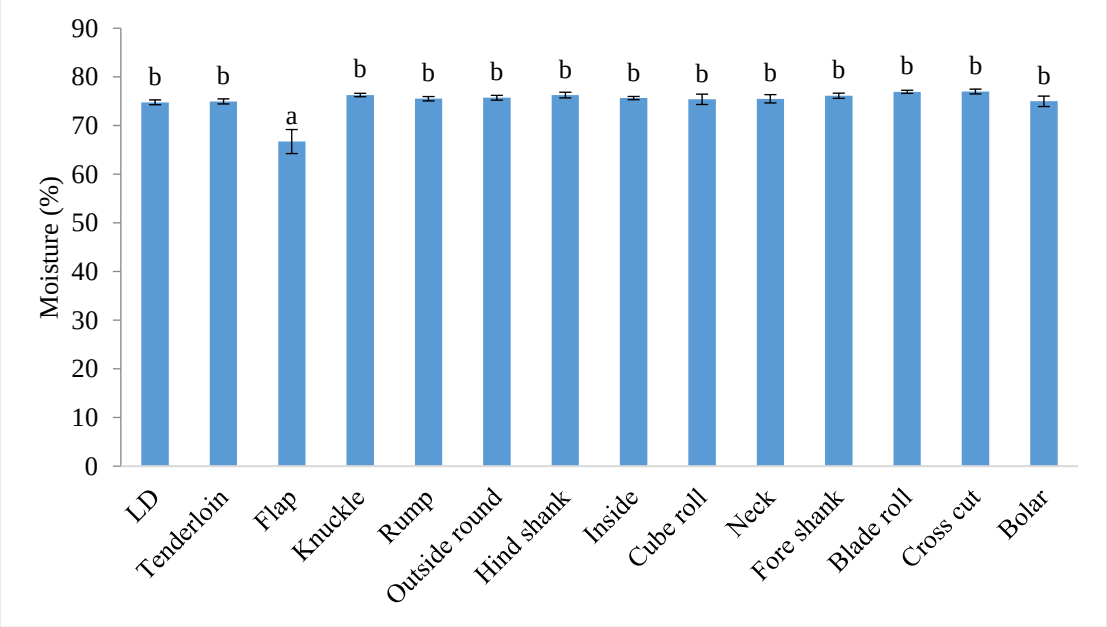

Figure 8. Moisture content of 14 goat kid cuts (% of raw meat).

Mean values of 9 goat kid animals are plotted with error bars representing standard deviations within the 9 observations of each cut. LD stands for *longissimus dorsi*.

4.1.2 Fat

For retail cuts, fat content is an important factor from the standpoint of a healthy diet. In most studies, the reported fat content means the intramuscular fat (marbling) in lean

meat, but Sheridan et al. (2003) and Tshabalala et al. (2003) determined the dissectable fat content (subcutaneous fat and intermuscular fat). Traditionally, meat with a high total fat content is always found to contain more intramuscular fat (Jung, Hwang, & Joo, 2016).

The intramuscular fat content of 14 goat kid cuts is presented in Figure 9. Mean values of 9 goat kid meats are plotted with error bars representing standard deviations within each cut. Flap had the remarkably highest fat content amongst all the 14 cuts, which had reached 12.81%. Bolar (4.63%) was not significantly different with cube roll (3.09%), knuckle (2.75%), neck (2.71%), *Longissimus dorsi* (LD, 2.55%) and rump (2.5%). But they were significantly higher in fat than that of the fore shank (2.45%), outside round (2.34%), tenderloin (2.12%), hind shank (2.08%), inside (2.07%), blade roll (1.99%) and cross cut (1.76%).

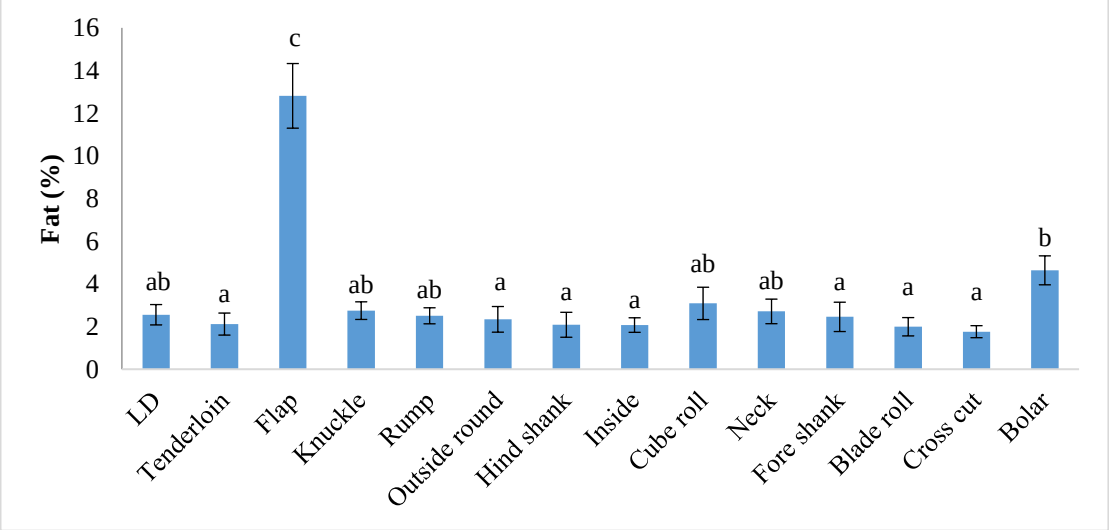

Figure 9. Fat content of 14 goat kid cuts (% of raw meat).

Mean values of 9 goat kid animals are plotted with error bars representing standard deviations within the 9 observations of each cut. LD stands for *longissimus dorsi*.

According to Hogg et al. (1992), goat leg and shoulder contain the highest lean content while flap and mid-breast had less of total lean. This conclusion is in line with the finding of my study where the cross cut and blade roll, as part of goat shoulder, had the lowest fat content while the flap contained the highest. Similar result was also reported by Tshabalala et al. (2003) that the ventral trunk of castrated Boer goats had the highest subcutaneous fat content of 4.39%. The extremely high fat content of flap found in this

study suggested not only intramuscular fat, but also intermuscular and subcutaneous fat may have been included in the flap cut. Kirton et al. (1999) stated that the lamb flap and breast were low value cuts due to the higher subcutaneous and intermuscular fat proportion, and they also pointed out lamb loin and rack have similar subcutaneous fat cover, but rack has more intermuscular fat than loin (Kirton et al., 1999). The results from the current study of goat kids showed a consistency with that of the lamb. Although no significance was presented, cube roll which comes from rack had a numerically higher fat value of 3.09% than either LD (2.55%) or tenderloin (2.12%).

The differences of fat content in various cuts reflect the fat deposition pattern. Compared to sheep and cattle, goat tends to deposit more internal fat in visceral organs, alimentary tract, renal tract and mesenteries etc, rather than in carcass like subcutaneous fat (Tshabalala et al., 2003). Within goat carcass, deposition of fat occurs mostly in its ventral cuts, which is similar to sheep carcass. Gall (1981) found that the highest fat content in sheep was also in dorsal and ventral trunks. Beside that, fat affects meat sensory properties (Webb, Bosman and Casey, 1994). When cooking, the melted fat can spread out the meat, enhancing its tenderness. The volatile compounds which contribute to aroma and flavor are soluble in fat and can retain for a longer time in fat matrix (Schonfeldt, 1989), thus flap and bolar are speculated to be more tender with an intenser aroma than the other cuts.

4.1.3 Protein

As a vital source of essential amino acids for humans, raw meat provides approx. 20% ~ 25% protein (Véronique Santé-Lhoutellier et al., 2008). The majority cuts from goat kids in current study fall into this range.

The protein content of 14 goat kid cuts is plotted in Figure 10 with mean value from 9 goat kid meats and error bars representing standard deviations within each cut. The lowest protein content was found in flap (18.94%), followed by bolar (19.5%). *Longissimus dorsi* (LD) and tenderloin had the highest protein content of 22.77% and 22.71%, respectively. The remaining cuts did not differ with each other in significance level, and their protein amounts were all around 20%.

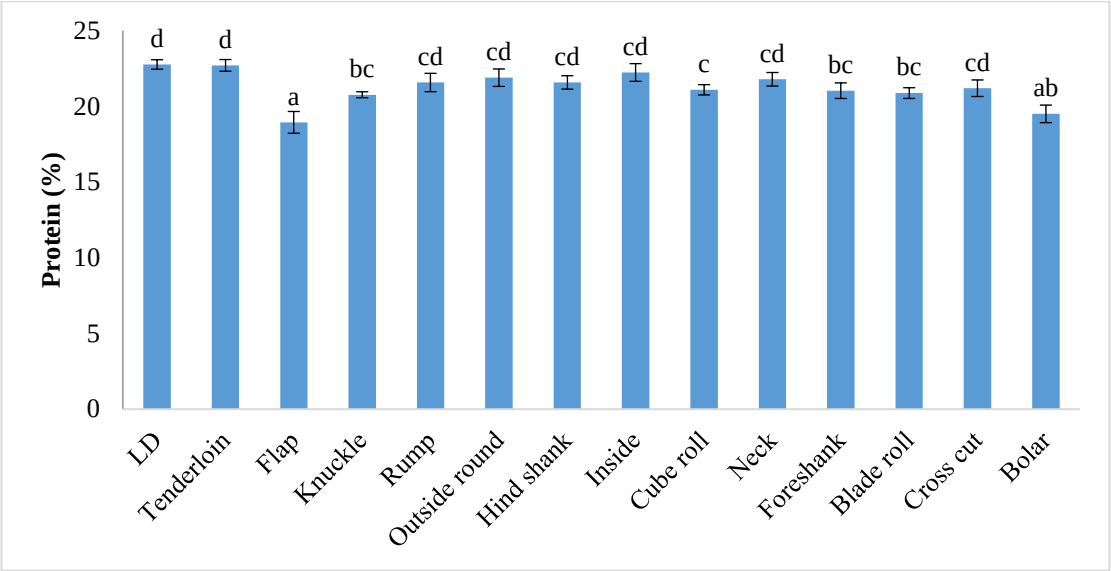

Figure 10. Protein content of 14 goat kid cuts (% of raw meat).

Mean values of 9 goat kid animals are plotted with error bars representing standard deviations within the 9 observations of each cut. LD stands for *longissimus dorsi*.

4.1.4 Ash

Ash, namely the inorganic residue, indicates the total mineral content of food products (Young et al., 2012). Different types and concentrations of minerals can affect food texture, taste, appearance, stability and some physicochemical properties so ash measurement is also an important part of nutritional proximate evaluation as well as the first step of individual elements determination (Milica et al., 2015). The ash content of these 14 cuts fell into the range of 0.97% ~ 1.09%, which were not significantly different to each other. The average ash content of goat meat was similar to other meat sources according to Table 2. Haem iron in Malaysia goat meat (2.1 mg/g) was reported to be comparable to the iron from other meat sources, e.g. beef (2.72 mg/g), lamb (1.74 mg/g) and veal (1.11 mg/g) (Webb et al., 2005). Sheridan et al. (2003) found, regardless of the diet offered, higher Ca, K, Mg, Na and P levels were present in goat carcasses compared to that in sheep carcasses,.

4.1.5 Proximate composition of goat cuts

Proximate analysis is an easy and cheap way to analyze the nutritional composition of meat. But the results may not be 100% accurate, only remaining at a proximate level. For example, in moisture determination, possibly there could have been some other

volatile substances losing together with moisture. Although in meat, nitrogen dominantly presents in protein, many vitamins also contain nitrogen so the result of crude protein may be inaccurate (Young et al., 2012). In crude fat determination, low quantity fat components such as free fatty acids and phospholipids cannot be extracted by petroleum ether while some pigments, vitamins and other fat-soluble molecules can be dissolved in petroleum ether (Petracci & BaÉZa, 2011). As for ash determination, elements like iron (Fe), copper (Cu) or zinc (Zn) can easily be lost by volatilization, which causes inaccuracy (Milica et al., 2015). Although there are so many abovementioned drawbacks, proximate analysis is still widely accepted and consistently followed by people all over the world (Young et al., 2012).

The chemical composition is different by the type of cut (Oh et al., 2016). Figure 11 gives a proximate overview of all of the 14 cuts. As for the moisture, fat and protein content that showed significant differences among cuts, the specific numerical value of each cut was listed below and compared regardless of significance.

The moisture content declined as follows: Cross cut (76.99%) > blade roll (76.93%) > knuckle (76.27%) > hind shank (76.25%) > fore shank (76.12%) > outside round (75.71%) > Inside (75.64%) > rump (75.50%) > neck (75.49%) > cube roll (75.39%) > tenderloin > bolar (74.98%) > (74.95%) > LD (74.77%) > flap (66.71%).

The content of fat decreased as follows: flap (12.81%) > bolar (4.63%) > cube roll (3.09%) > knuckle (2.75%) > neck (2.71%) > LD (2.55%) > rump (2.50%) > fore shank (2.45%) >outside round (2.34%) > tenderloin (2.12%) > hind shank (2.08%) > inside (2.07%) > blade roll (1.99%) > cross cut (1.76%).

Protein content followed the sequence below: LD (22.77%) > tenderloin (22.71%) > inside (22.23%) > outside round (21.89%) > neck (21.79%) > hind shank (21.58%) > rump (21.57%) > cross cut (21.20%) > cube roll (21.09%) > fore shank (21.03%) > blade roll (20.87%) > knuckle (20.76%) > bolar (19.50%) > flap (18.94%).

It can be clearly seen that the difference in protein was small and the main variation was found in moisture and fat where most cuts followed the principle that when moisture decreased in value the fat content increased, with their total contribution to composition being around 77.5%. As for protein and fat, Jung et al. (2016) clarified that protein

content tended to decrease with the increasing of intramuscular fat content. The protein and fat contents determined in the current study generally followed this negatively correlated trend. For example, flap and bolar that had the highest fat content were found to have the lowest protein proportion.

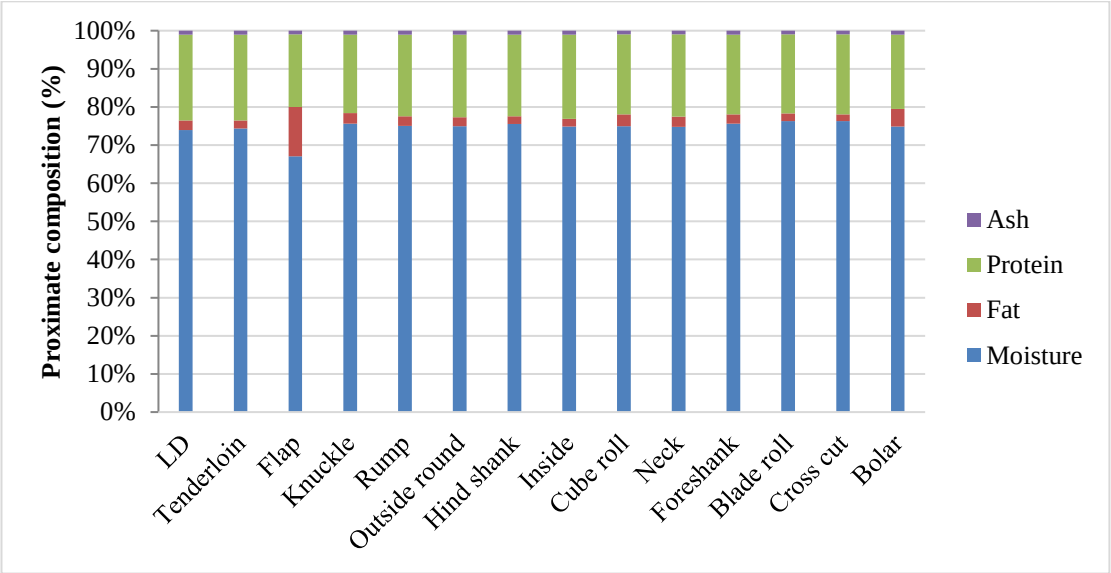

Figure 11. Proximate composition of 14 goat kid cuts (% of raw meat).

Mean values of 9 goat kid animals are plotted with error bars representing standard deviations within the 9 observations of each cut. LD stands for *longissimus dorsi*.

The results from the current study were similar to the results of 10 Hanwoo steer beef cuts reported by Cho et al. (2013) that the proximate composition of those 10 beef cuts ranged from 65.3% to 72.8% (moisture), 4.1% to 12.5% (crude fat), 18.7% to 21.88% (protein) and 0.7% to 1.4% (ash) on fresh meat basis. The *Longissimus dorsi* cut of milk-fed Saanen male goat kids slaughtered at 31 days with a live weight of 8.2 kg in the current study had 74.77% moisture, 22.77% protein, 2.55% fat and 1.08% ash, which is consistent with the proximate values of *Longissimus dorsi-lumborum* muscle of milk-fed Murciano-Granadina male goat kids slaughtered at 35 days with a live weight 7.6 kg who was reported to have 75.6% moisture, 22.2% protein, 1.19% intramuscular fat and 1.11% ash by Bañón et al. in 2006. The proximate values of rump cut in this study were 75.50% moisture, 21.57% protein, 2.50% fat and 1.01% ash, in which the first two contents had higher values compared to the 74.6% moisture and 19.63% protein in *gluteus superficialis* muscle of Balkan goat aged 4 years with an average live weight of 45.76 kg (Ivanovic et al., 2016), and the fat proportion (3.76%) in the adult Balkan goat was higher than that of Saanen young goat kids. Although

different breeds, feeding management and lab conditions will contribute to lack of agreement on the chemical composition of specific cut between studies, it cannot be denied that the age is a big factor in affecting the final result.

In summary, cuts from ventral trunk (flap and bolar) had a higher fat portion but lower protein and moisture portion, and the remaining 12 cuts all had similar proximate composition to one another. Thus a moisture proportion of 75%, a protein proportion of 20%, a fat proportion of 2.5% and an ash proportion of 1% are present in almost all of the cuts from Saanen goat kid carcasses except the ventral cuts. This is in accordance with Webb et al. (2005) who found that a standard composition of an adult mammalian muscle is 75% water, 19% protein, 2.5% fat and 0.65% minerals. Cut with a low fat content and high protein content is always perceived as superior quality and is favored by consumers. Goat is known to have more lean meat and low intramuscular fat compared to that of the sheep at similar ages, and goat meat is a good source of low-energy, high value protein (Hogg et al., 1992). Within goat, the central trunk, particularly the flap cut, is regarded to have a relatively inferior quality in terms of proximate composition when compared to other cuts.

The proximate values of 9 goats were not statistically different with each other (data not shown), thus 3 goats were randomly chosen to conduct digestibility study after power calculation (section 3.3).

4.2 Free amino acids (FAAs) and peptides in goat kid meat

The nitrogen compounds such as protein, peptides and free amino acids (FAAs), are all good biochemical predictors of meat nutritive values (Moya et al., 2001). Protein quality not only depends on the composition and concentration of essential amino acids, but also depends on its digestibility because digestion is the first essential step to utilize dietary protein (Adibi & Mercer, 1973; Escudero et al., 2010). Ingested proteins must be broken down into small peptides or FAAs to pass through the small intestine wall and enter into the bloodstream so that proteins can be absorbed and utilized by humans (Véronique Santé-Lhoutellier et al., 2008). The digestibility of meat protein is about 94% ~ 95%, higher than the protein digestibility of beans (78%) and whole wheat (86%) (Bhutta, 1999). Protein quality can also be evaluated by Protein Digestibility

Corrected Amino Acid Score which has the highest score of 1.0. Meat protein such as beef is scored 0.9, higher compared to the 0.5 ~ 0.7 of most plant proteins (Williams, 2007).

In this study, protein digestibility of different goat kid cuts was compared by monitoring the initial FAAs and peptides present in the meat before undergoing the simulated *in vitro* digestion. The release of FAAs and peptides after gastric and intestinal phases of digestion was determined. Stuknytė et al. (Stuknytė et al., 2014) also measured the release of free amino acids to compare and predict the digestibility of spaghetti. In current study, 21 free amino acids were quantified by LC-MS system (section 3.3.4.2). All of 9 essential amino acids, which are VAL, THR, PHE, LEU, ILE, MET, HIS, LYS, TRP, 5 conditionally essential amino acids, which are PRO, GLY, ARG, TYR and GLN, and all of 5 non-essential amino acids, which are GLU, ASN, ALA, SER and ASP, were identified. Besides, cystine which is formed from the oxidation of two cysteine molecules, and hydroxyproline (HO-PRO) which is hydroxylated from proline were also included. SDS-PAGE (section 3.3.5.2) was used to separate proteins and large peptides according their molecular weight.

4.2.1 FAAs in goat kid meat

4.2.1.1 Presence of FAAs prior to the simulated digestion

During post-mortem aging process, proteolytic degradation catalyzed by endogenous muscle enzymes (cathepsins, calpains and multicatalytic proteinase complex) occurs, producing polypeptide fragments (Moya et al., 2001). Then peptidyl peptidases and aminopeptidases from both muscle and microorganisms further degrade polypeptides to release smaller dipeptides and individual FAAs which partly characterize the meat flavour (Bauchart et al., 2006; López, Bru, Vignolo, & Fadda, 2015; Yi et al., 2016). A lot of structural proteins, such as filamin, titin, troponin T, nebulin and desmin, degrade during the proteolysis process, forming myofibril fragments and enhancing meat tenderness (Lametsch, Roepstorff, & Bendixen, 2002).

The concentrations of FAAs in 14 different Sous-vide cooked Saanen goat kid cuts prior to the digestion simulation are presented in Table 7. The results are given as mean

(mg/100 g wet weight) and the standard error of difference (SED) to compare the variability of the means among different cuts.

As Table 7 demonstrates, statistical differences in almost all of the individual FAAs except HO-PRO, ASN, GLY and cystine, were found between the cuts. Total free amino acids (TAAs) and total essential free amino acids (EAAs) also showed significant differences with p values under 0.01 and 0.001, respectively. The TAAs and EAAs content of 14 cooked goat cuts are plotted in Figure 12. In terms of TAAs, all the goat cuts from hindquarter fraction (*longissimus dorsi*, tenderloin, flap, knuckle, rump, outside round, hind shank and inside) were not significantly different from each other. The forequarter cuts had a relatively large variation where cross cut had the highest level of TAAs (395.12 mg/100 g), while statistically lower TAAs amount were found in cube roll (279.62 mg/100 g), fore shank (289.96 mg/100 g) and bolar (301.86 mg/100 g). As for EAAs, it can be observed from Figure 12 that the average EAAs amount from hindquarter cuts (varying from 88.79 to 125.07 mg/100 g) was greater than that from the forequarter (64.91 ~ 98.75 mg/100 g), and so did the EAAs percentage (%EAAs) out of TAAs (27.62% ~ 35.03% versus 23.21% ~ 28.82%). Inside contained the highest EAAs (125.07 mg/100 g) as well as the highest %EAAs (35.03%). *Longissimus dorsi* (LD) had a lower EAAs value (101.40 mg/100 g) compared to inside, but their %EAAs did not have significant differences (35.03% versus 32.26%). Same as the TAAs, the lowest level of EAAs was found in cube roll (64.91 mg/100 g occupying 23.21% of TAAs).

Table 7. Amounts of FAAs in 14 goat kid cuts prior to digestion (mg/100 g of cooked meat).

	LD	Tenderloin	Flap	Knuckle	Rump	Outside round	Hind shank	Inside	Cube roll	Neck	Fore shank	Blade roll	Cross cut	Bolar	SED	P-value
HIS	6.71^{bcd}	6.73^{bcd}	6.05^{abcd}	6.87^{bcd}	7.03^{cd}	6.26^{abcd}	6.73^{bcd}	6.34^{bcd}	4.76^{a}	5.81^{abcd}	5.44^{ab}	5.85^{abcd}	7.27^{d}	5.49^{abc}	0.45	***
HO-PRO	1.96^{a}	2.37^{a}	2.38^{a}	2.27^{a}	2.10^{a}	1.98^{a}	2.08^{a}	1.45^{a}	1.64^{a}	2.04^{a}	1.92^{a}	2.03^{a}	2.27^{a}	1.78^{a}	0.54	ns
ASN	8.71^{a}	8.98^{a}	10.41^{a}	13.52^{a}	15.32^{a}	10.51^{a}	9.53^{a}	15.27^{a}	8.79^{a}	10.46^{a}	7.64^{a}	7.77^{a}	11.35^{a}	10.22^{a}	2.94	ns
ARG	15.64^{gh}	13.56^{fg}	9.80^{bcd}	10.48^{cde}	12.92^{f}	11.91^{def}	12.53^{ef}	16.05^{h}	6.92^{a}	7.25^{a}	7.86^{ab}	7.47^{a}	8.39^{abc}	9.07^{abc}	0.65	***
SER	19.58^{bcde}	20.06^{bcde}	17.23^{abcd}	20.67^{cde}	23.01^{e}	20.21^{bcde}	21.00^{de}	21.76^{e}	13.30^{a}	15.96^{ab}	15.89^{ab}	16.84^{abcd}	20.28^{bcde}	16.57^{abc}	1.27	***
GLY	32.15^{a}	32.02^{a}	33.81^{a}	32.32^{a}	34.00^{a}	33.00^{a}	32.76^{a}	30.23^{a}	27.20^{a}	31.40^{a}	30.18^{a}	28.94^{a}	31.89^{a}	28.96^{a}	3.39	ns
GLN	37.39^{a}	51.95^{ab}	59.19^{ab}	64.92^{ab}	47.82^{ab}	41.72^{a}	53.00^{ab}	35.99^{a}	63.14^{ab}	72.27^{ab}	53.91^{ab}	63.06^{ab}	87.36^{b}	48.58^{ab}	12.4	**
ASP	3.21^{ab}	4.45^{abc}	7.35^{e}	5.76^{cde}	4.06^{abc}	2.65^{ab}	4.53^{abc}	2.30^{a}	3.47^{abc}	4.45^{abc}	4.17^{abc}	4.82^{bcd}	7.02^{de}	4.14^{abc}	0.7	***
THR	13.33^{ab}	13.53^{ab}	9.86^{a}	10.86^{a}	12.14^{ab}	11.81^{ab}	19.46^{b}	17.51^{ab}	9.90^{a}	11.71^{ab}	12.56^{ab}	14.10^{ab}	16.64^{ab}	13.66^{ab}	2.41	**
GLU	17.36^{a}	19.04^{ab}	13.12^{a}	15.18^{a}	15.07^{a}	14.05^{a}	31.56^{ab}	25.62^{ab}	23.87^{ab}	24.51^{ab}	23.83^{ab}	26.92^{ab}	41.87^{b}	24.56^{ab}	6.89	**
ALA	55.66^{ab}	60.04^{ab}	56.21^{ab}	59.90^{ab}	66.41^{b}	60.66^{ab}	57.15^{ab}	57.95^{ab}	51.08^{a}	57.79^{ab}	49.19^{a}	51.87^{ab}	61.60^{ab}	50.48^{a}	4.25	**
PRO	8.71^{ab}	9.26^{ab}	12.47^{b}	11.35^{ab}	11.19^{ab}	10.15^{ab}	11.34^{ab}	8.46^{ab}	7.73^{a}	9.23^{ab}	9.33^{ab}	9.97^{ab}	12.81^{b}	9.30^{ab}	1.32	**
LYS	10.61^{a}	10.38^{a}	11.68^{ab}	10.73^{a}	12.02^{abc}	10.85^{a}	14.85^{cd}	15.82^{d}	9.94^{a}	11.22^{ab}	9.88^{a}	10.92^{a}	14.36^{bcd}	12.25^{abc}	0.91	***
Cystine	0.03^{a}	0.02^{a}	0.05^{a}	0.06^{a}	0.02^{a}	0.04^{a}	0.05^{a}	0.04^{a}	0.04^{a}	0.06^{a}	0.05^{a}	0.05^{a}	0.06^{a}	0.05^{a}	0.014	ns
MET	9.01^{abcd}	8.49^{abcd}	8.07^{abc}	9.57^{abcd}	11.97^{cd}	10.24^{bcd}	9.24^{abcd}	12.57^{d}	5.70^{a}	6.56^{ab}	6.71^{ab}	6.87^{ab}	8.37^{abc}	7.70^{ab}	1.19	***
VAL	13.59^{abcd}	13.32^{abcd}	13.29^{abcd}	15.27^{cde}	18.08^{e}	14.69^{bcde}	15.28^{cde}	17.13^{de}	9.24^{a}	10.87^{ab}	11.60^{abc}	12.30^{abc}	14.97^{bcde}	12.73^{abc}	1.25	***
TYR	12.52^{bcde}	11.91^{bcd}	10.69^{abcd}	13.57^{cdef}	16.80^{ef}	14.82^{def}	13.31^{bcdef}	16.84^{f}	7.53^{a}	9.18^{ab}	9.76^{abc}	9.85^{abc}	11.46^{abcd}	11.17^{abcd}	1.24	***
ILE	9.77^{de}	8.67^{bcde}	8.33^{bcd}	9.32^{cde}	11.98^{f}	10.55^{ef}	9.09^{cde}	11.79^{f}	5.67^{a}	6.71^{ab}	6.73^{ab}	7.09^{ab}	8.30^{bcd}	7.69^{bc}	0.57	***
LEU	23.37^{efgh}	21.62^{defg}	18.69^{bcdef}	20.60^{cdefg}	28.63^{h}	23.93^{fgh}	17.83^{abcdef}	26.35^{gh}	11.79^{a}	13.93^{ab}	13.61^{ab}	14.68^{abc}	17.14^{abcde}	16.23^{abcd}	1.88	***
PHE	12.13^{cdef}	11.07^{bcde}	10.41^{abcde}	12.73^{def}	16.53^{f}	14.18^{ef}	10.44^{abcde}	14.64^{ef}	6.19^{a}	7.54^{ab}	7.68^{abc}	7.95^{abc}	9.22^{abcd}	8.98^{abcd}	1.31	***
TRP	2.89^{def}	2.88^{def}	2.40^{bcd}	2.86^{def}	3.24^{f}	3.03^{ef}	2.58^{cde}	2.92^{def}	1.74^{a}	2.05^{abc}	2.01^{ab}	2.08^{abc}	2.48^{bcd}	2.26^{abc}	0.16	***
TAAs	314.31^{ab}	330.36^{ab}	321.48^{ab}	348.82^{ab}	370.36^{ab}	327.24^{ab}	354.36^{ab}	357.01^{ab}	279.62^{a}	320.99^{ab}	289.96^{a}	311.43^{ab}	395.12^{b}	301.86^{a}	26.24	**
EAAs	101.40^{cd}	96.69^{cd}	88.79^{bcd}	98.82^{cd}	121.62^{e}	105.54^{de}	105.51^{de}	125.07^{e}	64.91^{a}	76.40^{ab}	76.22^{ab}	81.85^{abc}	98.75^{cd}	86.99^{bcd}	5.78	***
EAAs (%)	32.26%^{fg}	29.27%^{de}	27.62%^{cde}	28.33%^{de}	32.84%^{fg}	32.25%^{fg}	29.78%^{ef}	35.03%^{g}	23.21%^{a}	23.80%^{ab}	26.29%^{bcd}	26.28%^{bcd}	24.99%^{abc}	28.82%^{de}	0.0085	***

Results are presented as Mean and Standard Error of Difference (SED). TAAs stand for total free amino acids. EAAs stand for total essential amino acids (In bold). LD stands for *longissimus dorsi*.

Values with different superscripts (^{a,b,c,d,e,f,g,h}) in the same row for the same amino acids differ significantly across the cuts.

P > 0.05 presented as no significance (ns). P < 0.05 presented as ∗ for level of significance. P < 0.01 presented as ∗∗ for level of significance. P < 0.001 presented as ∗∗∗ for level of significance.

% EAAs was calculated by (EAAs/TAAs) x 100.

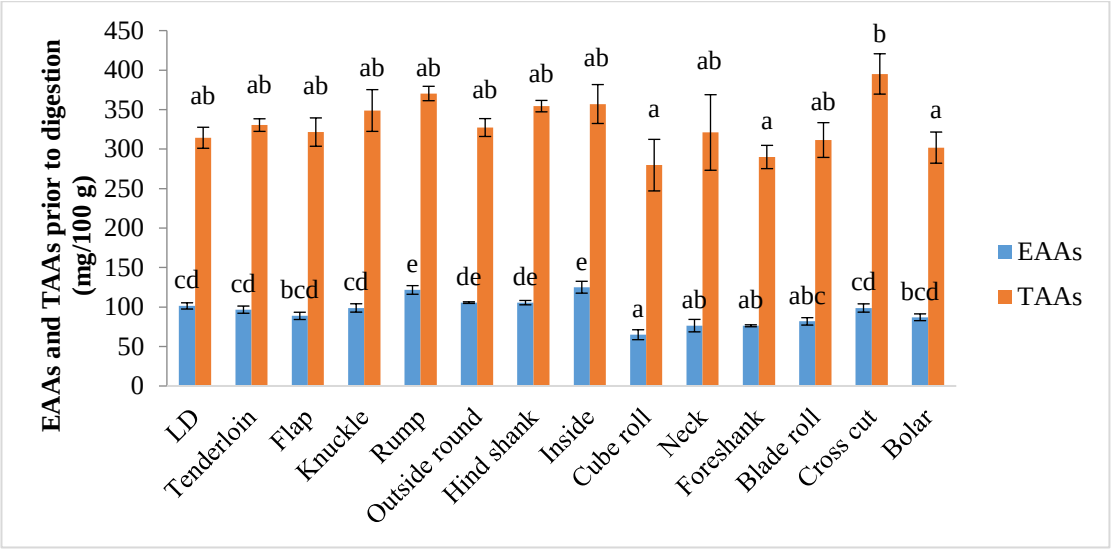

Figure 12. Amounts of EAAs and TAAs in 14 goat kid cuts prior to digestion (mg/100 g of cooked meat).

Mean values are plotted with error bars representing standard deviations. Different superscripts ($^{a, b, c, d, e}$) differ significantly among cuts prior to digestion. LD stands for *longissimus dorsi*.

FAAs fluctuated variously in different cuts and this fluctuation could not be statistically explained. But in general, among all the amino acids, ALA and GLN were the most abundant amino acids found in all 14 cuts, varying from 49.19 mg/100 g to 66.41 mg/100 g and from 37.39 mg/100 g to 87.36 mg/100 g. The following plentiful FAAs were GLY (27.20 ~ 34.00 mg/100 g), GLU (13.12 ~ 41.87 mg/100 g), LEU (11.79 ~ 28.63 mg/100 g) and SER (13.30 ~ 23.01 mg/100 g). The amounts of ASN, ARG, THR, PRO, LYS, MET, VAL, TYR, ILE and PHE were quite similar among different cuts, of around 10 mg/100 g. And approximately 5 mg/100 g of HIS and ASP were found in all cuts of the goat kid meat. The contents of HO-PRO and TRP were even lower with an average amount of 2.02 and 2.5 mg/100 g, respectively. HO-PRO is mainly present in collagen and elastin, which both of them only occupy a small portion of muscle protein (2% and 0.1%, respectively) (Feidt, Petit, Bruas-Reignier, & Brun-Bellut, 1996), thus a low level of HO-PRO ranging from 1.45 to 2.38 mg/100 g was found. Cystine varied from 0.02 to 0.06 mg/100 g in all 14 cuts without statistical significance, which fell in the range of cysteine (0.03 to 2.1 mg/100 g in meat) reported by Madruga et al. (Madruga et al., 2010).

Field et al. (1971) reported that greater amounts of VAL, LEU, LYS, MET, PHE, HIS, GLU, ALA and TYR were present in *biceps femoris* muscle of bull than that in the

longissimus dorsi (LD) muscle. In this study, VAL, MET, HIS, GLU, ALA and TYR in the hind shank of goat meat also had a higher amount than that in the goat LD cut. Cornet and Bousset (1999) investigated the variations of FAAs and dipeptides among 3 pig muscles *longissimus dorsi, masseter* and *trapezius*. They found that these 3 muscles had similar types of FAAs and dipeptides but in different amounts. GLN, GLY, ALA and carnosine were found to have high contents in all 3 muscles (>100 μmol/ g wet weight). Their finding supports the results of the current study where ALA, GLN and GLY were found to be the top three most abundant amino acids in most of the cuts. Madruga et al. (2010) investigated the FAAs content on raw and cooked rump steaks of Saanen castrated goats slaughtered at six months of age. They reported GLY, ARG, GLU, ALA and GLN had the largest quantities amongst 21 FAAs they have measured, which was mostly in agreement with the results of the current study, which showed rump cut had more ALA, GLN, GLY, LEU and SER than other FAAs. However, the total FAAs contents in raw and cooked goat meat given by Madruga et al. (2010) were 220 mg/100 g and 151mg/100 g, respectively. The cooked rump samples measured in the current study had a bit higher TAAs content which was 370.36 mg/100 g. This difference could be partially attributed to age. The Saanen male goats used in this study were only 31 days old with a living weight of 8.2 kg, which is younger than the goats used by Madruga et al. And younger animals have been reported to have a greater protein synthesis and degradation than older ones (Watanabe et al., 2004), thus the results of the current study are reasonable and in expectation. Franco et al. (2010) found a large variety in 6 different muscles from Blonde Galician male veal calves, in terms of FAAs content, where the total value ranged from 215 to 278 mg per 100 g of fresh meat. Their result was lower than my result of total FAAs which varied from 279.62 to 395.12 mg per 100 g of cooked goat meat. They obtained the highest values for HIS, ALA and GLU, the sum of them occupying around 50% of the total FAAs. In goat kid meat, the sum of ALA, GLN, GLY and GLU also accounted for about 50% out of the TAAs.

Inconsistencies and variations of FAAs content in the literatures may arise from various factors that contribute to alter the meat proteolytic activity. For example, animal diet, breed, age, storage time (aging time) after slaughter, muscle pHu, cuts, cooking and even different analytical methods would matter (Feidt et al., 1996; Watanabe et al., 2004). A long aging time is known to increase the amount of FAAs, and HIS was reported to maintain unchanged during aging while VAL increased more than other

FAAs (Feidt et al., 1996; Field et al., 1971). Cooking can cause the hydrolysis of proteins and peptides (Madruga et al., 2010). The release of peptides and FAAs is muscle-dependent because different muscles have different types of proteases and enzyme activities (Watanabe et al., 2004). In addition, various post-mortem muscles have different pH decline rate which can affect the proteolytic activity and myofibril fragmentation even though all the animals are raised under the same conditions (Koutsidis et al., 2008). Feidt et al. (1996) confirmed both the content of amino-acids and their increases with storage time differed among the *longissimus dorsi*, *triceps bruchii caput longum* and *rectus femoris* muscles from bulls. The different FAAs content in various goat kid cuts could be attributed to the mutual interactions of these factors.

4.2.1.2 FAAs released at the end of gastric digestion

Table 8 exhibits the concentration of FAAs in 14 cooked goat kid cuts released at the end of gastric phase during simulated *in vitro* digestion.

Seven free amino acids (HIS, SER, GLY, GLU, ALA, LYS and cystine) did not have significant differences amongst cuts, while the remaining 14 FAAs as well as the TAAs and EAAs showed significance in various cuts. Similar to before digestion, the TAAs content among the hindquarter cuts quantified after gastric phase were not significantly different to each other as shown in Figure 13, varying form the highest amount of 555.97 mg/100 g in flap to 465.51 mg/100 g in tenderloin. Within the forequarter, except for the lowest amount found in blade roll (437.28 mg/100 g) and bolar (420.64 mg/100 g), the remaining forequarter cuts ranging from 454.51 mg/100 g in neck to 514.35 mg/100 g in fore shank were comparable to hindquarter cuts. Regarding to EAAs level, the highest amount of 251.88 mg/100 g was found in flap while the highest %EAAs was proved to be occupied by inside (48.60%). Similar to TAAs, all the hindquarters cuts did not possess much variations in either EAAs amount or proportion, but all the forequarter cuts with the exception of fore shank have shown a significantly lower quantity of EAAs than the flap and lower %EAAs than the inside ($P < 0.001$). Cross cut had both the lowest EAAs amount of 182.13 mg/100 g and %EAAs of 39.80%. It is worth noting that, regardless of significance, all the cuts from the

hindquarter of goat kids had higher proportion of EAAs (44.98% ~ 48.60%) than all the forequarter cuts (39.80% ~ 44.78%) after gastric digestion.

Table 8. Amounts of FAAs in 14 goat kid cuts at the end of gastric digestion (mg/100 g of cooked meat).

	LD	Tenderloin	Flap	Knuckle	Rump	Outside round	Hind shank	Inside	Cube roll	Neck	Fore shank	Blade roll	Cross cut	Bolar	SED	P-value
HIS	6.51^{a}	6.66^{a}	7.54^{a}	6.46^{a}	6.82^{a}	8.48^{a}	7.16^{a}	6.27^{a}	6.08^{a}	6.50^{a}	6.91^{a}	5.79^{a}	5.01^{a}	5.34^{a}	1.12	ns
HO-PRO	0.96^{abc}	1.12^{bcd}	1.37^{d}	1.02^{bc}	0.91^{ab}	0.92^{ab}	1.12^{bcd}	0.75^{a}	1.11^{bc}	1.06^{bc}	1.18^{cd}	0.98^{abc}	1.08^{bc}	1.00^{abc}	0.073	***
ASN	12.79^{ab}	12.27^{ab}	15.21^{b}	12.99^{ab}	14.79^{b}	14.42^{ab}	14.60^{b}	13.76^{ab}	12.82^{ab}	12.64^{ab}	14.02^{ab}	11.38^{ab}	12.51^{ab}	9.30^{a}	1.5	*
ARG	12.94^{abc}	15.92^{bcd}	18.64^{d}	15.82^{bcd}	17.77^{d}	15.59^{bcd}	18.32^{d}	16.12^{cd}	14.39^{abcd}	14.52^{abcd}	18.09^{d}	9.97^{a}	10.49^{a}	11.27^{ab}	1.38	***
SER	16.24^{a}	15.13^{a}	18.18^{a}	16.77^{a}	18.34^{a}	17.78^{a}	19.92^{a}	18.06^{a}	15.36^{a}	15.26^{a}	17.45^{a}	15.35^{a}	16.99^{a}	14.72^{a}	1.72	ns
GLY	23.85^{a}	18.72^{a}	24.18^{a}	19.27^{a}	19.84^{a}	20.99^{a}	23.52^{a}	19.12^{a}	20.03^{a}	19.19^{a}	21.80^{a}	20.63^{a}	22.58^{a}	21.72^{a}	2.9	ns
GLN	25.22^{a}	36.41^{bcd}	45.44^{defg}	41.75^{cdef}	32.91^{abc}	29.82^{ab}	41.07^{cdef}	27.49^{ab}	50.13^{fg}	46.20^{efg}	44.73^{defg}	40.33^{cde}	52.71^{g}	29.14^{ab}	2.72	***
ASP	13.60^{a}	18.37^{abcd}	25.28^{e}	20.04^{bcde}	20.66^{bcde}	18.21^{abcd}	22.36^{cde}	18.25^{abcd}	19.99^{bcde}	20.48^{bcde}	23.01^{de}	16.96^{abc}	16.79^{abc}	16.22^{ab}	1.73	***
THR	13.28^{ab}	12.56^{ab}	14.91^{b}	12.95^{ab}	13.96^{ab}	14.42^{ab}	14.75^{b}	13.71^{ab}	12.47^{ab}	11.88^{ab}	13.22^{ab}	11.58^{ab}	12.21^{ab}	10.87^{a}	1.06	**
GLU	54.10^{a}	49.14^{a}	59.70^{a}	54.48^{a}	54.12^{a}	51.21^{a}	53.28^{a}	48.49^{a}	52.27^{a}	49.58^{a}	57.25^{a}	60.23^{a}	60.36^{a}	55.62^{a}	3.51	ns
ALA	50.10^{a}	40.45^{a}	48.69^{a}	42.36^{a}	43.31^{a}	45.51^{a}	45.99^{a}	42.31^{a}	43.06^{a}	40.99^{a}	44.86^{a}	45.73^{a}	49.05^{a}	48.06^{a}	5.52	ns
PRO	8.43^{a}	10.16^{abcd}	14.05^{d}	12.04^{abcd}	11.84^{abcd}	11.68^{abcd}	13.83^{cd}	10.80^{abcd}	11.40^{abcd}	10.75^{abcd}	13.27^{bcd}	9.24^{ab}	9.65^{abc}	10.15^{abcd}	1.23	***
LYS	46.69^{a}	43.18^{a}	50.74^{a}	45.50^{a}	44.68^{a}	43.33^{a}	50.83^{a}	44.40^{a}	41.08^{a}	41.00^{a}	48.93^{a}	34.91^{a}	32.14^{a}	47.73^{a}	10.01	ns
Cystine	0.44^{a}	0.77^{a}	1.20^{a}	0.95^{a}	0.83^{a}	0.74^{a}	1.15^{a}	0.59^{a}	1.47^{a}	1.36^{a}	1.69^{a}	0.81^{a}	0.97^{a}	0.43^{a}	0.322	ns
MET	22.60^{d}	20.41^{abcd}	21.43^{bcd}	20.30^{abcd}	21.00^{bcd}	21.85^{cd}	19.87^{abcd}	20.43^{abcd}	19.53^{abcd}	17.45^{ab}	19.96^{abcd}	17.47^{ab}	17.95^{abc}	16.64^{a}	1.23	***
VAL	16.55^{abc}	15.25^{a}	19.62^{c}	17.50^{abc}	18.15^{abc}	18.18^{abc}	19.43^{bc}	18.14^{abc}	15.71^{abc}	15.09^{a}	17.71^{abc}	15.16^{a}	15.48^{ab}	15.10^{a}	1.14	***
TYR	26.34^{bc}	27.42^{cd}	32.16^{d}	28.05^{cd}	28.95^{cd}	28.62^{cd}	27.57^{cd}	28.11^{cd}	25.36^{bc}	24.13^{abc}	27.17^{cd}	21.91^{ab}	21.68^{ab}	19.58^{a}	1.49	***
ILE	11.29^{abcd}	12.66^{bcde}	14.47^{de}	13.45^{cde}	15.27^{e}	15.24^{e}	13.76^{de}	15.08^{e}	11.90^{abcde}	10.35^{abc}	12.38^{bcde}	8.88^{a}	9.38^{ab}	8.95^{a}	0.98	***
LEU	55.33^{c}	52.08^{bc}	58.15^{c}	52.38^{bc}	57.00^{c}	56.26^{c}	53.23^{bc}	53.83^{bc}	50.53^{abc}	46.77^{ab}	55.77^{c}	46.50^{ab}	42.46^{a}	42.39^{a}	2.46	***
PHE	46.15^{bc}	48.55^{bc}	54.27^{c}	45.38^{bc}	47.55^{bc}	48.73^{bc}	44.34^{bc}	49.67^{bc}	48.02^{bc}	42.06^{abc}	46.71^{bc}	37.83^{ab}	41.14^{ab}	31.60^{a}	3.6	***
TRP	7.36^{bcd}	8.29^{d}	10.75^{e}	8.04^{cd}	9.07^{de}	8.95^{de}	7.65^{cd}	8.94^{de}	8.26^{d}	7.25^{bcd}	8.23^{d}	5.66^{ab}	6.36^{abc}	4.80^{a}	0.54	***
TAAs	470.77^{bc}	465.51^{bc}	555.97^{c}	487.50^{bc}	497.76^{bc}	490.92^{bc}	513.72^{bc}	474.32^{bc}	480.97^{bc}	454.51^{bc}	514.35^{bc}	437.28^{ab}	457.01^{bc}	420.64^{a}	31.63	***
EAAs	225.77^{cde}	219.63^{bcde}	251.88^{e}	221.95^{cde}	233.50^{cde}	235.44^{de}	231.00^{cde}	230.47^{cde}	213.58^{abcd}	198.35^{abc}	229.83^{cde}	183.77^{ab}	182.13^{a}	183.41^{ab}	10.59	***
EAAs (%)	48.05%^{de}	47.11%^{cde}	45.35%^{bcde}	45.40%^{bcde}	46.75%^{cde}	48.18%^{de}	44.98%^{bcde}	48.60%^{e}	44.44%^{bcd}	43.64%^{abc}	44.78%^{bcde}	42.03%^{ab}	39.80%^{a}	43.50%^{abc}	0.012	***

Results are presented as Mean and Standard Error of Difference (SED). TAAs stand for total free amino acids. EAAs stand for total essential amino acids (In bold). LD stands for *longissimus dorsi*.

Values with different superscripts (^{a,b,c,d,e,f,g}) in the same row for the same amino acids differ significantly across the cuts.

P > 0.05 presented as no significance (ns). P < 0.05 presented as ∗ for level of significance. P < 0.01 presented as ∗∗ for level of significance. P < 0.001 presented as ∗∗∗ for level of significance.

% EAAs was calculated by (EAAs/TAAs) x 100.

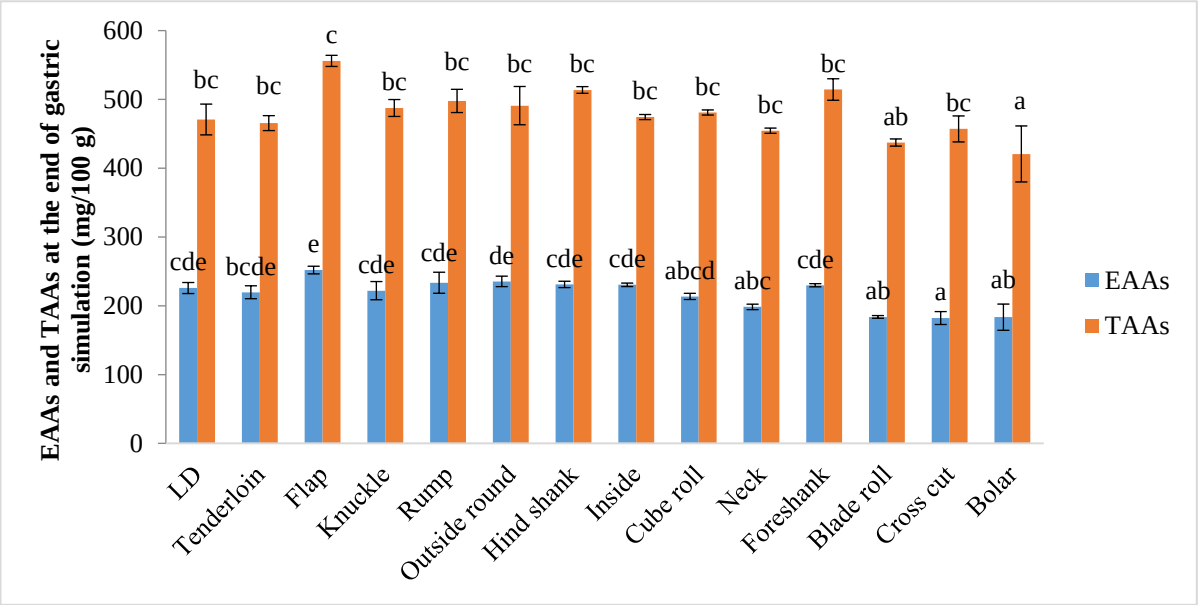

Figure 13. Amounts of EAAs and TAAs in 14 goat kid cuts at the end of gastric digestion (mg/100 g of cooked meat).

Mean values are plotted with error bars representing standard deviations. Different superscripts ($^{a, b, c, d, e}$) differ significantly among cuts at the end of gastric phase. LD stands for *longissimus dorsi.*

By comparison with the results at before digestion phase, 13 amino acids (ASN, ARG, ASP, GLU, LYS, cystine, MET, VAL, TYR, ILE, LEU, PHE, and TRP) increased in quantity in almost all of the cuts. 4 amino acids (HIS, THR, PRO and SER) did not exhibit large increase, while the remaining 4 amino acids (HO-PRO, GLY, GLN and ALA) showed a slightly decreased trend when comparing to the before digestion phase. Within those 13 amino acids, the increase of ASN, ARG, VAL and ILE was negligible, and GLU, MET, TYR, LEU and TRP showed a moderate growth, where concentrations were approximately two to three times larger than before digestion. ASP, LYS, and PHE indicated a considerable increasing with a four to five times higher amount. This is in accordance with Santé-Lhoutellier et al. (2008) who reported that pepsin preferentially hydrolyzes the carboxylic side of MET, LEU, TRP, TYR and PHE. And it is most active at the peptide bonds between PHE-PHE, PHE-TYR and TYR-LEU (Krehbiel & Matthews, 2003).

4.2.1.3 FAAs released at the end of intestinal digestion

Table 9 presents the concentrations of FAAs in 14 cooked goat kid cuts released at the end of the intestinal phase during simulated *in vitro* digestion.

As is shown in Table 9, there was a statistically significant difference in the amounts of FAAs and TAAs obtained from different cuts, except SER, ASP, THR and cystine. The amount of EAAs did not significantly differ across the cuts but the %EAAs did. Figure 14 gives an overview about the TAAs and EAAs content in goat meat after intestinal digestion. TAAs had a large variation among 14 cuts where the lowest amount was in the flap (3935.13 mg/100 g) and the highest amount was in the fore shank (5784.48 mg/100 g). The forequarter cuts were comparable to hindquarters in the intestinal phase, and cuts from the fore shank and shoulder (cross cut and blade roll) were numerically higher than all of the hindquarter cuts. Same pattern was observed with the EAAs, although no significance was found. In respect of %EAAs, different cuts varied in a small range around 54%. Interestingly, the fore shank was found to have the lowest %EAAs of 53.00%, which was significantly lower than that of the inside, which had the highest %EAAs of 55.93%.

Table 9. Amounts of FAAs in 14 goat kid cuts at the end of intestinal digestion (mg/100 g of cooked meat).

	LD	Tenderloin	Flap	Knuckle	Rump	Outside round	Hind shank	Inside	Cube roll	Neck	Fore shank	Blade roll	Cross cut	Bolar	SED	P-value
HIS	115.96^{abc}	141.40^{abc}	103.13^{a}	111.97^{ab}	90.37^{a}	117.12^{abc}	100.39^{a}	117.09^{abc}	101.73^{a}	123.55^{abc}	157.53^{c}	164.79^{c}	161.32^{bc}	117.14^{abc}	15.17	***
HO-PRO	2.76^{a}	5.23^{abc}	3.68^{abc}	3.56^{abc}	2.67^{a}	3.36^{ab}	3.72^{abc}	3.28^{ab}	4.20^{abc}	5.17^{abc}	5.41^{bc}	5.70^{bc}	6.04^{c}	4.72^{abc}	0.76	***
ASN	62.81^{abc}	80.85^{cd}	60.17^{ab}	66.02^{abc}	62.21^{ab}	71.10^{bcd}	57.71^{ab}	71.62^{bcd}	47.75^{a}	57.08^{ab}	85.54^{d}	65.46^{abc}	67.65^{bcd}	54.07^{ab}	5.26	***
ARG	689.96^{ab}	878.66^{bcde}	576.18^{a}	730.92^{abcd}	657.77^{ab}	716.94^{abc}	702.03^{abc}	856.30^{bcde}	623.44^{ab}	785.98^{abcde}	1012.57^{e}	952.40^{cde}	983.46^{de}	847.48^{bcde}	1.38	***
SER	32.90^{a}	42.03^{a}	38.13^{a}	38.16^{a}	40.18^{a}	44.42^{a}	41.26^{a}	49.15^{a}	12.67^{a}	29.06^{a}	38.31^{a}	33.61^{a}	33.67^{a}	14.47^{a}	13.23	ns
GLY	86.60^{a}	128.40^{bc}	113.61^{ab}	117.52^{abc}	108.28^{ab}	121.62^{abc}	114.61^{abc}	115.07^{abc}	116.97^{abc}	109.48^{ab}	151.58^{c}	140.30^{bc}	141.15^{bc}	128.96^{bc}	10.74	***
GLN	140.07^{a}	193.40^{abc}	145.96^{abc}	187.88^{abc}	144.37^{ab}	158.65^{abc}	164.05^{abc}	175.03^{abc}	145.45^{abc}	183.41^{abc}	214.70^{bc}	199.95^{abc}	217.51^{c}	130.38^{a}	30	***
ASP	12.81^{a}	16.15^{a}	20.33^{a}	16.26^{a}	15.82^{a}	14.43^{a}	16.27^{a}	17.10^{a}	2.11^{a}	16.42^{a}	21.77^{a}	24.77^{a}	25.13^{a}	3.69^{a}	9.55	ns
THR	56.69^{a}	65.13^{a}	50.28^{a}	60.46^{a}	55.26^{a}	64.69^{a}	60.27^{a}	74.01^{a}	36.29^{a}	66.55^{a}	86.48^{a}	78.68^{a}	74.98^{a}	32.48^{a}	16.9	ns
GLU	90.22^{a}	104.39^{ab}	102.34^{a}	109.43^{ab}	93.80^{a}	96.43^{a}	89.51^{a}	97.10^{a}	90.54^{a}	102.41^{a}	159.94^{c}	146.75^{bc}	157.53^{c}	123.41^{abc}	12.43	***
ALA	163.74^{a}	196.92^{abc}	168.52^{ab}	185.53^{abc}	166.40^{ab}	183.76^{abc}	163.98^{a}	179.12^{abc}	165.90^{ab}	172.82^{abc}	219.19^{bc}	209.77^{abc}	226.06^{c}	194.99^{abc}	15.63	***
PRO	13.92^{a}	20.68^{abcde}	20.67^{abcde}	22.29^{bcdef}	19.17^{abcd}	21.08^{abcde}	21.10^{abcde}	19.08^{abcd}	17.84^{ab}	18.99^{abc}	28.56^{f}	26.37^{def}	27.16^{ef}	26.20^{cdef}	2.12	***
LYS	539.34^{a}	687.96^{abcd}	570.30^{a}	708.20^{abcd}	656.57^{abc}	693.10^{abcd}	598.83^{ab}	729.87^{abcd}	617.46^{ab}	704.99^{abcd}	886.98^{d}	844.32^{cd}	803.32^{bcd}	689.40^{abcd}	10.01	***
Cystine	0.68^{a}	1.30^{a}	1.30^{a}	1.02^{a}	0.41^{a}	0.94^{a}	0.73^{a}	1.10^{a}	0.40^{a}	0.58^{a}	1.21^{a}	1.01^{a}	0.82^{a}	0.66^{a}	0.39	ns
MET	152.80^{a}	213.94^{c}	147.34^{a}	175.81^{abc}	151.15^{a}	175.79^{abc}	146.63^{a}	190.76^{abc}	154.25^{a}	156.49^{ab}	204.10^{bc}	207.51^{c}	220.48^{c}	188.96^{abc}	13.9	***
VAL	118.92^{a}	152.15^{abcd}	129.34^{a}	137.61^{ab}	128.63^{a}	144.49^{abc}	125.71^{a}	146.43^{abcd}	125.44^{a}	137.33^{ab}	183.97^{cd}	176.83^{bcd}	187.99^{d}	160.15^{abcd}	12.27	***
TYR	652.26^{ab}	728.36^{ab}	537.09^{a}	588.41^{ab}	570.54^{a}	688.18^{ab}	553.62^{a}	589.04^{ab}	572.73^{a}	605.35^{ab}	780.27^{b}	703.98^{ab}	685.53^{ab}	623.46^{ab}	57.28	***
ILE	88.68^{abc}	105.35^{abcde}	87.50^{ab}	101.06^{abcde}	85.94^{ab}	97.14^{abcd}	90.34^{abcd}	100.56^{abcde}	74.53^{a}	88.65^{abc}	139.36^{e}	129.56^{de}	129.17^{cde}	120.29^{bcde}	11.67	***
LEU	584.48^{abcd}	696.15^{cde}	509.65^{a}	655.26^{bcde}	551.46^{ab}	605.66^{abcd}	533.40^{ab}	664.60^{bcde}	532.33^{ab}	568.62^{abc}	688.57^{cde}	704.99^{de}	773.87^{e}	664.40^{bcde}	38.65	***
PHE	526.59^{ab}	664.89^{b}	445.20^{a}	528.76^{ab}	454.03^{a}	557.61^{ab}	465.03^{a}	581.42^{ab}	478.05^{a}	482.78^{a}	578.78^{ab}	556.49^{ab}	642.43^{b}	584.48^{ab}	44.96	***
TRP	105.19^{a}	139.42^{ab}	104.40^{a}	118.26^{ab}	108.89^{ab}	129.55^{ab}	105.66^{a}	134.30^{ab}	97.28^{a}	115.22^{ab}	129.66^{ab}	124.01^{ab}	148.45^{b}	112.73^{ab}	12.22	**
TAAs	4237.38^{ab}	5262.74^{bcd}	3935.13^{a}	4664.37^{abcd}	4163.92^{ab}	4706.06^{abcd}	4154.82^{ab}	4912.03^{abcd}	4017.33^{a}	4530.93^{abc}	5784.48^{d}	5497.27^{cd}	5713.40^{d}	4822.53^{abcd}	332.05	***
EAAs	2288.65^{a}	2866.39^{a}	2147.14^{a}	2597.39^{a}	2282.31^{a}	2585.16^{a}	2226.25^{a}	2739.04^{a}	2217.36^{a}	2444.18^{a}	3065.43^{a}	2987.18^{a}	3141.70^{a}	2670.02^{a}	413.07	ns
EAAs (%)	54.00%^{abc}	54.41%^{abc}	54.57%^{abc}	55.69%^{bc}	54.87%^{abc}	54.93%^{abc}	53.59%^{ab}	55.92%^{c}	55.38%^{bc}	53.92%^{abc}	53.00%^{a}	54.05%^{abc}	54.95%^{abc}	55.33%^{bc}	0.0065	***

Results are presented as Mean and Standard Error of Difference (SED). TAAs stand for total free amino acids. EAAs stand for total essential amino acids (In bold). LD stands for *longissimus dorsi*.

Values with different superscripts (^{a,b,c,d,e,f,g}) in the same row for the same amino acids differ significantly across the cuts.

P > 0.05 presented as no significance (ns). P < 0.05 presented as ∗ for level of significance. P < 0.01 presented as ∗∗ for level of significance. P < 0.001 presented as ∗∗∗ for level of significance.

% EAAs was calculated by (EAAs/TAAs) x 100.

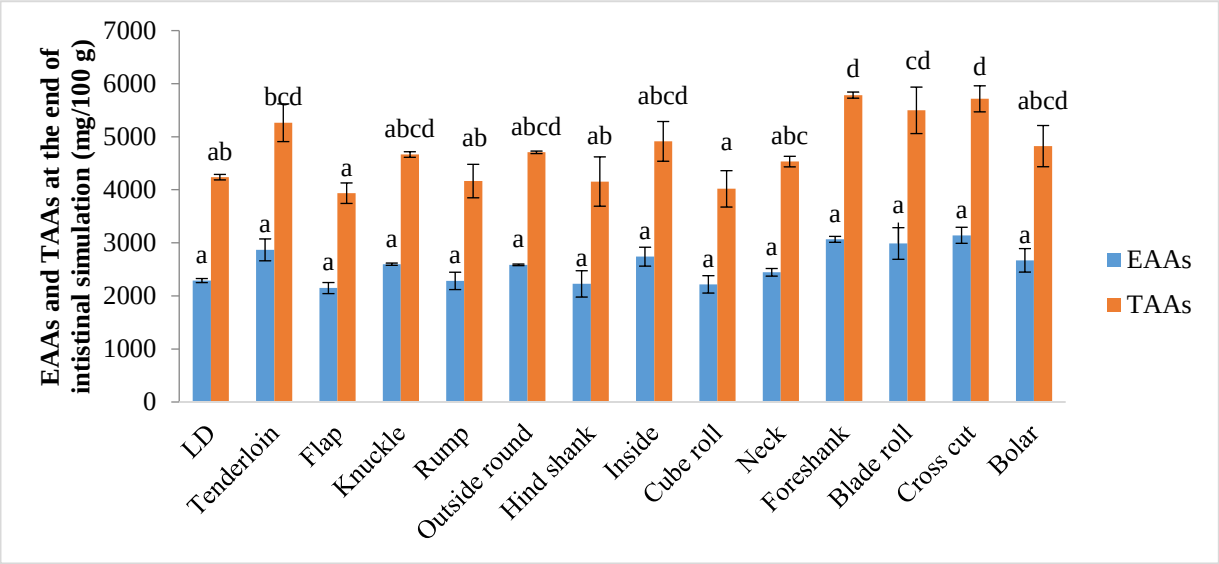

Figure 14. Amounts of EAAs and TAAs in 14 goat kid cuts at the end of intestinal digestion (mg/100 g of cooked meat).

Mean values are plotted with error bars representing standard deviations. Different superscripts ($^{a, b, c, d, e,f}$) differ significantly among cuts at the end of intestinal phase. LD stands for *longissimus dorsi*.

Compared to the gastric phase, almost all of the FAAs apart from ASP and cysteine showed an increase in FAAs after the intestinal digestion. ARG which was the most abundant amino acid in all 14 cuts (ranging from 576.18 to 1012.57 mg/100 g) showed the highest jump; the concentration of which was 50 times higher than that in the gastric phase. Apart from ARG, the amounts of LYS, TYR, LEU and PHE all exceeded 500 mg/100 g and were more than 10 times higher than the corresponding concentrations in the gastric phase.

Pancreatin that was used to simulate intestinal phase contains different peptidases, such as endopeptidase (chymotrypsin, trypsin and elastase) (Véronique Santé-Lhoutellier et al., 2008), and exopeptidases (carboxypeptidase A and B) (Szterk, 2013), which can break down proteins and polypeptides into smaller peptides and FAAs. Different peptide linkages between amino acids are preferentially hydrolyzed by specific pancreatic enzymes. Trypsin attacks the peptide bond on the N-terminal side of LYS and ARG (Lametsch et al., 2002; Sante-Lhoutellier, Aubry, & Gatellier, 2007). Chymotrypsin recognizes peptide bonds formed by carboxyl group of hydrophobic aromatic amino acids like PHE, TYR and TRP, and aliphatic amino acids such as LEU

and MET (Liu & Xiong, 2000). ALA and GLY are specifically hydrolyzed by elastase. Carboxypeptidase A and B exhibit sensitivity and specificity to C-ended amino acids (e.g. PHE,TPR, TYR) but they can be inhibited by PRO (Krehbiel & Matthews, 2003; Szterk, 2013). The extensive activity of these enzymes could explain the large increase of FAAs released during the intestinal digestion in the current study. In addition, trypsin was reported to have a more effective proteolytic performance than α-chymotrypsin, resulting in more release of ARG and LYS (Neuhoff, Arold, Taube, & Ehrhardt, 1988), which explains why the top two amino acids in most of the studied cuts were ARG and LYS. ARG can stimulate blood flood circulation and improve immune function, and LYS can help with the body growth and bone maintenance (Paddon-Jones & Rasmussen, 2009).

The amount of PRO did not change much from before digestion phase to gastric phase, fluctuating at 10 mg/100 g in all of the cuts, and only increased to around 20 mg/100 g at the end of intestinal phase. According to Bauchart et al. (2007), proline-containing peptides were reported to be generally resistant to proteolysis by digestive enzymes, which could probably lead to a low increase in PRO after digestion. Cystine is a limiting amino acid in meat and it was estimated to have the lowest bioavailability (Ravindran et al., 2002). This may have resulted in the lowest cystine concetration in all of the three digestion phases, despite a general increasing pattern seen after the gastric digestion.

EAAs play an important role in regulating human health. Muscle anabolism is primarily stimulated by EAAs (Volpi, Kobayashi, Sheffield-Moore, Mittendorfer, & Wolfe, 2003). LEU can promote the secretion of insulin and facilitate muscle protein synthesis. The scarcity of LEU in diet can result in similar symptoms to hypoglycaemia, like lack of mental stability, giddiness and headache (Paddon-Jones & Rasmussen, 2009). LYS is also an important protein function regulator and inadequate intake of LYS leads to dizziness, hair loss and anemia (Norziah & Ching, 2000). In the current study, goat kid meat was found to liberate a large amount of LEU (532.33 ~ 773.87 mg/100 g), LYS (539.34 ~ 886.98 mg/100 g) and other EAAs as well at the end of intestinal digestion, which indicates a beneficial effect to the human health.

4.2.1.4 The comparison of FAAs in three phases

TAAs, EAAs and %EAAs of 14 goat kid cuts before and after gastric and intestinal digestion are plotted in Figures 15, 16 and 17, respectively. As shown in Figures 15 and 16, the gastric phase only contributed to a small portion in liberating FAAs from the protein matrix, while the intestinal phase showed a huge increase in FAAs generation. The amount of intestinal TAAs (varying from 3935 to 5784 mg/100 g) and EAAs (2147 ~ 3141 mg/100 g) were almost 10 times and 12 times higher than the corresponding TAAs (421 ~ 555 mg/100 g) and EAAs (182 ~ 251 mg/100 g) contents in the gastric phase. Pepsin can maximally break down about 15% of dietary proteins and the peptic digestion in the stomach mainly transforms proteins into large polypeptides to make them more susceptible to further hydrolysis by intestinal enzymes, and approx. 1% of the total amino acids are released turning into free amino acids in stomach (Egger et al., 2016; Krehbiel & Matthews, 2003), which support the findings of this study. Krehbiel and Matthews (2003) reported that 40% of the final intestinal digesta were in the form of FAAs and 60% were oligopeptides of up to six amino acid residues after pancreatin digestion. Though all EAAs are known to be present in meat protein, composition of EAAs has been reported to differ in different muscles (Jung et al., 2016) and this was also observed in the current study. From Figure 17, it can be seen that %EAAs varied in before digestion phase (23.21% ~ 35.03%), gastric phase (39.80% ~ 48.60%) and intestinal phase (53.00% ~ 55.92%). A larger increase from the before digestion phase to the gastric phase (13.57% ~ 21.22%) than that from the gastric phase to the intestinal phase (5.95% ~ 15.15%) can be observed in all 14 cuts, which shows a high proportion of EAAs was released in gastric digestion while a high percentage of non-essential amino acids was released in intestinal phase.

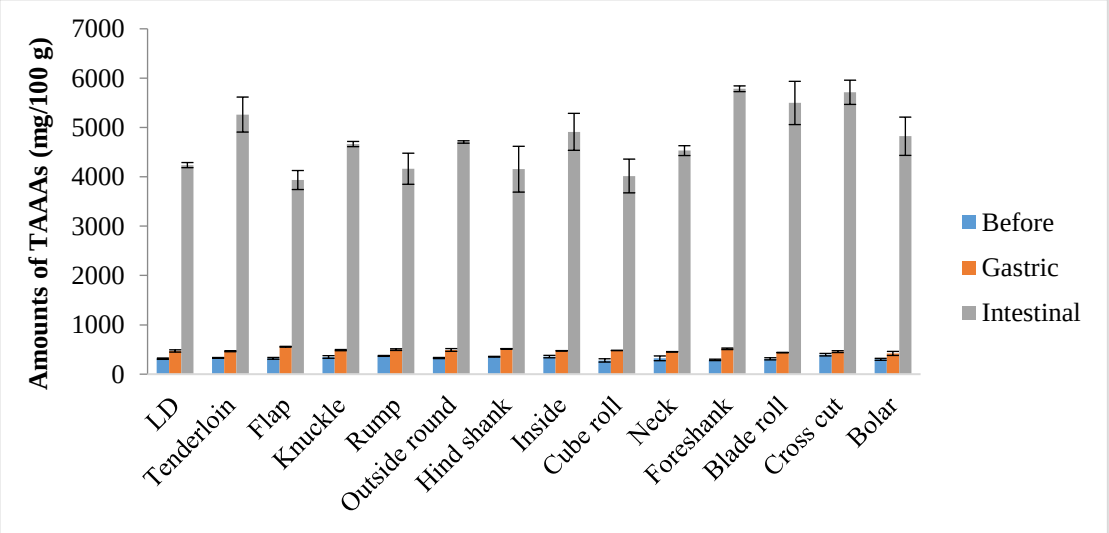

Figure 15. TAAs in 14 goat kid cuts before and after digestion (mg/100 g of cooked meat).

Mean values were plotted with error bars representing standard deviations. LD stands for *longissimus dorsi*.

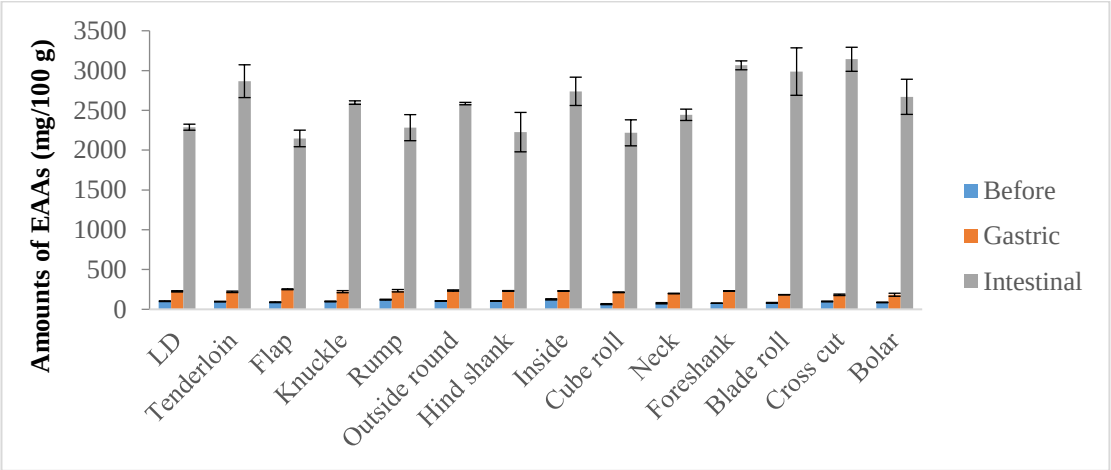

Figure 16. EAAs in 14 goat kid cuts before and after digestion (mg/100 g of cooked meat).

Mean values were plotted with error bars representing standard deviations. LD stands for *longissimus dorsi*.

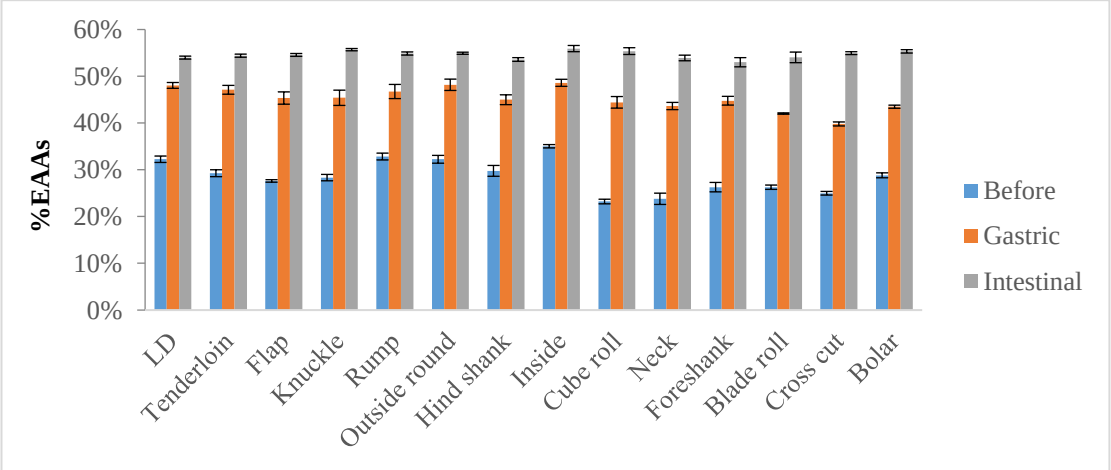

Figure 17. %EAAs in 14 goat kid cuts before and after digestion.

Mean values were plotted with error bars representing standard deviations. LD stands for *longissimus dorsi*.

Before digestion, the average value of goat cuts from hindquarter (LD, tenderloin, flap, knuckle, rump, outside round, hind shank and inside) was numerically greater than that of forequarter cuts in terms of either TAAs, EAAs or EAAs percentage. The FAAs present in the before digestion phase primarily depended on the activity of muscle endogenous enzymes catalyzing proteolytic degradation as mentioned before (section 4.2.1.1). But initial FAAs amounts are also related to the original protein content (Oh et al., 2016). According to the results of the proximate composition (Table 6), the average value of crude protein from the hindquarter cuts (21.56%) was slightly higher than the mean value from the forequarter cuts (20.91%), which probably have affected the enzymatic degradation. After the gastric digestion, the TAAs and EAAs amounts in hindquarter were still higher than that of most forequarter cuts, with the exception of fore shank and cube roll, which have shown higher susceptibility to pepsin degradation compared to the other forequarter cuts studied in this project. All the hindquarter cuts possessed a higher %EAA than that of the forequarter cuts. After the intestinal phase, the forequarter cuts became more comparable with the hindquarters. It is obvious from Figures 15 and 16 that one hindquarter cut (tenderloin) and three forequarter cuts (fore shank, blade roll and cross cut) were relatively higher in TAAs and EAAs amount and the other cuts were not statistically different with each other. This indicates that these 4 cuts were more susceptible to the intestinal digestion. The %EAAs were comparable between the two fractions (hind- and fore- quarter), of around 54%.

Before digestion, cross cut had the highest TAAs while inside had the highest EAAs as well as %EAAs. Cube roll was found to have the lowest level in all three aspects before digestion. After gastric phase, flap had the highest TAAs and EAAs amount while inside still occupied the highest EAAs percentage. Bolar owned the lowest TAAs content, and cross cut had the lowest EAAs amount as well as EAAs proportion. After intestinal phase, fore shank had the highest TAAs and cross cut turned out to possess the highest EAAs. Inside was consistently proved to have the largest EAAs proportion. Interestingly, flap was found to have the lowest amount of TAAs and EAAs as well after intestinal digestion, and the lowest EAAs percentage was in fore shank. The lowest TAAs in flap after the whole digestion could possibly be related to the highest content of crude fat and the lowest protein content in flap. But flap also showed the highest TAAs after gastric digestion, which suggests that, compared to other cuts, flap was more digestible in gastric phase and more susceptible by pepsin.

4.2.2 Peptides in goat kid meat

Apart from the FAAs, the biologically active peptides, either released by endogenous enzymes or produced during digestion, are also the vital criteria to evaluate dietary protein quality (Bauchart et al., 2006). The presence or absence of protein bands was qualitatively compared throughout the before digestion, gastric and intestinal phases of simulated *in vitro* digestion.

4.2.2.1 Peptides found prior to digestion simulation

The 1 D glycine-SDS-PAGE image of proteins and peptides in 14 cooked goat kid cuts before digestion can be seen from Figure 18.

The visualized protein bands were separated into two fractions using the dot line as shown in Figure 18: over 14 kDa as a representative of large protein molecules and below 14 kDa as a representative of peptides, as suggested by Kramer et al. (1993). However, the > 14 kDa part were analyzed preferentially because FAAs and small peptides with low amounts formed during proteolysis, especially those under 5 kDa, may not be able to be detected due to the difficulties of glycine system to stack short peptides and the insensitive staining of Coomassie blue (Liu & Xiong, 2000; Véronique Santé-Lhoutellier et al., 2008; Schägger & von Jagow, 1987).

The referenced standard marker had a wide range of protein molecular weights ranging from 3.5 to 260 kDa. The upper part of the protein bands of most cuts were well-resolved except for *longissimus dorsi*, tenderloin and neck, which showed a darker smudge along the gel lane. This indicated these three cuts remained in the form of more undegraded or partially degraded proteins until analyzed. The < 14 kDa part of those 14 cuts were observed to be similar to one another, and they all showed a band below the smallest marker band of 3.5 kDa, which may have been derived from the sarcoplasmic protein of goat meat. López et al. (2015) characterized the proteolysis and small peptides of commercial Argentinean fermented sausages, and have shown that myofibrillar fraction was less susceptible to proteolysis than the sarcoplasmic fraction that normally releases small peptides less than 3 kDa. Band in a molecular mass around 30 kDa is probably derived from the degraded polypeptides of troponin T, as confirmed

by many authors of the increase of 30 kDa component in myofibrils and the concurrent decrease of troponin T during postmortem conditioning (Negishi, Yamamoto, & Kuwata, 1996; Okumura, Yamada, & Nishimura, 2003). The post-mortem degradation of troponin T is known to be closely related to meat tenderness.

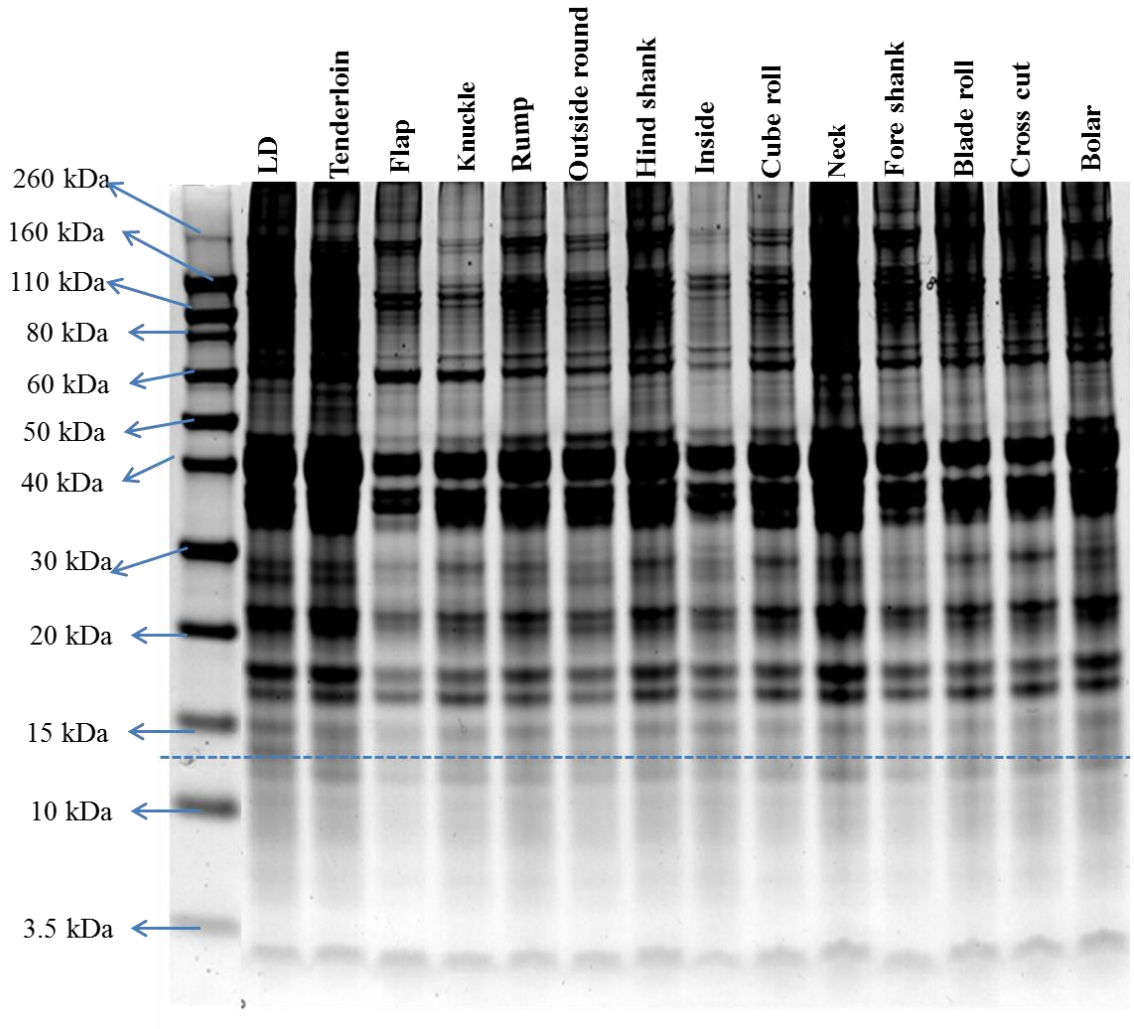

Figure 18. SDS-PAGE profile of 14 cooked goat kid cuts before digestion.

The pre-stained standard marker showed 12 protein bands with molecular weights ranging from 3.5 kDa to 260 kDa. 1 × sample loading buffer (50mM Tris-HCl, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol and 0.002% bromophenol blue) was used to dilute samples. About 40 µg protein was loaded in each wall. LD stands for *longissimus dorsi*.

In muscular tissue, the most abundant protein myofibrils are mainly composed of myosin heavy chain/light chain (~43 %), actin (~20 %) and other minor proteins such as troponins (~5 %), tropomyosin (~5 %) and α-actinin (~2 %) (Yi et al., 2016). Myosin

has a large molar mass of about 520 kDa, comprising two myosin heavy chains (MHC, 220 kDa) and four myosin light chains (MLC, 17 ~ 22 kDa) (Wick, 1999). Myosin heavy chain (MHC) which goes through oxidization and denaturation under cooking can break down to intermediate and small peptides (Kaur et al., 2016). Actin which has a molecular weight of 45 kDa is said to only contribute to a small part of proteolytic degradation (Lametsch et al., 2002). Besides, albumin (68 kDa), creatine kinase (42 kDa) and myoglobin (18 kDa) are the proteins mostly present in the sarcoplasmic fraction (Véronique Santé-Lhoutellier et al., 2008). Hence, the wide range of muscle proteins with different molecular weights and their degraded fragments could explain the dark and intense gel bands over 14 kDa.

During protease hydrolysis, small peptides produced are mainly composed of LYS, GLU, ALA, GLY and ARG. Anserine (β-Ala-1-methyl-His), carnosine (β-Ala-His) and glutathione (γ-Glu-Cys-Gly) are three main endogenous dipeptides present in mammalian skeletal muscle, accounting for 89% of all the peptidic amino acids in fresh muscle (Bauchart et al., 2006). Glycine-rich peptides are preferentially generated during protein degradation (Bauchart et al., 2006). With the processing of hydrolysis, peptides will further degrade to FAAs (Wu, Chen, & Shiau, 2003). Therefore, in this study, the fact that some of these amino acids such as ARG, GLY and LYS did not present a large amount as FAAs in before digestion and gastric phase as shown in Table 7 and 8, could probably be attributed to their main format of peptides. But after intestinal digestion, they exhibited a large increase in quantity as displayed in Table 9, which could be partially related to the degradation of peptides releasing more corresponding FAAs.

Apart from muscle peptidases which play a major role in meat protein degradation, microbiota also contributes to proteolysis, resulting in a more complicated composition of FAAs and small peptides (López et al., 2015).

4.2.2.2 Peptides after the gastric digestion

Figure 19 reflects the SDS-PAGE profiles of digests from the 14 goat cuts after *in vitro* gastric digestion simulation. Compared to the before digestion phase, there was an obvious decrease in band intensity which indicated the protein degradation after pepsin

digestion. At least half of the proteins leaving the stomach was reported to be in the form of peptides (Boisen & Eggum, 1991).

The whole band pattern was in a range of < 160 kDa, and protein bands can be found in the molecular weight about 160 ~ 80, 60 ~ 30, 20 and 15 ~ 3.5 kDa. Thereinto, the prominent bands distributed at around 50, 45, 40 and 30 kDa. Kaur et al. (2016) reported that the large peptides with molecular weights of 74 ~ 91 kDa in raw beef might be the pepsin digestion products of myosin heavy chain (MHC), and cooked beef had more peptides with intermediate molecular weights of 15 ~ 30 kDa. They concluded meat cooking could lead to a greater and faster digestion of protein and polypeptides with a molecular weight over 25 kDa, which could be verified in the current study. The band situated at 40 kDa was probably an interference band from pepsin whose molecular weight is approx. 39 kDa (Takagi, Teshima, Okunuki, & Sawada, 2003).

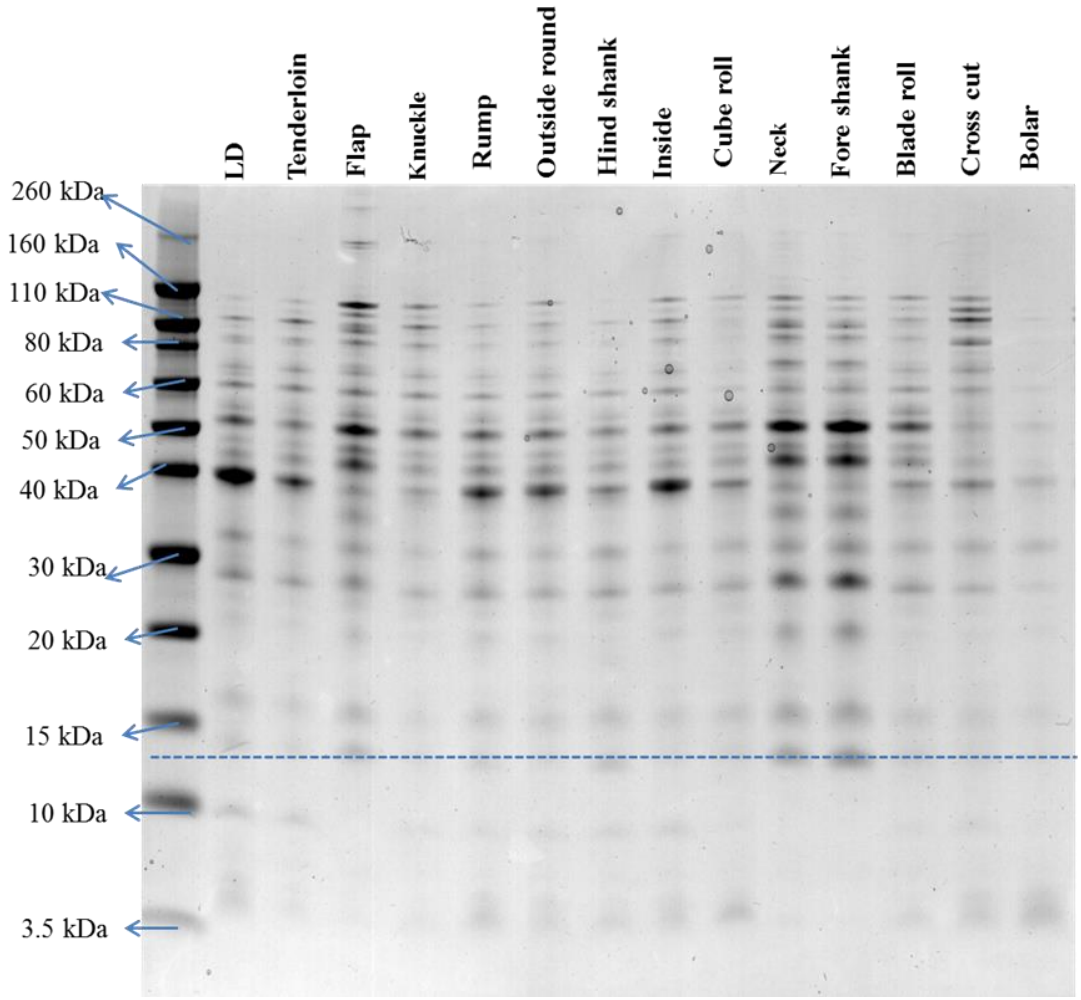

Figure 19. SDS-PAGE profile of 14 cooked goat kid cuts after gastric digestion.

The pre-stained standard marker showed 12 protein bands with molecular weights ranging from 3.5 kDa to 260 kDa. 1 × sample loading buffer (50mM Tris-HCl, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol and 0.002% bromophenol blue) was used to dilute samples. About 40 µg protein was loaded in each wall. LD stands for *longissimus dorsi*.

4.2.2.3 Peptides after intestinal digestion

From Figure 20 it can be seen that the intensity of all of the bands have become lighter upon the intestinal digestion. The bands over 80 kDa have completely disappeared after the intestinal digestion, while protein bands between 30 ~ 40 kDa (around 35 kDa) remained prominently. The bands below 30 kDa, though blurred, could still be observed.

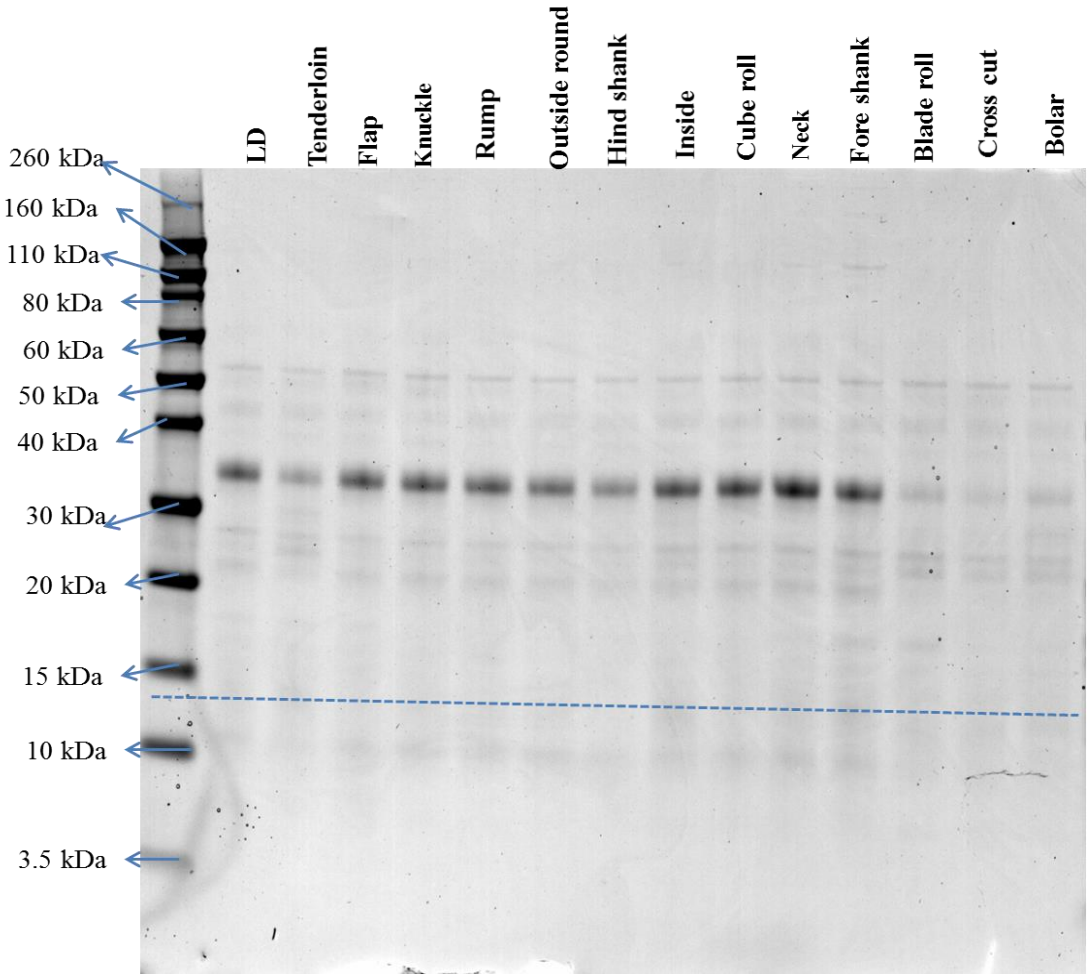

Figure 20. SDS-PAGE profile of 14 cooked goat kid cuts after intestinal digestion.

The pre-stained standard marker showed 12 protein bands with molecular weights ranging from 3.5 kDa to 260 kDa. 1 × sample loading buffer (50mM Tris-HCl, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol and 0.002% bromophenol blue) was used to dilute samples. About 40 µg protein was loaded in each wall. LD stands for *longissimus dorsi*.

Yi et al. (2016) observed a disappearance of protein bands of 80 ~ 95 kDa after duodenal digestion of frozen insects. Kaur et al. (2016) reported that the parent proteins with a molecular weight > 50 kDa produced in gastric beef digests were further degraded and digested upon pancreatin digestion. This phenomenon also happened in the goat kid meat of the current study. After pancreatin digestion, the goat meat showed fewer proteins or peptides of high molecular weight than the gastric phase, which suggests more breakdown and degradation of parent protein from gastric phase, forming smaller peptides and free amino acids.

During *in vitro* digestion, except the proteolysis caused by the endogenous muscle enzymes, digestive enzymes (pepsin and pancreatin) can also cleavage the peptide linkage within the proteins, producing a mixture of FAAs and peptides of various molecular sizes. In the simulated gastric phase, the protein hydrolysis with endopeptidase pepsin occurs randomly, and relatively large peptides are produced. Pepsin also changes protein structure by opening protein and offering more accessible sites for subsequent pancreatin hydrolysis. Sarcoplasmic proteins are preferentially cleaved in gastric phase, while contractile myofibrillar protein such as myosin, actin, troponin, tropomyosin and creatine kinase are more susceptible in intestinal phase (Sayd et al., 2016).

As an intermediate product of the *in vitro* digestion, peptides accumulated in the before digestion phase showing intensely dark bands (Figure 18), and then were hydrolyzed by digestive enzymes pepsin in the gastric phase and pancreatin in the intestinal phase, exhibiting more and more lighter and sparser bands and appearing in more and more lower sites of smaller molecular weights (Figure 19 and 20). To sum up, the SDS-PAGE profiles of the goat kid meat have shown an obvious decrease in band intensity as the digestion progressed, particularly the proteins and large peptides of over 14 kDa, which indicated an almost completed disintegration of sarcoplasmic and myofibrillar protein, namely, a high digestibility catalyzed by pepsin and pancreatin.

Many bioactive peptides from meat are known to be generated during the human digestion process. Inhibitor of angiotensin I-converting enzyme (ACE) is one of the most widely-studied bioactive peptide which can be generated from muscle proteins by digestive enzymes (i.e. pepsin, α-chymotrypsin and trypsin) (Arihara, Nakashima, Mukai, Ishikawa, & Itoh, 2001; Tsai, Chen, & Pan, 2008). ACE is a dipeptidyl carboxypeptidase which can convert angiotensin I to angiotensin II leading to the increase of blood pressure. ACE inhibitory peptides that have antihypertensive effects derive form the protease-digestion of myosin. Myopentapeptides A and B which had the sequences of Met-Asn-Pro-Pro-Lys and Ile-Thr-Thr-Asn-Pro, respectively, were the two ACE inhibitors found from the myosin heavy chain of porcine skeletal muscle by Arihara et al. (2001). Other bioactive peptides such as antioxidant, antimicrobial and antiproliferative peptides have been found and quantified in different meat sources like chicken, oyster and tuna (Ryan, Ross, Bolton, Fitzgerald, & Stanton, 2011). The bioactive peptides in goat meat can be further studied to better understand the goat nutritional quality.

4.3 The digestibility of different cuts

Protein digestibility can be regarded as the amount of amino acids (either in the form of peptides or FAAs) absorbed across the gut wall, which is calculated by the difference between consumed amount of amino acids and the excreted amount of amino acids into the fecal matter (Firman, 1992). The *in vitro* digestibility of some traditional animal sources was studied by other authors, which was found to be 90% for pork, 89% for beef, 85% for salmon and 78% for turkey (Yi et al., 2016).

In this study, the protein digestibility was calculated based on the amount of FAAs released during the simulated *in vitro* gastrointestinal digestion. This approach cannot account for the actual *in vivo* digestibility and it gave a lower value than the digestibility measured with N-balances using *in vitro* assay because the small peptides generated during digestion were not measured and taken into the calculation. Most digesta are in the form of small peptides after digestion rather than free amino acids. Adibi and Mercer (1973) investigated the *in vivo* concentration changes of free amino acids and peptides in jejunal contents before and 3 h after a protein meal. They found a lower increase in FAAs content (26.08 μmol/ml) compared to peptide amino acids which

increased by 102.03 µmol/ml, and there were less amino acids in free form than in small peptides. Cheng (2016) also stated that pancreatic proteases can release 3 ~ 4 times more of small peptides than FAAs. In addition, many nutritional physiologists agreed that 70 ~ 85% amino acids are absorbed in the form of small peptides (di- and tri-peptides) with the remaining being assimilated as FAAs (Krehbiel & Matthews, 2003). Hence the digestibility values presented based on FAAs in this study are quite low and should be used as a relative comparison of the different digestibility in various cuts rather than the actual protein digestibility itself.

The %digestibility of 14 different goat kid cuts evaluated through the release of TAAs and EAAs after *in vitro* gastrointestinal simulation are listed in Table 10. Digestibility was calculated based on using the difference between the amounts of TAAs determined after intestinal phase (Table 9) and before digestion phase (Table 7) in each cut (net increase) divided by the corresponding crude protein content (Table 6) measured in proximate analysis. The calculation was repeated for the EAAs. The results were given as % of meat protein representing how much protein had been digested into free amino acids (either total or essential) and the standard error of difference (SED) to compare the variability of digestibility among different cuts.

As illustrated in Table 10, after gastrointestinal digestion, the 14 cuts exhibited a similar digestibility pattern, for both TAAs and EAAs. The 14 cuts could be classified into 3 levels of digestibility. Firstly, the four forequarter cuts (fore shank, cross cut, blade roll and bolar) had higher digestibility than all the other cuts, in terms of the generation of TAAs (ranging from 26.12% in fore shank to 23.18% in bolar) and EAAs (varying from 14.36% in cross cut to 13.24% in bolar). Secondly, although the digestibility in all the hindquarter cuts was not significantly different with each other, a numerically higher digestibility (either TAAs or EAAs) can be observed in the four hindquarter cuts (tenderloin, knuckle, inside and outside round), which possessed a moderate range of TAAs digestibility (21.72% in tenderloin ~ 20.00% in outside round) and EAAs digestibility (12.20% in tenderloin ~ 11.33% in outside round). Thirdly, the remaining 6 cuts which were composed of two forequarter cuts (neck and cube roll) and four hindquarter cuts (LD, flap, rump and hind shank) owned a relatively lower TAAs digestibility (all below 20%) as well as EAAs digestibility (all below 11%) where *Longissimus dorsi* was found to have the lowest digestibility in regards to the release of TAAs (17.23%) and EAAs (9.61%). This conclusion could were confirmed by SDS-

PAGE profile in Figure 20 where the last three bands (representing blade roll, cross cut and bolar) were obviously lighter and less intense than other bands, indicating the production of more small peptides and FAAs as well as the higher digestibility in these three cuts.

Table 10. Digestibility of 14 different cooked goat kid cuts (% of meat protein).

Digestibility			
	Cut	**TAAs**	**EAAs**
Hindquarter	LD	17.23%^{a}	9.61%^{a}
	Tenderloin	21.72%^{abcd}	12.20%^{abcd}
	Flap	19.08%^{ab}	10.87%^{abc}
	Knuckle	20.78%^{abcd}	12.03%^{abcd}
	Rump	17.59%^{ab}	10.02%^{a}
	Outside round	20.00%^{abc}	11.33%^{abcd}
	Hind shank	17.61%^{a}	9.83%^{a}
	Inside	20.49%^{abc}	11.76%^{abcd}
Forequarter	Cube roll	17.72%^{a}	10.21%^{ab}
	Neck	19.32%^{ab}	10.87%^{abc}
	Fore shank	26.12%^{d}	14.21%^{d}
	Blade roll	24.84%^{cd}	13.92%^{cd}
	Cross cut	25.09%^{cd}	14.36%^{d}
	Bolar	23.18%^{bcd}	13.24%^{bcd}
SED		1.50%	0.89%
P-value		***	***

Results are presented as Mean and Standard Error of Difference (SED). Values with different superscripts (a,b,c,d) in the same column differ significantly across the cuts. $P \leq 0.001$ presented as *** for level of significance. %TAAs = (TAAs after intestinal digestion – TAAs before digestion)/protein *100. %EAAs = (EAAs after intestinal digestion – EAAs before digestion)/protein *100. LD stands for *longissimus dorsi*.

Digestibility of meat protein can be influenced by a variety of factors like species, breed, age, sex, feedstuffs, cooking, storage time, muscles, protease inhibitors, connective tissue, fat content and fiber content (Firman, 1992; Mosenthin, Sauer, & Ahrens, 1994; Véronique Santé-Lhoutellier et al., 2008). For example, the meat from

younger animals have equal or higher capacity of digestibility than the meat from old animals (Firman, 1992). Veronique et al. (2008) reported that the carbonylation and aggregation of myofibrillar protein induced by a long time cooking could reduce the proteolytic susceptibility to pepsin causing a decreased gastric digestibility, while no obvious effect of heat was observed on activity of pancreatic proteases. Véronique Santé-Lhoutellier et al. (2008) found myofibrillar protein digestibility by pepsin was not significantly affected by storage time but protein susceptibility to trypsin and α-chymotrypsin had improved during storage. Hence these factors can also influence the digestibility of goat meat to some extent.

The variability of the digestibility among different goat cuts shows the influential effect of individual cuts. Even though the environmental conditions, diet, slaughter age, storage time and cooking method were all similar for all the male Saanen goats used in this study, the proximate composition of nutrients and protein digestibility were still slightly different for various cuts, indicating a different rate of resource utilization of individual cuts. However, to my knowledge, the information on *in vitro* digestibility of animal cuts is scarce, thus there is no reference to compare with my results. According to the factors which can affect digestibility, two reasons that may cause the different protein bioavailability among goat cuts are proposed and discussed. These two factors are the composition of connective tissues and antioxidant capacity in the different cuts.

Connective tissues contain two major proteins: collagen and elastin, and both of them vary among muscles (Jeremiah, Dugan, Aalhus, & Gibson, 2003). Collagen that has the triple helix polypeptides chains is composed of one β band (200 kDa), one α_1 (100 kDa) band and one α_2 band (100 kDa), thus type I collagen has a molecular weight of about 300 kDa (Zhang, Li, & Shi, 2006). Collagen is resistant to proteinase hydrolysis due to its stable helix but the denatured collagen is easily attacked by proteinases. Collagen will start to denature when temperature reaches 37.5°C, and it will gelatinize and solubilize when temperature is about 40^{o}C ~ 50^{o}C (Boback et al., 2007). Gelatin (< 300 kDa) and collagen hydrolysates which are polypeptide composites (< 50 kDa) are the denatured products from collagen. Both of them only have coil conformation without triple helices, which can be attacked by pepsin because pepsin can only hydrolyze the non-triple helical domain of native collagen leading to a wide distribution of small peptides or amino acids with low molecular weights. Thus the denaturation and

gelatinization of collagen surrounding muscle fibers can facilitate the proteolytic actions of digestive enzymes (Boback et al., 2007; Zhang et al., 2006).

Connective tissue content differs in various animal cuts, depending on physiological functions and anatomic locations (Laser-ReuterswÄRd, Asp, BjÖRck, & RudËRus, 1982). Mitchell et al. (1928) indicated that the forequarter beef muscles contained considerably more total collagen, especially in the shank. Jeremiah et al. (2003) stated a less than 5% connective tissue was found in hindquarter cuts. Casey et al. (1985) reported the collagen content in beef carcass varied form from 1.9% in loin to 4.4% in shin, and the mean collagen value in hindquarter (2.2%) was significantly lower than that in forequarter (2.5%). In this study, sous-vide processed goat kid meat was cooked at 55^{o}C ~ 60^{o}C for 2 hours. During cooking, the collagen-rich connective tissues were softened, denatured and solubilized, forming gelatin and hydrolysates, which could have been broken down by the gastric pepsin before entering the intestinal phase and speed up the digestion process. According to the aforementioned references and the digestibility determined in this study, the relatively higher digestibility in most of the forequarter cuts of goat kids, particularly the fore shank, shoulder cuts (blade roll and cross cut) and bolar, could be partly attributed to having more collagen tissues in these cuts. And *longissimus dorsi* which had the lowest digestibility might contain the lowest level of connective tissues. Apart from fat, flap also had a lot of visible connective tissues, which possibly could be an important reason leading to the highest TAAs in flap after pepsin digestion. But its total digestibility after the intestinal phase was limited due to the low protein content. In summary, this estimation needs to be confirmed by further study with the determination of collagen content or the tenderness of each cut. Belew et al. (2003) measured the Warner–Bratzler shear force values of 40 cooked bovine muscles, and they found that generally, locomotive muscles were less tender than the support muscles. But some muscles from forequarter such as M. *biceps brachii* were more tender than some support muscles like M. *longissimus lumborum* and M. *longissimus thoracis.*

Protein conformation and hydrophobicity can be altered by either protein or fat oxidation. A mild protein oxidation can slightly unfold protein and enhance the susceptibility of peptide bonds to proteolytic enzymes, resulting in the increase of digestibility. But an intense protein oxidation can proceed from mere protein-unfolding to intermolecular cross-links and protein aggregates, leading to a reduced accessibility

of protein substrates to proteolytic enzymes (Friguet, Stadtman, & Szweda, 1994). In muscle protein, myosin is the most susceptible to reactive oxygen species, followed by troponin T (Morzel, Gatellier, Sayd, Renerre, & Laville, 2006). In amino acids, PHE, HIS, PRO, CYS, TYR, TRP, MET, LYS and ARG are more sensitive to oxidation. Hence a lot of essential amino acids lose and digestibility decreases when protein undergoes oxidative modifications (Lund, Heinonen, Baron, & Estévez, 2011). Fat oxidation could decrease the utilization of diet protein and lower the digestibility. Fat hydroperoxides can initiate the blocking and crosslinking of proteins, and react with the sulphur-containing amino acid residues, e.g. CYS or MET. Carbonylic lipid oxidation products, such as hydroxyketones and aldehydes, can attack the amine groups of lysine, resulting in a decrease in EAAs content as well as the digestibility (Korczak, Hęś, Gramza, & Jędrusek-Golińska, 2004). Kim et al. (2010) investigated the fat oxidation of 3 bovine muscles: *longissimus lumborum* (LL), *semimembranosus* (SM), and *adductor* (AD). They found that among the cuts, LL had the least lipid oxidation followed by AD and SM. However, some peptides and amino acids in meat can act as antioxidants to scavenge free radicals. For example, sulfur-containing and labile amino acids such as HIS, MET, TYR, PHE, and TRP have proven antioxidant activity and can be oxidized by low-energy radical species, acting as endogenous antioxidants (Oh et al., 2016). Dipeptide arnosine also possess antioxidant attributes and can prevent the autoxidation of fatty acids (Koutsidis et al., 2008). In this study, after the intestinal digestion, the HIS, MET, PHE and TRP quantity were found to be higher in the fore shank, blade roll, cross cut, bolar and tenderloin compared to those in the other cuts (Table 9). This suggests these cuts had higher radical scavenging activity, less protein and fat oxidation, and hence relatively higher digestibility than the other cuts. But this conclusion needs to be confirmed by further studies on the radical scavenging activity assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH), N,N-Dimethyl-p-phenylendiamine (DMPD) or 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic-acid (ABTS), or Ferric reducing antioxidant power assay (FRAP). Oh et al. (2016) also found a higher antioxidant activity in low-preference cuts (shank, brisket and topside) than in high-preference cuts (rib and loin).

In summary, the digestibility modulated by oxidation is very complex and further investigations to better understand the protein digestibility in relation to oxidative modifications in meat are required (Véronique Santé-Lhoutellier et al., 2008). In goats, digestibility study of specific muscles is insufficient. In the current study, the different digestibility observed in the goat cuts might be related to the different levels of

connective tissues present and the different degree of antioxidant activity against the protein and fat oxidation. Other factors like muscle fiber type could also play a role in affecting the protein digestibility. Based on the results of the current research, after gastrointestinal digestion, fore shank and shoulder cuts (cross cut and blade roll) had higher TAAs (5784.48 ~ 5497.27 mg /100 g) and digestibility (26.12% ~ 24.84%). Bolar, tenderloin and leg cuts (knuckle, inside and outside round) had a slightly lower TAAs (4912.03 ~ 4664.37 mg/100 g) and digestibility (23.18% ~ 20.00%). Neck, flap, cube roll, hind shank, rump and *longissimus dorsi* had relatively lowest TAAs (4530.93 ~ 3935.13 mg/100g) as well as digestibility (19.32% ~ 17.23%). In respect of the whole carcass, most forequarter cuts (fore shank, cross cut, blade roll and bolar) possessed a relatively higher amounts of total free amino acids and digestibility than that of the hindquarter cuts after intestinal digestion.

Chapter 5 Conclusion

In the current study, 14 different cuts from the milk-fed Saanen male goat kids were studied for their proximate composition and digestibility, where the specific results are plotted in Figure 21.

The proximate composition and digestibility of Saanen goat kid differ by cuts. As for the proximate composition, the flap had a significantly lower moisture content (66.71%) than all of the other cuts. The moisture content varied around 75% except for the flap and there was no significant difference. The highest fat content was concentrated in the ventral trunk (flap and bolar), where the flap had the highest fat content (12.81%) than all of the other cuts ($P < 0.001$). The fat proportion in the bolar (4.63%) was higher than that of the fore shank, tenderloin, hind shank, leg cuts (inside and outside round) and shoulder cuts (blade roll and cross cut) ($P < 0.001$). The remaining 12 cuts all had similar fat content, varying around 2.5% ($P > 0.05$). The lower protein content was also found in the ventral trunk where the flap had the lowest protein (18.94%) than all of the cuts except for the bolar ($P < 0.001$). The protein content in the bolar (19.50%) was significantly lower than all of the other cuts apart from the knuckle, fore shank and blade roll. The *longissimus dorsi* (LD) and tenderloin had the highest protein content of 22.77% and 22.71%, respectively. The ash content of all the cuts was around 1% and did not show statistical difference among the 14 cuts, fluctuating at 1%.

After *in vitro* gastrointestinal digestion, the fore shank and the cross cut had the highest released TAAs of 5784.48 and 5713.40 mg/100 g, respectively. There were not significantly different with the other two forequarter cuts (blade roll and bolar), tenderloin and leg cuts (knuckle, inside and outside round), but significantly higher than that of the LD, flap, rump, hind shank, cube roll, and neck ($P < 0.001$). The 14 cuts did not have significant differences in EAAs content at the end of the intestinal phase of the digestion simulation. The %EAAs of all of the 14 cuts had exceeded 50% of the TAAs released after the intestinal digestion. No significance was observed among the %EAAs of 12 cuts except for the fore shank with the lowest %EAAs of 53.00% and the inside with the highest %EAAs of 55.93%. The digestibility of all of the hindquarter cuts and two forequarters cuts (cube roll and neck) were not statistically different from each other, either evaluated by TAAs or EAAs. The highest TAAs digestibility (26.12%) was in the fore shank, which was similar to the digestibility of the three forequarter cuts

(cross cut, blade roll and bolar) and two hindquarter cuts (tenderloin and knuckle), but higher than that of all of the other cuts ($P < 0.001$). The highest EAAs digestibility (14.36%) was in the cross cut, which was statistically higher than that of the four hindquarter cuts (LD, flap, rump and hind shank) and two forequarter cuts (cube roll and neck). The peptides were analyzed qualitatively by SDS-PAGE in the current study. The decrease in band intensity indicated the efficient digestion and breakdown of protein catalyzed by digestive enzymes. Future study on peptidomics can be applied to quantitatively analyze the specific peptides in goat kid meat to better characterize its nutritional profile.

Different nutritional composition and digestibility among various cuts undoubtedly influence their flavor characteristics, which largely decides consumer preference when choosing meat cuts. Retail cuts from loin and rib are highly preferred in the market with a relatively higher price, compared to the cuts with low-preference like shank. This could be attributed to the difference in tenderness where the higher fat content in loin results in a more tender ranking. From the results of the current study, the fore shank and shoulder cuts (blade roll and cross cut) do not have significant difference with LD, tenderloin and cube roll in fat content, but their protein digestibilities were higher than that of the LD and the cube roll ($P < 0.001$). Thus the three forequarter cuts (fore shank, blade roll and cross cut) with a high digestibility may have the similar organoleptic attributes with the LD and tenderloin, which needs to be confirmed by further sensory study. With a better understanding of the tenderness and flavour of individual goat kid cuts, consumers can make an informed decision when purchasing meat cut. However, from the standpoint of nutrients, fore shank and shoulder cuts (blade roll and cross cut) are recommended due to the high protein digestibility and similar proximate content with other cuts except for ventral trunk (flap and bolar). Consumers can take these three forequarter cuts into consideration when choosing goat meat cuts in the market. Meat industry may make better use of these under-utilized meat cuts to promote their marketability and to improve the consumption of low-preference meat cuts for stabilization of goat market.

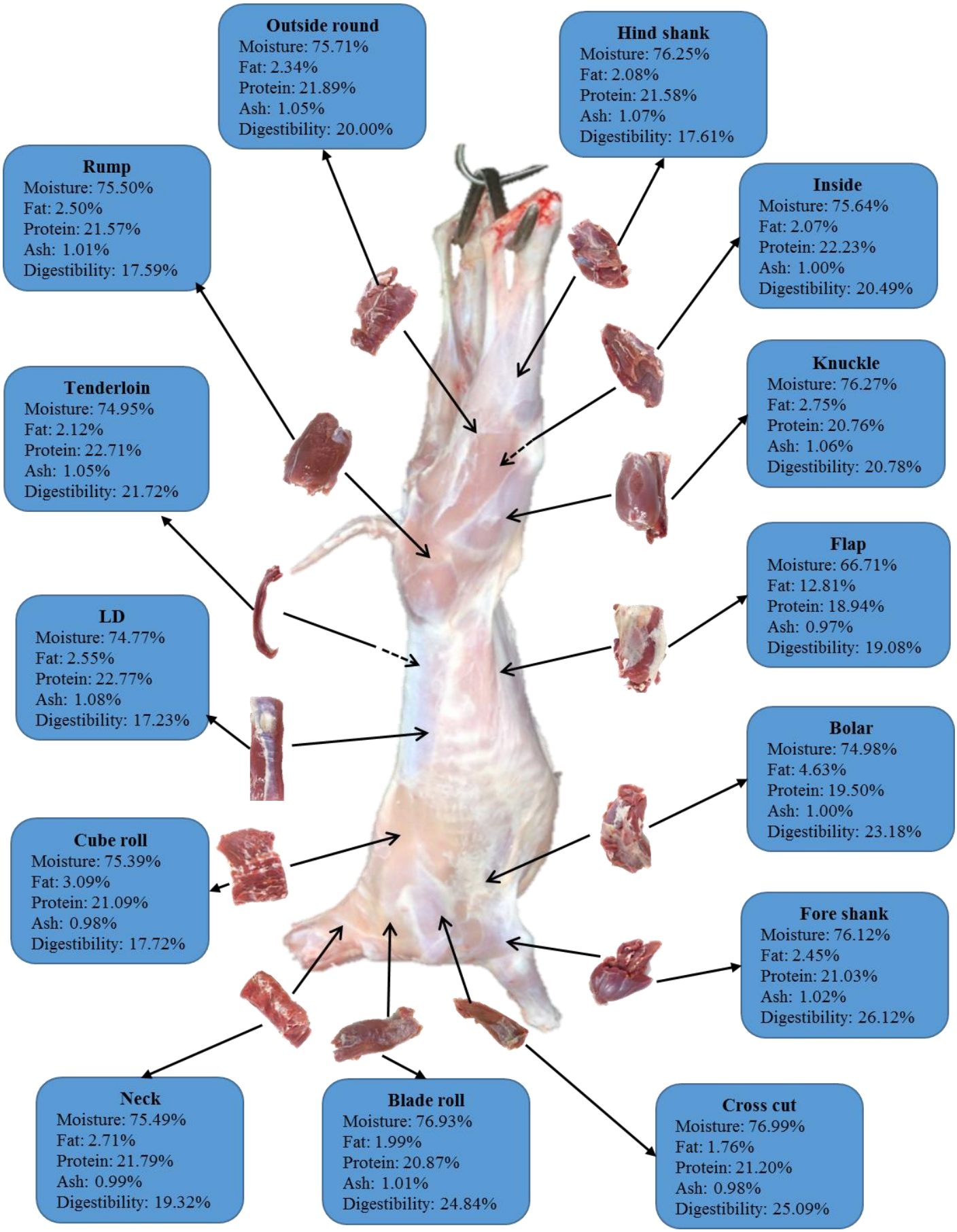

Figure 21. Nutritional anatomy of 14 goat kid cuts.

References

Adibi, S. A., & Mercer, D. W. (1973). Protein Digestion in Human Intestine as Reflected in Luminal, Mucosal, and Plasma Amino Acid Concentrations after Meals. *Journal of Clinical Investigation, 52*(7), 1586-1594.

Ahmed, S. T., Lee, J. W., Mun, H. S., & Yang, C. J. (2015). Effects of supplementation with green tea by-products on growth performance, meat quality, blood metabolites and immune cell proliferation in goats [Article]. *Journal of Animal Physiology & Animal Nutrition, 99*(6), 1127-1137. doi:10.1111/jpn.12279

Arihara, K., Nakashima, Y., Mukai, T., Ishikawa, S., & Itoh, M. (2001). Peptide inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins. *Meat Science, 57*(3), 319-324. https://doi.org/10.1016/S0309-1740(00)00108-X

Armenta, J. M., Cortes, D. F., Pisciotta, J. M., Shuman, J. L., Blakeslee, K., Rasoloson, D., . . . Shulaev, V. (2009). Sensitive and rapid method for amino acid quantitation in malaria biological samples using AccQ• Tag ultra performance liquid chromatography-electrospray ionization-MS/MS with multiple reaction monitoring. *Analytical Chemistry, 82*(2), 548-558.

Atti, N., Rouissi, H., & Mahouachi, M. (2004). The effect of dietary crude protein level on growth, carcass and meat composition of male goat kids in Tunisia. *Small Ruminant Research, 54*(1), 89-97. http://dx.doi.org/10.1016/j.smallrumres.2003.09.010

Azilawati, M. I., Dzulkifly, M. H., Jamilah, B., Shuhaimi, M., & Amin, I. (2016). Estimation of uncertainty from method validation data: Application to a reverse-phase high-performance liquid chromatography method for the determination of amino acids in gelatin using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate reagent. *Journal of Pharmaceutical and Biomedical Analysis, 129*, 389-397. https://doi.org/10.1016/j.jpba.2016.07.012

Babiker, S. A., El Khider, I. A., & Shafie, S. A. (1990). Chemical composition and quality attributes of goat meat and lamb. *Meat Science, 28*(4), 273-277. http://dx.doi.org/10.1016/0309-1740(90)90041-4

Bañón, S., Vila, R., Price, A., Ferrandini, E., & Garrido, M. (2006). Effects of goat milk or milk replacer diet on meat quality and fat composition of suckling goat kids. *Meat Science, 72*(2), 216-221.

Banskalieva, V., Sahlu, T., & Goetsch, A. L. (2000). Fatty acid composition of goat muscles and fat depots: a review. *Small Ruminant Research, 37*(3), 255-268. http://dx.doi.org/10.1016/S0921-4488(00)00128-0

Bas, P., Dahbi, E., El Aich, A., Morand-Fehr, P., & Araba, A. (2005). Effect of feeding on fatty acid composition of muscles and adipose tissues in young goats raised in the Argan tree forest of Morocco. *Meat Science, 71*(2), 317-326. http://dx.doi.org/10.1016/j.meatsci.2005.04.018

Batten, G. (1987). A new meat goat breed Symposium conducted at the meeting of the Proc. 4th Int. Conf. on Goats. Brasilia, Brazil

Bauchart, Rémond, D., Chambon, C., Patureau Mirand, P., Savary-Auzeloux, I., Reynès, C., & Morzel, M. (2006). Small peptides (<5kDa) found in ready-to-eat beef meat. *Meat Science, 74*(4), 658-666. http://dx.doi.org/10.1016/j.meatsci.2006.05.016

Bauchart, C., Morzel, M., Chambon, C., Mirand, P. P., Reynès, C., Buffière, C., & Rémond, D. (2007). Peptides reproducibly released by in vivo digestion of beef meat and trout flesh in pigs. *British journal of nutrition, 98*(6), 1187-1195.

Bax, M.-L., Aubry, L., Ferreira, C., Daudin, J.-D., Gatellier, P., Rémond, D., & Santé-Lhoutellier, V. (2012). Cooking Temperature Is a Key Determinant of in Vitro Meat Protein Digestion Rate: Investigation of Underlying Mechanisms. *J Agric Food Chem, 60*(10), 2569-2576. doi:10.1021/jf205280y

Belew, J., Brooks, J., McKenna, D., & Savell, J. (2003). Warner–Bratzler shear evaluations of 40 bovine muscles. *Meat Science, 64*(4), 507-512.

Bhutta, Z. (1999). Protein: digestibility and availability (pp. 1646-1656): San Diego: Academic Press.

Boback, S. M., Cox, C. L., Ott, B. D., Carmody, R., Wrangham, R. W., & Secor, S. M. (2007). Cooking and grinding reduces the cost of meat digestion. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 148*(3), 651-656. doi:https://doi.org/10.1016/j.cbpa.2007.08.014

Boisen, S., & Eggum, B. (1991). Critical evaluation of in vitro methods for estimating digestibility in simple-stomach animals. *Nutrition research reviews, 4*(1), 141-162.

Bordoni, A., Laghi, L., Babini, E., Di Nunzio, M., Picone, G., Ciampa, A., . . . Capozzi, F. (2014). The foodomics approach for the evaluation of protein bioaccessibility in processed meat upon in vitro digestion. *Electrophoresis, 35*(11), 1607-1614.

Borrisser-Pairó, F., Panella-Riera, N., Gil, M., Kallas, Z., Linares, M. B., Egea, M., . . . Oliver, M. A. (2017). Consumers' sensitivity to androstenone and the evaluation of different cooking methods to mask boar taint. *Meat Science, 123*, 198-204. doi:https://doi.org/10.1016/j.meatsci.2016.10.006

Boyazoglu, J., & Morand-Fehr, P. (2001). Mediterranean dairy sheep and goat products and their quality. *Small Ruminant Research, 40*(1), 1-11. http://dx.doi.org/10.1016/S0921-4488(00)00203-0

Brewer, M. S., Zhu, L. G., Bidner, B., Meisinger, D. J., & McKeith, F. K. (2001). Measuring pork color: effects of bloom time, muscle, pH and relationship to instrumental parameters. *Meat Science, 57*(2), 169-176. doi:https://doi.org/10.1016/S0309-1740(00)00089-9

Brunelle, J. L., & Green, R. (2014). Chapter Twelve - One-dimensional SDS-Polyacrylamide Gel Electrophoresis (1D SDS-PAGE). In L. Jon (Ed.), *Methods in Enzymology* (Vol. Volume 541, pp. 151-159): Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/B9780124201194000124. https://doi.org/10.1016/B978-0-12-420119-4.00012-4

Brzostowski, H., Niżnikowski, R., & Tański, Z. (2008). Quality of goat meat from purebred French Alpine kids and Boer crossbreeds. *Arch Tierz, 51*(4), 381-388.

Buszewski, B., & Noga, S. (2012). Hydrophilic interaction liquid chromatography (HILIC)—a powerful separation technique. *Anal Bioanal Chem, 402*(1), 231-247. doi:10.1007/s00216-011-5308-5

Casey, J. C., Crosland, A. R., & Patterson, R. L. S. (1985). Collagen content of meat carcasses of known history. *Meat Science, 12*(4), 189-203. https://doi.org/10.1016/0309-1740(86)90051-3

Castellanos, M., Van Eendenburg, C. V., Gubern, C., & Sanchez, J. M. (2016). Ethyl-bridged hybrid column as an efficient alternative for HPLC analysis of plasma amino acids by pre-column derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. *Journal of Chromatography B, 1029–1030*, 137-144. https://doi.org/10.1016/j.jchromb.2016.07.004

Cawthorn, D.-M., & Hoffman, L. C. (2014). The role of traditional and non-traditional meat animals in feeding a growing and evolving world. *Animal Frontiers, 4*(4), 6-12. doi:10.2527/af.2014-0027

Cheng, H. M. (2016). Digestion of Carbohydrates, Proteins and Fats. In *Physiology Question-Based Learning* (pp. 149-158): Springer.

Cho, S., Kang, G., Seong, P., Kang, S., Park, K., Kim, Y., & Park, B. (2013). Physico-chemical meat quality and nutritional composition of 10 cuts for Hanwoo steer beef of quality grade 1. *Ann. Anim. Resour. Sci, 24*, 147-156.

Chou, C.-C., Lin, S.-P., Lee, K.-M., Hsu, C.-T., Vickroy, T. W., & Zen, J.-M. (2007). Fast differentiation of meats from fifteen animal species by liquid chromatography with electrochemical detection using copper nanoparticle plated electrodes. *Journal of Chromatography B, 846*(1), 230-239. http://dx.doi.org/10.1016/j.jchromb.2006.09.006

Claeys, E., De Smet, S., Balcaen, A., Raes, K., & Demeyer, D. (2004). Quantification of fresh meat peptides by SDS–PAGE in relation to ageing time and taste intensity. *Meat Science, 67*(2), 281-288. http://dx.doi.org/10.1016/j.meatsci.2003.11.001

Cohen, S. A., & Michaud, D. P. (1993). Synthesis of a Fluorescent Derivatizing Reagent, 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate, and Its Application for the Analysis of Hydrolysate Amino Acids via High-Performance Liquid Chromatography. *Anal Biochem, 211*(2), 279-287. http://dx.doi.org/10.1006/abio.1993.1270

Cornet, M., & Bousset, J. (1999). Free amino acids and dipeptides in porcine muscles: differences between `red' and `white' muscles. *Meat Science, 51*(3), 215-219. doi:https://doi.org/10.1016/S0309-1740(98)00104-1

Denis, S., Sayd, T., Georges, A., Chambon, C., Chalancon, S., Santé-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.

Dhanda, J. S., Taylor, D. G., McCosker, J. E., & Murray, P. J. (1999). The influence of goat genotype on the production of Capretto and Chevon carcasses. 3. Dissected carcass composition. *Meat Science, 52*(4), 369-374. http://dx.doi.org/10.1016/S0309-1740(99)00014-5

Diana, M., Rafecas, M., & Quílez, J. (2014). Free amino acids, acrylamide and biogenic amines in gamma-aminobutyric acid enriched sourdough and commercial breads. *Journal of Cereal Science, 60*(3), 639-644. doi:https://doi.org/10.1016/j.jcs.2014.06.009

Dubeuf, J. P., Morand-Fehr, P., & Rubino, R. (2004). Situation, changes and future of goat industry around the world. *Small Ruminant Research, 51*(2), 165-173. http://dx.doi.org/10.1016/j.smallrumres.2003.08.007

Ebrahimi, M., Rajion, M., & Goh, Y. (2014). Effects of Oils Rich in Linoleic and α-Linolenic Acids on Fatty Acid Profile and Gene Expression in Goat Meat. *Nutrients, 6*(9), 3913-3928.

Egger, L., Ménard, O., Delgado-Andrade, C., Alvito, P., Assunção, R., Balance, S., . . . Portmann, R. (2016). The harmonized INFOGEST in vitro digestion method: From knowledge to action. *Food Research International, 88*, 217-225. http://dx.doi.org/10.1016/j.foodres.2015.12.006

Elgasim, E. A., & Alkanhal, M. A. (1992). Proximate composition, amino acids and inorganic mineral content of Arabian Camel meat: comparative study. *Food Chemistry, 45*(1), 1-4. http://dx.doi.org/10.1016/0308-8146(92)90002-J

Escudero, E., Sentandreu, M. Á., & Toldrá, F. (2010). Characterization of Peptides Released by in Vitro Digestion of Pork Meat. *J Agric Food Chem, 58*(8), 5160-5165. doi:10.1021/jf904535m

FAOSTAT. (2014). Food and Agriculture Organisation Statistics Database. http://www.fao.org/faostat/en/#compare

Feidt, Petit, Bruas-Reignier, & Brun-Bellut. (1996). Release of free amino-acids during ageing in bovine meat. *Meat Science, 44*(1), 19-25. https://doi.org/10.1016/S0309-1740(96)00088-5

Field, R., Riley, M., & Chang, Y. O. (1971). Free amino acid changes in different aged bovine muscles and their relationship to shear values. *Journal of Food Science, 36*(4), 611-612.

Firman, J. D. (1992). Amino Acid Digestibilities of Soybean Meal and Meat Meal in Male and Female Turkeys of Different Ages1. *The Journal of Applied Poultry Research, 1*(3), 350-354. doi:10.1093/japr/1.3.350

Franco, D., GonzÁLez, L., Bispo, E., RodrÍGuez, P., Garabal, J. I., & Moreno, T. (2010). Study of Hydrolyzed Protein Composition. Free Amino Acid, and Taurine Content in Different Muscles of Galician Blonde Beef. *Journal of Muscle Foods, 21*(4), 769-784. doi:10.1111/j.1745-4573.2010.00218.x

Friguet, B., Stadtman, E. R., & Szweda, L. I. (1994). Modification of glucose-6-phosphate dehydrogenase by 4-hydroxy-2-nonenal. Formation of cross-linked protein that inhibits the multicatalytic protease. *Journal of Biological Chemistry, 269*(34), 21639-21643.

Geissler, C., & Powers, H. J. (2005). *Human nutrition* [Bibliographies Non-fiction Computer File]: Edinburgh ; New York : Elsevier/Churchill Livingstone, 2005. 11th ed. / edited by Catherine A. Geissler, Hilary J. Powers. Retrieved from http://ezproxy.aut.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=cat05020a&AN=aut.b1106982x&site=eds-live. Retrieved from cat05020a database.

Gillingham, A. (2008). *'Goats and goat farming - Meat production', Te Ara - the Encyclopedia of New Zealand,*. Retrieved 18 July, 2017,

Gray, N., Zia, R., King, A., Patel, V. C., Wendon, J., McPhail, M. J. W., . . . Nicholson, J. K. (2017). High-Speed Quantitative UPLC-MS Analysis of Multiple Amines in Human Plasma and Serum via Precolumn Derivatization with 6-Aminoquinolyl-N-

hydroxysuccinimidyl Carbamate: Application to Acetaminophen-Induced Liver Failure. *Analytical Chemistry, 89*(4), 2478-2487. doi:10.1021/acs.analchem.6b04623

Gutheil, R. A., & Bailey, M. E. (1993). Calculation of Molecular Weights and Kjeldahl Nitrogen-to-protein Conversion Factors for Myofibrillar Proteins from Amino Acid Sequences *Journal of Muscle Foods, 4*(2), 109-118. doi:10.1111/j.1745-4573.1993.tb00496.x

Hogg, B. W., Mercer, G. J. K., Mortimer, B. J., Kirton, A. H., & Duganzich, D. M. (1992). Carcass and meat quality attributes of commercial goats in New Zealand. *Small Ruminant Research, 8*(3), 243-256. http://dx.doi.org/10.1016/0921-4488(92)90045-6

Huff-Lonergan, E., & Sosnicki, A. (2002). Water-holding capacity of fresh meat. *Fact Sheet, 4669*.

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. http://dx.doi.org/10.1016/j.foodchem.2010.08.036

Intakes, I. R. D. (2005). Nutrient Reference Values for Australia and New Zealand.

Ivanovic, S., Nesic, K., Pisinov, B., & Pavlovic, I. (2016). The impact of diet on the quality of fresh meat and smoked ham in goat. *Small Ruminant Research, 138*, 53-59. http://dx.doi.org/10.1016/j.smallrumres.2016.04.005

Jeremiah, L. E., Dugan, M. E. R., Aalhus, J. L., & Gibson, L. L. (2003). Assessment of the chemical and cooking properties of the major beef muscles and muscle groups. *Meat Science, 65*(3), 985-992. https://doi.org/10.1016/S0309-1740(02)00308-X

Jiménez-Colmenero, F., Carballo, J., & Cofrades, S. (2001). Healthier meat and meat products: their role as functional foods. *Meat Science, 59*(1), 5-13. https://doi.org/10.1016/S0309-1740(01)00053-5

Johnson, Eastridge, J. S., Neubauer, D. R., & McGowan, C. H. (1995). Effect of sex class on nutrient content of meat from young goat. *Journal of animal science, 73*(1), 296-301. doi:10.2527/1995.731296x

Jung, E. Y., Hwang, Y. H., & Joo, S. T. (2016). Muscle profiling to improve the value of retail meat cuts. *Meat Science, 120* (Supplement C), 47-53. https://doi.org/10.1016/j.meatsci.2016.04.012

Karakok, S. G., Ozogul, Y., Saler, M., & Ozogul, F. (2010). Proximate analysis. Fatty acid profiles and mineral contents of meats: a comparative study. *Journal of Muscle Foods, 21*(2), 210-223.

Kaur, L., Astruc, T., Vénien, A., Loison, O., Cui, J., Irastorza, M., & Boland, M. (2016). High pressure processing of meat: effects on ultrastructure and protein digestibility. *Food & function, 7*(5), 2389-2397.

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. http://dx.doi.org/10.1016/j.lwt.2013.09.023

Keeton, J. T. (1994). Low-fat meat products—technological problems with processing. *Meat Science, 36*(1), 261-276. https://doi.org/10.1016/0309-1740(94)90045-0

Kim, Y. H., Huff-Lonergan, E., Sebranek, J. G., & Lonergan, S. M. (2010). Effects of lactate/phosphate injection enhancement on oxidation stability and protein degradation in early postmortem beef cuts packaged in high oxygen modified atmosphere. *Meat Science, 86*(3), 852-858. https://doi.org/10.1016/j.meatsci.2010.07.008

Kirton. (1970). Body and carcass composition and meat quality of the New Zealand feral goat. *New Zealand Journal of Agricultural Research, 13*(1), 167-181.

Kirton, Mercer, G. J. K., Duganzich, D. M., Clarke, J. N., & Woods, E. G. (1999). Composition of lamb carcasses and cuts based on the October 1983 to 1998 export lamb carcass classification standards in New Zealand. *New Zealand Journal of Agricultural Research, 42*(1), 65-75. doi:10.1080/00288233.1999.9513354

Korczak, J., Hęś, M., Gramza, A., & Jędrusek-Golińska, A. (2004). Influence of fat oxidation on the stability of lysine and protein digestibility in frozen meat products. *EJPAU, 7*(1), 02.

Koutsidis, G., Elmore, J. S., Oruna-Concha, M. J., Campo, M. M., Wood, J. D., & Mottram, D. S. (2008). Water-soluble precursors of beef flavour: I. Effect of diet and breed. *Meat Science, 79*(1), 124-130. https://doi.org/10.1016/j.meatsci.2007.08.008

Kramer, W., Girbig, F., Gutjahr, U., Kowalewski, S., Jouvenal, K., Müller, G., . . . Wess, G. (1993). Intestinal bile acid absorption. Na(+)-dependent bile acid transport activity in rabbit small intestine correlates with the coexpression of an integral 93-kDa and a peripheral 14-kDa bile acid-binding membrane protein along the duodenum-ileum axis. *Journal of Biological Chemistry, 268*(24), 18035-18046.

Krehbiel, C., & Matthews, J. (2003). Absorption of amino acids and peptides. *Amino acids in animal nutrition, 2*, 41-70.

Laack, R. (2000). Determination of ultimate pH of meat and poultry Symposium conducted at the meeting of the Proc. 53rd Recip. Meat Conf., Columbus, OH. Am. Meat Sci. Assoc., Savoy, IL

Labconco, C. (1998). A guide to Kjeldahl nitrogen determination methods and apparatus. *Labconco Corporation: Houston, TX, USA*.

Laemmli, U. K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4 [10.1038/227680a0]. *Nature, 227*(5259), 680-685.

Lametsch, R., Roepstorff, P., & Bendixen, E. (2002). Identification of Protein Degradation during Post-mortem Storage of Pig Meat. *J Agric Food Chem, 50*(20), 5508-5512. doi:10.1021/jf025555n

Laser-ReuterswÄRd, A., Asp, N. G., BjÖRck, I., & RudËRus, H. (1982). Effect of collagen content and heat treatment on protein digestibility and biological value of meat products. *International Journal of Food Science & Technology, 17*(1), 115-123. doi:10.1111/j.1365-2621.1982.tb00166.x

Lee, J. H., Kannan, G., Eega, K. R., Kouakou, B., & Getz, W. R. (2008). Nutritional and quality characteristics of meat from goats and lambs finished under identical dietary regime. *Small Ruminant Research, 74*(1), 255-259. http://dx.doi.org/10.1016/j.smallrumres.2007.05.004

Li, L., Liu, Y., Zhou, G., Xu, X., & Li, C. (2017). Proteome Profiles of Digested Products of Commercial Meat Sources [Original Research]. *Frontiers in Nutrition, 4*(8). doi:10.3389/fnut.2017.00008

Li, L., Liu, Y., Zou, X., He, J., Xu, X., Zhou, G., & Li, C. (2017). In vitro protein digestibility of pork products is affected by the method of processing. *Food Research International, 92*, 88-94. https://doi.org/10.1016/j.foodres.2016.12.024

Liméa, L., Bocage, B., Arquet, R., Mahieu, M., & Alexandre, G. (2010). Carcass conformation and cut composition of Creole goat from Guadeloupe. *Tropical Animal Health and Production, 42*(3), 507-514. doi:10.1007/s11250-009-9451-3

Liu, G., & Xiong, Y. L. (2000). Electrophoretic Pattern, Thermal Denaturation, and in Vitro Digestibility of Oxidized Myosin. *J Agric Food Chem, 48*(3), 624-630. doi:10.1021/jf990520h

Lombardi-Boccia, G., Lanzi, S., & Aguzzi, A. (2005). Aspects of meat quality: trace elements and B vitamins in raw and cooked meats. *Journal of Food Composition and Analysis, 18*(1), 39-46. https://doi.org/10.1016/j.jfca.2003.10.007

Longobardi, F., Sacco, D., Casiello, G., Ventrella, A., Contessa, A., & Sacco, A. (2012). Garganica kid goat meat: Physico-chemical characterization and nutritional impacts. *Journal of Food Composition and Analysis, 28*(2), 107-113. http://dx.doi.org/10.1016/j.jfca.2012.08.007

López, C. M., Bru, E., Vignolo, G. M., & Fadda, S. G. (2015). Identification of small peptides arising from hydrolysis of meat proteins in dry fermented sausages. *Meat Science, 104*, 20-29. http://dx.doi.org/10.1016/j.meatsci.2015.01.013

Lu, W., Lv, X., Gao, B., Shi, H., & Yu, L. (2015). Differentiating Milk and Non-milk Proteins by UPLC Amino Acid Fingerprints Combined with Chemometric Data Analysis Techniques. *J Agric Food Chem, 63*(15), 3996-4002. doi:10.1021/acs.jafc.5b00702

Lund, M. N., Heinonen, M., Baron, C. P., & Estévez, M. (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research, 55*(1), 83-95. doi:10.1002/mnfr.201000453

Madruga, & Bressan, M. C. (2011). Goat meats: Description, rational use, certification, processing and technological developments. *Small Ruminant Research, 98*(1–3), 39-45. http://dx.doi.org/10.1016/j.smallrumres.2011.03.015

Madruga, Dantas, I., Queiroz, A., Brasil, L., & Ishihara, Y. (2013). Volatiles and Water- and Fat-Soluble Precursors of Saanen Goat and Cross Suffolk Lamb Flavour. *Molecules, 18*(2), 2150.

Madruga, Elmore, J. S., Oruna-Concha, M. J., Balagiannis, D., & Mottram, D. S. (2010). Determination of some water-soluble aroma precursors in goat meat and their enrolment on flavour profile of goat meat. *Food Chemistry, 123*(2), 513-520. doi:https://doi.org/10.1016/j.foodchem.2010.04.004

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science, 71*(1), 100-121. http://dx.doi.org/10.1016/j.meatsci.2005.03.003

McGowan, C. H., Nurse, G., & Anous, M. R. (1995). Breed type and sex effects on carcass traits, composition and tenderness of young goats. *Small Ruminant Research, 17*(1), 57-63. http://dx.doi.org/10.1016/0921-4488(95)00661-4

Meischke, M., Van Laack, R., & Smulders, F. (1997). The water-holding capacity of fresh meat. *Veterinary quarterly, 19*(4), 175-181.

Milica, P., Snezana, K., & Zorica, S. (2015). Analytical Methods for Determination of Moisture and Ash in Foodstuffs. In *Handbook of Food Analysis, Third Edition - Two Volume Set* (pp. 275-295): CRC Press. Retrieved from http://dx.doi.org/10.1201/b18668-18. Retrieved 2016/10/30. doi:10.1201/b18668-1810.1201/b18668-18

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., . . . Dupont, D. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. *Food & function, 5*(6), 1113-1124.

Mitchell, H., Hamilton, T., & Haines, W. (1928). Some factors affecting the connective tissue content of beef muscle. *The Journal of nutrition, 1*(2), 165-178.

Mohammed, I. (2011). Consumer willingness to pay a premium for Halal Goat meat. *Food Distribution Research, 42*(1).

Morand-Fehr, P., Boutonnet, J. P., Devendra, C., Dubeuf, J. P., Haenlein, G. F. W., Holst, P., . . . Capote, J. (2004). Strategy for goat farming in the 21st century. *Small Ruminant Research, 51*(2), 175-183. http://dx.doi.org/10.1016/j.smallrumres.2003.08.013

Morzel, M., Gatellier, P., Sayd, T., Renerre, M., & Laville, E. (2006). Chemical oxidation decreases proteolytic susceptibility of skeletal muscle myofibrillar proteins. *Meat Science, 73*(3), 536-543. https://doi.org/10.1016/j.meatsci.2006.02.005

Mosenthin, R., Sauer, W. C., & Ahrens, F. (1994). Dietary pectin's effect on ileal and fecal amino acid digestibility and exocrine pancreatic secretions in growing pigs. *The Journal of nutrition, 124*(8), 1222-1229.

Mottram, D. S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry, 62*(4), 415-424. http://dx.doi.org/10.1016/S0308-8146(98)00076-4

Moya, V. J., Flores, M., Aristoy, M. C., & Toldrá, F. (2001). Pork meat quality affects peptide and amino acid profiles during the ageing process. *Meat Science, 58*(2), 197-206. http://dx.doi.org/10.1016/S0309-1740(00)00152-2

Mustafa, A., Åman, P., Andersson, R., & Kamal-Eldin, A. (2007). Analysis of free amino acids in cereal products. *Food Chemistry, 105*(1), 317-324. http://dx.doi.org/10.1016/j.foodchem.2006.11.044

Nakamura, H., Karakawa, S., Watanabe, A., Kawamata, Y., Kuwahara, T., Shimbo, K., & Sakai, R. (2015). Measurement of 15N enrichment of glutamine and urea cycle amino acids derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate using liquid chromatography–tandem quadrupole mass spectrometry. *Anal Biochem, 476*(Supplement C), 67-77. https://doi.org/10.1016/j.ab.2015.02.002

Naveena, B., Khansole, P. S., Kumar, M. S., Krishnaiah, N., Kulkarni, V. V., & Deepak, S. (2017). Effect of sous vide processing on physicochemical, ultrastructural, microbial and sensory changes in vacuum packaged chicken sausages. *Food Science and Technology International, 23*(1), 75-85. doi:10.1177/1082013216658580

Nediani, M., García, L., Saavedra, L., Martínez, S., López Alzogaray, S., & Fadda, S. (2017). Adding Value to Goat Meat: Biochemical and Technological Characterization of Autochthonous Lactic Acid Bacteria to Achieve High-Quality Fermented Sausages. *Microorganisms, 5*(2), 26.

Negishi, H., Yamamoto, E., & Kuwata, T. (1996). The origin of the 30 kDa component appearing during post-mortem ageing of bovine muscle. *Meat Science, 42*(3), 289-303. https://doi.org/10.1016/0309-1740(95)00044-5

Neuhoff, V., Arold, N., Taube, D., & Ehrhardt, W. (1988). Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. *Electrophoresis, 9*(6), 255-262. doi:10.1002/elps.1150090603

Norziah, M. H., & Ching, C. Y. (2000). Nutritional composition of edible seaweed Gracilaria changgi. *Food Chemistry, 68*(1), 69-76. https://doi.org/10.1016/S0308-8146(99)00161-2

Nuñez Gonzalez, F. A., Owen, J. E., & Arias Cereceres, M. T. (1983). Studies on the Criollo goat of Northern Mexico: Part 2—Physical and chemical characteristics of the musculature. *Meat Science, 9*(4), 305-314. http://dx.doi.org/10.1016/0309-1740(83)90040-2

Official Methods of Analysis of AOAC International. (2012). (19th ed.)

Oh, M., Kim, E.-K., Jeon, B.-T., Tang, Y., Kim, M. S., Seong, H.-J., & Moon, S.-H. (2016). Chemical compositions, free amino acid contents and antioxidant activities of Hanwoo (Bos taurus coreanae) beef by cut. *Meat Science, 119*(Supplement C), 16-21. https://doi.org/10.1016/j.meatsci.2016.04.016

Okumura, T., Yamada, R., & Nishimura, T. (2003). Survey of conditioning indicators for pork loins: changes in myofibrils, proteins and peptides during postmortem conditioning of vacuum-packed pork loins for 30 days. *Meat Science, 64*(4), 467-473. https://doi.org/10.1016/S0309-1740(02)00224-3

Oliveira, R. L., Palmieri, A. D., Carvalho, S. T., Leao, A. G., de Abreu, C. L., Ribeiro, C. V., . . . Bezerra, L. R. (2015). Commercial cuts and chemical and sensory attributes of meat from crossbred Boer goats fed sunflower cake-based diets. *Anim Sci J, 86*(5), 557-562. doi:10.1111/asj.12325

Ouali, A., Herrera-Mendez, C. H., Coulis, G., Becila, S., Boudjellal, A., Aubry, L., & Sentandreu, M. A. (2006). Revisiting the conversion of muscle into meat and the underlying mechanisms. *Meat Science, 74*(1), 44-58. doi:http://doi.org/10.1016/j.meatsci.2006.05.010

Paddon-Jones, D., & Rasmussen, B. B. (2009). Dietary protein recommendations and the prevention of sarcopenia: Protein, amino acid metabolism and therapy. *Current opinion in clinical nutrition and metabolic care, 12*(1), 86-90. doi:10.1097/MCO.0b013e32831cef8b

Paeschke, T. M., & Aimutis, W. R. (2010). *Digestive health and nondigestible carbohydrates* [Electronic document]: Ames, IA : Wiley-Blackwell, 2010. Retrieved from http://ezproxy.aut.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=cat05020a&AN=aut.b23755064&site=eds-live Retrieved from cat05020a database.

Paleari, M. A., Moretti, V. M., Beretta, G., Mentasti, T., & Bersani, C. (2003). Cured products from different animal species. *Meat Science, 63*(4), 485-489. http://dx.doi.org/10.1016/S0309-1740(02)00108-0

Pearce, K. L., Rosenvold, K., Andersen, H. J., & Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes — A review. *Meat Science, 89*(2), 111-124. https://doi.org/10.1016/j.meatsci.2011.04.007

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. http://dx.doi.org/10.1016/j.meatsci.2012.09.018

Petracci, M., & BaÉZa, E. (2011). Harmonization of methodologies for the assessment of poultry meat quality features. *World's Poultry Science Journal, 67*(1), 137-151. doi:10.1017/S0043933911000122

Prinsen, H. C. M. T., Schiebergen-Bronkhorst, B. G. M., Roeleveld, M. W., Jans, J. J. M., de Sain-van der Velden, M. G. M., Visser, G., . . . Verhoeven-Duif, N. M. (2016). Rapid quantification of underivatized amino acids in plasma by hydrophilic interaction liquid chromatography (HILIC) coupled with tandem mass-spectrometry. *Journal of Inherited Metabolic Disease, 39*(5), 651-660. doi:10.1007/s10545-016-9935-z

Priolo, A., Micol, D., Agabriel, J., Prache, S., & Dransfield, E. (2002). Effect of grass or concentrate feeding systems on lamb carcass and meat quality. *Meat Science, 62*(2), 179-185. http://dx.doi.org/10.1016/S0309-1740(01)00244-3

Ravindran, V., Hendriks, W. H., Camden, B. J., Thomas, D. V., Morel, P. C. H., & Butts, C. A. (2002). Amino acid digestibility of meat and bone meals for broiler chickens. *Australian Journal of Agricultural Research, 53*(11), 1257-1264. doi:https://doi.org/10.1071/AR02055

Rhee, K. S., Cho, S. H., & Pradahn, A. M. (1999). Composition, storage stability and sensory properties of expanded extrudates from blends of corn starch and goat meat, lamb, mutton, spent fowl meat, or beef. *Meat Science, 52*(2), 135-141. http://doi.org/10.1016/S0309-1740(98)00157-0

Robert, G. K. (2012). Meat Composition. In *Handbook of Meat and Meat Processing, Second Edition* (pp. 45-62): CRC Press. Retrieved from http://dx.doi.org/10.1201/b11479-6.

Rodrigues, D. B., Mariutti, L. R., & Mercadante, A. Z. (2016). An in vitro digestion method adapted for carotenoids and carotenoid esters: moving forward towards standardization. *Food Funct, 7*(12), 4992-5001. doi:10.1039/c6fo01293k

Ryan, J. T., Ross, R. P., Bolton, D., Fitzgerald, G. F., & Stanton, C. (2011). Bioactive Peptides from Muscle Sources: Meat and Fish. *Nutrients, 3*(9), 765.

Sadeghi Ekbatan, S., Sleno, L., Sabally, K., Khairallah, J., Azadi, B., Rodes, L., . . . Kubow, S. (2016). Biotransformation of polyphenols in a dynamic multistage gastrointestinal model. *Food Chemistry, 204*, 453-462. doi:http://dx.doi.org/10.1016/j.foodchem.2016.02.140

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. *J Agric Food Chem, 56*(4), 1488-1494. doi:10.1021/jf072999g

Sante-Lhoutellier, V., Aubry, L., & Gatellier, P. (2007). Effect of Oxidation on In Vitro Digestibility of Skeletal Muscle Myofibrillar Proteins. *J Agric Food Chem, 55*(13), 5343-5348. doi:10.1021/jf070252k

Santé-Lhoutellier, V., Engel, E., Aubry, L., & Gatellier, P. (2008). Effect of animal (lamb) diet and meat storage on myofibrillar protein oxidation and in vitro digestibility. *Meat Science, 79*(4), 777-783. https://doi.org/10.1016/j.meatsci.2007.11.011

Savell, J., Miller, R., Wheeler, T., Koohmaraie, M., Shackelford, S., Morgan, B., . . . McKeith, F. (1994). Standardized Warner-Bratzler shear force procedures for genetic evaluation Symposium conducted at the meeting of the National Beef Tenderness Plan Conference, 1-3.

Sayd, T., Chambon, C., & Santé-Lhoutellier, V. (2016). Quantification of peptides released during in vitro digestion of cooked meat. *Food Chemistry, 197*(Part B), 1311-1323. https://doi.org/10.1016/j.foodchem.2015.11.020

Schagger, H. (2006). Tricine-SDS-PAGE.. *Nat. Protocols, 1*(1), 16-22. doi:10.1038/nprot.2006.4

Schägger, H., & von Jagow, G. (1987). Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem, 166*(2), 368-379. http://dx.doi.org/10.1016/0003-2697(87)90587-2

Schönfeldt, Naude, R., Bok, W., Van Heerden, S., Smit, R., & Boshoff, E. (1993). Flavour-and tenderness-related quality characteristics of goat and sheep meat. *Meat Science, 34*(3), 363-379.

Schönfeldt, Naudé, R. T., Bok, W., van Heerden, S. M., Sowden, L., & Boshoff, E. (1993). Cooking- and juiciness-related quality characteristics of goat and sheep meat. *Meat Science, 34*(3), 381-394. http://dx.doi.org/10.1016/0309-1740(93)90085-V

Schonfeldt, H. C. (1989). *A comparison of the quality characteristics of goat meat with those of sheep meat*. Universiteit van Pretoria.

Sen, A. R., Santra, A., & Karim, S. A. (2004). Carcass yield, composition and meat quality attributes of sheep and goat under semiarid conditions. *Meat Science, 66*(4), 757-763. http://dx.doi.org/10.1016/S0309-1740(03)00035-4

Sharma, & Rajput, Y. (2015). SDS-PAGE–Principle and Applications. *Advanced Techniques and Novel Approaches for Quality and Safety Evaluation of Dairy Foods*, 36.

Sharma, G., Attri, S. V., Behra, B., Bhisikar, S., Kumar, P., Tageja, M., . . . Singhi, S. (2014). Analysis of 26 amino acids in human plasma by HPLC using AQC as derivatizing agent and its application in metabolic laboratory [journal article]. *Amino Acids, 46*(5), 1253-1263. doi:10.1007/s00726-014-1682-6

Sheridan, R., Hoffman, L., & Ferreira, A. (2003). Meat quality of Boer goat kids and Mutton Merino lambs. 1. Commercial yields and chemical composition. *ANIMAL SCIENCE-GLASGOW THEN PENICUIK-, 76*(1), 63-72.

Shija, D. S., Mtenga, L. A., Kimambo, A. E., Laswai, G. H., Mushi, D. E., Mgheni, D. M., . . . Safari, J. G. (2013). Chemical Composition and Meat Quality Attributes of Indigenous Sheep and Goats from Traditional Production System in Tanzania. *Asian-Australas J Anim Sci, 26*(2), 295-302. doi:10.5713/ajas.2012.12432

Silva, F. A. P., Ferreira, V. C. S., Madruga, M. S., & Estévez, M. (2016). Effect of the cooking method (grilling, roasting, frying and sous-vide) on the oxidation of thiols, tryptophan, alkaline amino acids and protein cross-linking in jerky chicken [journal article]. *Journal of Food Science and Technology, 53*(8), 3137-3146. doi:10.1007/s13197-016-2287-8

Smith, G., Pike, M., & Carpenter, Z. (1974). Comparison of the palatability of goat meat and meat from four other animal species. *Journal of Food Science, 39*(6), 1145-1146.

Stuknytė, M., Cattaneo, S., Pagani, M. A., Marti, A., Micard, V., Hogenboom, J., & De Noni, I. (2014). Spaghetti from durum wheat: Effect of drying conditions on heat damage, ultrastructure and in vitro digestibility. *Food Chemistry, 149*(Supplement C), 40-46. doi:https://doi.org/10.1016/j.foodchem.2013.10.071

Swan, J. E., Esguerra, C. M., & Farouk, M. M. (1998). Some physical, chemical and sensory properties of chevon products from three New Zealand goat breeds. *Small Ruminant Research, 28*(3), 273-280. http://doi.org/10.1016/S0921-4488(97)00087-4

Szterk, A. (2013). Chemical state of heterocyclic aromatic amines in grilled beef: Evaluation by in vitro digestion model and comparison of alkaline hydrolysis and organic solvent for extraction. *Food and Chemical Toxicology, 62*(Supplement C), 653-660. https://doi.org/10.1016/j.fct.2013.09.036

Takagi, K., Teshima, R., Okunuki, H., & Sawada, J.-i. (2003). Comparative study of in vitro digestibility of food proteins and effect of preheating on the digestion. *Biological and Pharmaceutical Bulletin, 26*(7), 969-973.

Titgemeyer, E. C., Merchen, N., Berger, L., & Deetz, L. (1988). Estimation of lysine and methionine requirements of growing steers fed corn silage-based or corn-based diets. *Journal of dairy science, 71*(2), 421-434.

Titi, H. H., Tabbaa, M. J., Amasheh, M. G., Barakeh, F., & Daqamseh, B. (2000). Comparative performance of Awassi lambs and Black goat kids on different crude protein levels in Jordan. *Small Ruminant Research, 37*(1), 131-135. http://dx.doi.org/10.1016/S0921-4488(99)00136-4

Toldra, F., & Aristoy, M. a.-C. n. (2008). Amino Acids. In *Handbook of Muscle Foods Analysis* (pp. 11-39): CRC Press. Retrieved from http://dx.doi.org/10.1201/9781420045307.ch2.

Tomović, V. M., Jokanović, M. R., Švarc-Gajić, J. V., Vasiljević, I. M., Šojić, B. V., Škaljac, S. B., . . . Žujović, M. M. (2016). Physical characteristics and proximate and mineral composition of Saanen goat male kids meat from Vojvodina (Northern Serbia) as influenced by muscle. *Small Ruminant Research, 145*, 44-52. http://dx.doi.org/10.1016/j.smallrumres.2016.10.019

Tsai, J.-S., Chen, J.-L., & Pan, B. S. (2008). ACE-inhibitory peptides identified from the muscle protein hydrolysate of hard clam (Meretrix lusoria). *Process Biochemistry, 43*(7), 743-747. doi:https://doi.org/10.1016/j.procbio.2008.02.019

Tshabalala, P. A., Strydom, P. E., Webb, E. C., & Kock, H. L. (2003). Meat quality of designated South African indigenous goat and sheep breeds. *Meat Science, 65*(1), 563-570. http://dx.doi.org/10.1016/S0309-1740(02)00249-8

Turner, K. E., Belesky, D. P., Cassida, K. A., & Zerby, H. N. (2014). Carcass merit and meat quality in Suffolk lambs, Katahdin lambs, and meat-goat kids finished on a grass–legume pasture with and without supplementation. *Meat Science, 98*(2), 211-219. http://doi.org/10.1016/j.meatsci.2014.06.002

Vetharaniam, I., & Daly, C. (2000). Sensitivity of ultimate meat pH to initial metabolite concentration when glycogen is not limiting. *New Zealand Society of Animal*

Production; 1999. Symposium conducted at the meeting of the Proceedings-New Zealand Society of Animal Production.

Volpi, E., Kobayashi, H., Sheffield-Moore, M., Mittendorfer, B., & Wolfe, R. R. (2003). Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *The American journal of clinical nutrition, 78*(2), 250-258.

Watanabe, A., Ueda, Y., & Higuchi, M. (2004). Effects of slaughter age on the levels of free amino acids and dipeptides in fattening cattle. *Animal Science Journal, 75*(4), 361-367. doi:10.1111/j.1740-0929.2004.00198.x

Webb. (2014). Goat meat production, composition, and quality. *Animal Frontiers, 4*(4), 33-37. doi:10.2527/af.2014-0031

Webb, Casey, N. H., & Simela, L. (2005). Goat meat quality. *Small Ruminant Research, 60*(1–2), 153-166. http://dx.doi.org/10.1016/j.smallrumres.2005.06.009

Wick, M. (1999). Filament assembly properties of the sarcomeric myosin heavy chain. *Poultry Science, 78*(5), 735-742. doi:10.1093/ps/78.5.735

Williams, P. (2007). Nutritional composition of red meat. *Nutrition & Dietetics, 64*, S113-S119. doi:10.1111/j.1747-0080.2007.00197.x

Wu, Chen, H.-M., & Shiau, C.-Y. (2003). Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus). *Food Research International, 36*(9), 949-957. https://doi.org/10.1016/S0963-9969(03)00104-2

Wu, G., Farouk, M. M., Clerens, S., & Rosenvold, K. (2014). Effect of beef ultimate pH and large structural protein changes with aging on meat tenderness. *Meat Science, 98*(4), 637-645. https://doi.org/10.1016/j.meatsci.2014.06.010

Yi, L., Van Boekel, M. A. J. S., Boeren, S., & Lakemond, C. M. M. (2016). Protein identification and in vitro digestion of fractions from Tenebrio molitor [journal article]. *European Food Research and Technology, 242*(8), 1285-1297. doi:10.1007/s00217-015-2632-6

Young, O. A., Frost, D. A., & Agnew, M. (2012). Analytical Methods for Meat and Meat Products. In *Handbook of Meat and Meat Processing, Second Edition* (pp. 139-160): CRC Press. Retrieved from http://dx.doi.org/10.1201/b11479-11. Retrieved 2016/10/24.

Zhang, Z., Li, G., & Shi, B. (2006). Physicochemical properties of collagen, gelatin and collagen hydrolysate derived from bovine limed split wastes. Journal-Society of Leather Technologists and Chemists, 90(1), 23.