

Review of Literature

2.1. History of domestication of pigs from wild

The wild boar (*Sus scrofa cristatus*), which is one of the most extensively dispersed mammalian species, is the only ancestor of the modern-day domestic pig. It is distributed all over the Europe and Asia, from Europe to Near East, including South and East Asia, spreading to North Africa (Choi et al., 2020). With the settlement of human and travelling for business on agricultural purposes, the evidence regarding the history of domestication of wild boar have provided an anthropogenic and biogeographical history on domestic pigs (Epstein and Bichard, 1984). Keuling et al. (2018) has defined based on the morphological characteristics, like the hair colour, size and body proportions, shape of the skull of 16 wild boar subspecies. These subspecies belong to the groups of European, Asian and South-Asian.

However, according to molecular phylogeny-based studies, wild boar is classified into Asian and European clades (Giuffra et al., 2000; Larson et al., 2005; Scandura et al., 2008). Based on mt-DNA and nuclear studies, the wild boar from east and south east Asia are known to have high genetic variation than European wild boars (Ramirez et al., 2009). The mainland South-Eastern Asia (MSEA) and Island South-Eastern Asia (ISEA) are known to be the regions of origin of wild boar, and also regarded as the hotspot where other species of *Sus* are also present (Amills et al., 2018). Wu et al. (2007) mentioned MSEA as the 'Mekong region' due to the fact that it covers nearly all the East Asian wild pigs that are genetically diverse than regions of Asia. Therefore, it is evident that the study of occurrence of domestication of wild boar was possible through molecular phylogeny analysis. This method not only reveal the origin of domestication of wild boar but also how diverse they were from each other including its distribution in different parts of the world.

The initial domestication of pig is believed to have occurred in the Near East approximately 9000 YBP (years before the present) from local population of wild boar (Bokonyi, 1947). It remains uncertain till today whether the present-day domestic pig exhibits single origin or multiple. Darwin, C. (1868) on the basis of phenotypic characters depicted the existence of two major variants of domestic pigs, namely a European variant (*Sus scrofa*) and an Asian variant (*Sus indicus*). It has been reported that Asian pigs were

used in the enhancement of European pigs in the early 18th and 19th century (Darwin, C. 1868; Jones, 1998). But to what extent the Asian pigs have genetically influenced the enhancement of European pigs is not known.

Studies done by Giuffra et al. (2000) and Kijas and Andersson, (2001) on mitochondrial DNA sequences of hair and blood samples of recent European and Asian wild boar and other domestic breeds unveiled a distinct Asian clade and two European clades. The Asian clade comprised of Japanese wild boar, Chinese Meishan domestic swine, and certain European domestic swine. Among the two European clades, the first clade consists of European wild boar which include pigs of Cook Islands and Israeli wild boars, while the second clade include the Italian wild boars. Again, another finding by Larson et al. (2005) based on several mitochondrial DNA (mt-DNA) sequences showed an evolutionary origin of pigs in the Island of South-East Asia (ISEA) followed by westward distribution, culminating in the western corners of the European countries. The findings stated that domestic pigs originating from Europe and Asia not only formed groups with European and Chinese wild boar, but also that certain domestic pigs formed groups with wild boar native to Italy, India, and peninsular South-east Asia. A distinct population of wild swine was also found in New Guinea, Halmahera (part of the Moluccan Islands), and Hawaii exhibited an additional distinctive genetic characteristic. Further studies also revealed that the wild boars of Near East had distinct characters that do not match with that of European domestic pigs (Larson et al., 2005). These data summarize where the domestication occurred, but not the time it occurred. Evidently, it is concluded that the major regions where the origin of domestic pigs might have occurred are Near East, Europe, China, ISEA, and Oceania.

2.1.1. The Near East (Middle East countries): The origination of domestication of pig was found to start in Near East, as demonstrated in the records of zooarchaeological survey. The records depicted demographic changes in the remains of pigs excavated from various significant archaeological sites, primarily located in parts of Turkey. The mt-DNA sequences of pigs found from the site Çayonu Tepesi (Pre-pottery and Pottery Neolithic period) presents an exceptional case showing pig slaughter at younger ages (Hongo and Meadow, 1998). Furthermore, alterations in skeletal structure and teeth such as reduction in snout length and modifications in overall shape, indications of physiological stresses (Ervynck et al., 2002; Dobney et al., 2004) suggested that, there was a close connection

between humans and animals. Their data also revealed alterations in the morphology and biometrics of pigs that have caused modifications in the rooting behaviour of wild boars due to human settlements introducing new food habits such as crops or human refusal.

It was suggested that the wild boars of Middle East countries belong to the European clade (Ramirez et al., 2009; Ottoni et al., 2013), however, recent findings from the wild boars of Iran showed that Near East domestic pigs have similar haplotypes with that of Asian clade (Khalilzadeh et al., 2016).

2.1.2. Europe: The domestication of domestic pigs in Europe took place in between the 19th and 18th millenniums before present (BP). As hypothesized, due to migration of the farmers from the Near East (known as demic diffusion), there might have occurred the transmission of pigs and cattle through trade and exchange programmes (Childe, 1958; Pinhasi et al., 2005; Sampietro et al., 2007). Larson and his colleagues (2007b) tried to provide elucidation to these inquiries by conducting sequencing of mt-DNA sequences from the archaeological remnants (specifically bone and teeth) of *S. scrofa* originating from Europe as well as the Near East. They found clear evidence that the Near East lineages have migrated to Europe as a result of human intervention. The evidence of Near East haplotype was found in the regions of Germany and the Paris Basin. The mt-DNA sequences extracted from Bercy revealed that, it was the earliest regions where both Near East and European domestic pig were found together. But after the mid- 6th millennium BP, the Near East pig lineages are reported to vanish, therefore the European domesticated pigs replaced the Near East origin. Not only in the Near East, but that of indigenous pig breeds of Armenia was also replaced by European domestic pigs (Larson et al., 2007a). There is also evidence of domestication of Italian wild boar that might have occurred separately (Albarella et al., 2006).

2.1.3. China: When compared with European domestic pigs, wild boars of China have high amount of genetic variation. Moreover, China is regarded as the primary centre of early pig domestication (Fang et al., 2009; Megens et al., 2008). When constructing a phylogenetic tree of European and Asian haplotypes, the western Eurasian and ISEA regions were composed of similar clades. While that of East Asian mainland was characterized by a significant polytomy of groupings and individual clades. The domestic clades found in the East Asia, represent the common Asian pigs found in the modern

breeds that is distributed globally (Larson et al., 2007b). However, the Asian haplotypes of wild boar were found only in islands of Japan, Okinawa, Taiwan and Hainan, South Korea. The samples where both the wild and domestic haplotypes were seen included the pigs from India and Nepal (Larson et al., 2005; Tanaka et al., 2008). Another clade, called as Mountainous and South-east Asian distribution (MTSEA) consists of both domestic and wild pig samples from Indochina, Bhutan and Taiwan (Tanaka et al., 2008). The genetic findings by Larson et al. (2007b) and Larson et al. (2010) states that the prevalent modern domestic pigs of central China represent the most common Asian lineages. These lineages are found throughout East Asia, in Australian feral pigs, and in modern European and American pig breeds.

The microsatellite and mt-DNA studies from pigs of Lanyu revealed that this population of pigs is different from all other pigs whether domestic or wild. Pigs of Lanyu located in the eastern part of China, are considered endemic, however it requires thorough investigation. Study performed by Cucchi et al. (2011) from the sites of Xinglongwa in Heibei Province, Jiahu in Henan Province and Zengpiyan in Guangxi Province supported the hypothesis that the early pig domestication were originated at Zengpiyan, as proposed by Nelson, (1998) and Yuan and Flad, (2002). There are evidences such as association of burial of human and *S. scrofa* skeletons found between 8000-7400 bp revealed through whole mitochondrial genome. There was also pig skulls found with circular holes in the frontal bone which might be due to poleaxing (Jing, 2006). Molar tooth shape of the decayed pigs helps to differentiate between wild and domestic pig (Cucchi et al., 2011). Through molar tooth decay study, it is suggested that Zengpiyan pigs were wild boar (Yuan and Flad, 2002). Zengpiyan pigs are found in the southern part of China with a wetter and warmer area type, they are described as 'fairly small but with a broad and high crowned skull' (Groves, 2007). It has also been revealed the presence of wild boar from Xinglongwa in Northeast part of China through morphometric studies (Cucchi et al., 2011). Xinglongwa pigs were hunted from locally found wild boar and that its wild boar phenotype characters are prevalent without going any major changes in last 8000 years. This supports the idea of economic value of wild boar which led to independent domestication of pigs in Northeast China. While that of pigs from Jiahu site appears to be different. Molar tooth decay analysis by Cucchi et al. (2011) stated that Jiahu pigs and pigs found at Xishuipo site display morphological changes that occurred due to

domestication. From the recent morphological and genetic studies, it has been confirmed that there was an independent domestication of wild boar in China.

2.1.4. Peninsular South-east Asia, Island South-east Asia (ISEA) and Oceania: This region is one of the most important and interesting to study the relationship between humans and pigs and its domestication process, and also that diversity at genetic level is found in this region than anywhere else (Groves, 1981). Domestication of pigs in mainland Asia is found to be simple as because only one species (*S. scrofa*) has been involved. But in context to ISEA, various species of *Sus* are found, suggesting to be hunted by human and domesticated. In this region, there are reports of inter-breeding between the wild *S. scrofa* and other indigenous species (Blouch and Groves, 1990). The feral and domestic pigs of New Guinea are the results of inter-breeding of *S. scrofa* and *S. celebensis* (Groves, 1981). Larson et al. (2005) supported the Groves (1981) conclusions of presence of various other species pigs. Pigs in Australia was nil, until introduced by European settlers, therefore the present feral pigs found in Australia were introduced by Europeans. Another difference reported from this region is that the *Sus* species represent a major biogeographical boundary called ‘Wallace’s Line’ and their haplotypes were similar to that of mainland Asia and was collectively called Pacific clade (Larson et al., 2005). Though there was no archaeological evidence for domestication of pigs in this part, but through genetic evidence it is found that some domestic pigs had similar haplotypes with wild boar currently found in Indochina (Larson et al., 2007b). And hence, it has been clear that once domestication occurred, the Pacific clade pigs were distributed into Pacific, Wallace and ISEA by humans. Two samples from northern Vietnam and from that of Laos and Yunnan Province of China have been found similar sequences with the present-day wild boar (Larson et al., 2010). Excluding the sequences of wild boar found in this region, all the Pacific clade pigs are distributed throughout the ISEA and the Pacific (Larson et al., 2010), however four such clade were also identified in Sumatra and Java Islands, including 15 to 19 samples from eight islands of Wallace’s Line and sequences of New Guinea had 100% similarity. This shows that Australian and that of New Guinea pigs are the descendants of pigs in mainland of Asia.

Verification of the presence of Wallacean type of pig in this region was done through morphometric studies involving the third molar teeth, the results were of two types being introduced by humans to these islands, one endemic *Sus celebensis* and other

Sus scrofa (Groves, 1983; Van den Bergh et al., 2009). The process of domestication in both South-East Asia and ISEA were done with the help of combination of genetic and morphogenetic analysis. It has been found that the dispersal or distribution of pigs had occurred through the early farmers during the Neolithic period and endemic to this region might have occurred due to hunting (Larson et al., 2007b; Cucchi et al., 2009).

2.1.5. North Africa: The populations of *Sus scrofa* in North Africa were reported involving that of the Mediterranean region. Wild pigs from Egypt were reported in late 19th century from the Nile Delta, the Gizeh marshes, Magrah oases and the Wadi-el-Gharand (Manilus and Gautier, 1999). The northern delta of Egypt has been claimed to be the centre of pig breeding (Hecker, 1982). Due to lack of limited fossil and archaeological records, the record for presence of wild boar remains unanswered during pre-history. However, recent samples of DNA suggested that wild boar samples of Morocco and Algeria had European haplotypes and that of Egypt and Sudan had Near Eastern haplotypes (Larson et al., 2005, 2007a).

2.1.6. India: The indication for pig domestication is very strong for regions of Near East, Europe, China and South-East Asia, however there are many centres where evidence for pig domestication or introduction of pigs remains unsolved. Compared to flora and fauna, the evidence of *Sus* remnants of India and Pakistan represents only a minor portion (Chattopadyaya, 2002). Genetic evidences have suggested that in India, independent domestication of Indian wild boar had occurred. Recent mt-DNA sequences reports have revealed that present-day pigs found in India and Bhutan have unique and different haplotypes similar to that of Indian wild boar (Larson et al., 2005, 2010; Tanaka et al., 2008). This depicts that domestic pigs of India are not migrated from Near East or South-East Asian or from Europe. They are the result of local domestication probably done through hunting, which is likely to occur during the Neolithic and Harrapan time. For this region, there are no detailed evidence for domestication of pigs either morphologically or genetically as of now (Meadow and Patel, 2002; Fuller, 2006).

2.2. Threats to domestic pigs due to crossbreeding

The concept of crossbreeding has been closely associated with pigs throughout history. In the 18th and 19th century when the industrial revolution began, to satisfy the rising demand of pig meat, they started importing and crossbreeding the European and Asian pigs. Though crossbreeding of pigs has yielded more lean meat, improved the production of sow, caused less death rates and high growth rates. However, due to extensive interbreeding between wild boars and domestic pigs as well as among the domestic breeds, it formed a multitude of phenotypic variations among domestic pig populations. Due to which many unique and native indigenous pig breeds are near to extinction. According to survey conducted by FAO, (2007), out of 742 native pig breeds, 137 are extinct and 130 are endangered.

Several attempts have been made, over the years to characterize the genetic diversity of domestic pig populations through different genetic techniques, such as AFLP markers, microsatellite and mitochondrial-DNA, in-order to identify their uniqueness and can be preserve. With the help of such techniques, most of the pigs were sequenced with complete genomes and made it easy to investigate the genetic variation of Asian and European pigs and domestic pig population. Such one study has revealed that the pigs of European clade are indigenous and rare breeds that have high variation among the breeds, like Mangalitsa from Hungary, Iberian pigs and Casertana from Italy. The whole genome sequencing revealed that these breeds consist of over 10,000 rare genetic variants that are not found in commercial breeds (M.A.M. Groenen, unpublished results). It can be concluded that conservation of such native pig breeds is important as they are the pool of unique genetic characters that is important in future breeding programs.

Likewise, populations of wild boar are also a pool of reservoir of genetic variation, which too needs to be conserved. Chances of extinction of wild pig populations are due to overhunting. In Western Europe, because of this reason, the local wild pig populations have gone extinct, and have been restocked with wild boars transporting from other places (Briedermann, 1990; Yalden, 2010). White, S. (2011) and Frantz et al. (2015) reported that pig breeds of Europe that were crossbred with wild boars were found to have genetic variation of wild boar in modern indigenous pig breeds. However, when analysed at molecular level, the whole genome sequence of 169 Asian and European pigs and 38 wild boars of Europe revealed 142,249 SNPs (single nucleotide polymorphisms) observed only in European wild boar (M.A.M. Groenen, unpublished results). It concludes that

wild boar of Asian populations possesses genetic variation not found in domestic pig stock. Therefore, conservation of wild pigs is not only necessary for their survival but also for breeding purposes in future for better and more resilient pigs.

2.3. Meat quality characteristics of pork

According to the, 'The Food Standards Australia New Zealand' (FSANZ) Food Standards Code, the definition of meat is elucidated as the entire portion or part of the carcass of ruminant and non-ruminant animals slaughtered with the exception of those in wild condition, but does not include eggs or foetuses. The definition of "meat" is only referred to the muscular tissue of animals (comprising skeletal muscle in addition to any attached connective tissue or adipose matter), however, the definition set forth by the Food Standards Australia New Zealand (FSANZ) also includes the internal organs called the offal or viscera (i.e., tissues other than muscular tissue, encompassing the brain, heart, kidney, liver, pancreas, spleen, thymus, tongue, and tripe) while excluding bone and bone marrow (Williams, 2007).

When speaking about the quality of a meat, it is a set of attributes that defines it, according to the need of consumers. Considering various factors, including the hygiene, nutritional significance and the organoleptic characters of a meat are also part of meat quality (Quali, 1991). The nutritional character of a meat can be defined as the one that guarantees the presence of nutritive constituents, such as proteins, essential amino acids, essential fatty acids, minerals, vitamins, and microelements, as well as their adequate bio-availability (Franco et al., 2010). They provide nourishment necessary for maintaining life and growth. In the present-day scenario, the consumers are aware of the need of nutrition and show great interest on nutritional value in food, particularly in the context of meat, the evaluation of nutritive quality depends on the presence and composition of nitrogenous constituents, such as high-grade protein, free amino acids (FAA), dipeptides, and other nonprotein nitrogenous compounds (NPN). These constituents are responsible for the flavour and taste in meat (Kato et al., 1989). Following are the attributes that have a huge impact on the meat quality of pig meat:

2.3.1. pH: According to meat scientist and technologist, the chemical properties of a meat depend on how it is stored, preserved and processed. Studying the level of pH is an important parameter that determines the quality of a meat (Kasprzyk et al., 2013). The pH

level studied at 45 min and 24 hr post-mortem help in the determination of defects in meat quality traits which are PSE (pale, soft, exudative) and DFD (dark, firm, dry) (Brewer et al., 2001). pH depicting the acidification of a muscle tissue, later plays a crucial role in glycolytic process (Choi and Kim, 2009). Acidity inhibits bacterial growth, preventing spoilage and ensuring quality and safety, therefore extending the shelf life of a meat (Kasprzyk and Bogucka, 2020). On the other hand, the acidity of a meat is greatly influenced by environmental and genetic factors (Sieczkowska et al., 2009) including the transportation and slaughter period. For the meat production industry, pH level at 24 hr post mortem is regarded an important and significant trait (Sieczkowska et al., 2009; Kasprzyk et al., 2013). The declining of pH level at 24 hr defines the normal meat maturation process (Kasprzyk and Bogucka, 2020).

2.3.2. Moisture: Moisture content plays an important role in all food products, including pig meat. Higher moisture content reduces shelf life, as it promotes the growth of microorganisms. Not only that, it also causes deterioration on the colour, flavour and texture of muscle tissues of meat. The younger and leaner pigs are known to have moisture content of 72% (Williams, 2007). The muscle fibres contain major portion of moisture, found in free state and smaller portion is said to exhibit in connective tissues. Small percentage of water is said to remain within the muscle tissue called as 'bound water' during the conditions such as heating, curing and storage period. During these processing conditions, the three-dimensional structure of the muscle fibres is influenced by temperature and pressure. The water that is lost during this process is called 'free water'. Water holding capacity content may disturb the muscle fibres of the meat, however it contributes to the improvement of the durability of meat. There are also many other processes that may alter the final moisture content in meat and its by-products such as grinding, chopping, freezing, salting, thawing, breakdown of connective tissue by chemical or enzymatic method, and other organic additives, altering the acidity (pH) of the meat too (Kamruzzaman et al., 2016).

2.3.3. Carbohydrates: Liver synthesizes the carbohydrate content present in the animal body, which constitutes about half of the total carbohydrate content. Carbohydrate is stored in the form of glycogen in the liver and muscles of animal body. It is also present in blood in the form of glucose. The colour, texture, tenderness and water holding capacity of meat is impacted by glycogen content indirectly. Glycogen is converted to glucose;

from glucose to lactic acid, all these complex processes are governed by the action of hormones and enzymes (Jensen et al., 2011). The lactic acid content present in the muscles increases with the aging, thus lowering the pH, therefore affecting the texture of the muscle, colour, tenderness and water holding capacity of the meat. The normal pH of muscle tissue is 5.6%, but whenever an animal suffers from stress due transportation and slaughter, due to which there is zero chance to regain its normal glycogen levels there is very less amount of glycogen to convert into lactic acid, that cause high pH levels and as a result, the meat quality becomes dark, firm and dry (DFD). DFD type of meat are due to exhaustion and therefore the glycogen content gets depleted before slaughter. This happens as because the muscles tend to absorb more water, and making them to absorb the incident light instead of reflecting it from the meat surface, making it look darker appearance with pH content. Such type of meat is not liked by customers and retailers, affecting their income source (Adzitey and Nurul, 2011). Another condition caused due to low water holding capacity is pale, soft and exudative (PSE) and low pH. For PSE type of meat, there is more reflectance of incident light making the colour look like pale yellow (Karunanayaka et al., 2016). Therefore, both the conditions of DFD and PSE are related to carbohydrate content of meat, that has a considerable amount of effect on the nutritional content of meat and its products.

2.3.4. Proteins and amino acids: Meat is one of the protein rich foods, thereby offering a significant biological worth to many. Proteins are organic substances that occur naturally and possess high molecular weight. These components are linked together to form different types of protein with different properties. The protein content variations are seen in tissues within a single living organism as well as in comparable tissues of different species. From that of carbohydrates and fats, the proteins are more complex in size and constituents. The meat protein content is different in different meat types (Marangoni et al., 2015). According to protein digestibility-corrected amino acid scores (PDCAAS), the protein content found in meat is high (0.92) than other protein sources like beans, lentils, peas and chickpea scoring around 0.57-0.71 (Barron-Hoyos et al., 2013). Protein content depends on the availability of amino acids present it.

Amino acids function as the fundamental constituents of proteins. The nutritional composition of meat can exhibit variability depending on the presence or absence of essential and non-essential amino acids. Only 20 amino acid make proteins, out of 192,

and of these 20 amino acids, 8 are essential amino acids required by the human body that must be obtained from diet and other 12 are non-essential amino acids that is synthesized by the human body, but only if the dietary sources that are rich in non-essential amino acids are ingested or else it could lead to protein malnutrition. Many studies have reported that the difference in the content of amino acids depends on the breed, age and location of the muscle (Ahmad et al., 2018). It has also been reported that the contents of essential amino acids like valine, isoleucine, phenylalanine, arginine and methionine in the animal muscle increase with age (Sakomura et al., 2015). The composition of the amino acids is also affected by the processing techniques such as heat and ionization radiations when applied for long time, including smoking and salting of meat and its products (Soladoye et al., 2015; Yu et al., 2017). It has also been reported that 50% of lysine was available at 160 °C, while 90% of it was present at 70°C.

2.3.5. Fats and Fatty acids: After carbohydrates and proteins, fat is one the major macro-nutrients. Triglycerides, which consist of three fatty acid chains and the alcohol glycerol, are commonly referred to as fat contents. Meat comprises of fatty tissues (adipocytes) that exhibit diverse proportions of fat. In meat, the fat content serves as a reservoir of energy, offering protection by serving as a padding layer around the skin and organs, particularly the heart and kidney. Additionally, it acts as an insulator against heat loss from the body (Wood et al., 2008). The composition of fatty acids and fats in adipose tissue varies considerably in different locations of the animal carcass (Ahmad et al., 2018). The internal fat of the animal that surrounds the organs is known to be harder than, that of external fat as it constituted of high content of unsaturated fatty acids (Ahmad et al., 2018). Cooking can exert an impact on the composition of fatty acids and content of fat in meat. Evidently, it has been demonstrated that loss in fat levels across various cuts of meat when subjected to broiling, grilling, and pan-frying without the addition of extra fat (Grunert et al., 2004).

The fatty acid is composed of unsaturated fatty acid that are essential such as oleic (C-18:1), linoleic (C-18:2), linolenic (C-18:3) and arachidonic (C-20:4) acid. They are the important constituents of cell wall, mitochondria, and other metabolic sites. Linoleic acid, (C-18:2) is found in large quantities in vegetable oils such as soya and corn oils, however its concentration in meat is 20 times lower. Eicosapentaenoic acid (C-20:5) and docosahexaenoic acid (C-22:6) are typically found in meat tissues at a low concentration compared to fish and fish oils (Woods and Fearon, 2009). It is said that the linoleic acid

concentration is found more in pig meat than in ox or sheep. These concentrations of fatty acids differ among the different species, also same with that of offal products. The liver is known to content high polyunsaturated fatty acids (PUFA), including the brain. The cholesterol content in brain is more than the muscle tissues (Wood et al., 2004). The omega 3 PUFA acids play an important role in the protection of cardiovascular diseases. Though seafood is the main source of omega 3 fatty acids, meat can also contribute around 20 % of omega 3 fatty acids. Omega 3 concentration in meat depends on the type of diet giving to domesticated animals. It has been also found that PUFA of animals are extremely important for the development of brain, mainly for the foetus. When linoleic and linolenic acids are consumed, they undergo digestion in the liver of animals and generate polyunsaturated fatty acids. Additionally, the elongation of the linoleic acid chain leads to the production of prostaglandins, which play a crucial role in the regulation of blood pressure. Prostaglandins are primarily found in various organs and tissues, that are synthesized within the cell through the utilization of essential fatty acids. Prostaglandins are generated by every nucleated cell referred to as autocrine and paracrine molecules, and exert their effects on the endothelium, uterine, and platelet cells (Aranceta and Pérez-Rodrigo, 2012). The composition of fatty acids in meat has the potential to be altered by modifying the diet of animals. Particularly for non-ruminant animals (single-stomach) as the levels of alpha-linolenic acid, linoleic acid, and long-chain polyunsaturated fatty acids demonstrate a rapid response to increased dietary intake. The digestive nature of animals has the potential to influence the fatty acid composition of meat. The presence of microbial enzymes stimulates the process of hydrolysis of unsaturated fatty acids, which ultimately results in a high concentration of stearic acid. This elevated concentration is observed in the small intestine, where it is subsequently absorbed (Lehnen et al., 2015).

2.3.6. Minerals: Minerals, which are non-carbon-containing nutrients found in food substances, are essential for the optimal growth, development and to maintain the human body. Minerals are of two types as required by the human body, they are macro- and micro-elements. Macro are the ones required by the body in large amounts that includes sodium, calcium, phosphorus, magnesium, chloride, potassium and sulphur, while micro-elements are the ones required in less quantity, such as iron, zinc, iodine, copper, cobalt, manganese, selenium and fluoride (Soetan et al., 2010). Potassium is found to be the most

dominant of all in terms of its concentration in meat as compared to other minerals, namely phosphorus, sodium, and magnesium. Meat also contains good amount of iron, zinc, and selenium. Each of these minerals fulfils diverse roles in the growth, development, and preservation of the human body, which shall now be explicated.

2.3.6.1 Potassium (K): The role of potassium in the human body composed of various physiological processes including metabolism, transmission of nerve impulses, promotion of growth, facilitation of muscle development, and maintenance of the acid-base balance. Potassium is the most abundant cation in the human body, representing around 98% of the total K content in the body found in tissue cells. Skeletal muscle, being the most prevalent form of tissue, serves as the principal source of potassium within the animal body. Potassium has two primary roles in cellular physiology: maintaining the resting potential of the cell membrane and regulating water and sodium metabolism within the cell. Moreover, it plays a crucial role in controlling nerve impulses in muscles and facilitating the activation of various enzymes, particularly those involved in energy production (Mohammadifard et al., 2018). Potassium serves as the primary intracellular constituent of muscular tissues, and its concentration increases with body growth (Mienkowska-Sterpniewska et al., 2007).

The recommended intake for potassium as suggested is 3500 mg/kg for adult men and women as per European Food Safety Authority (EFSA, 2016) and as per Dietary Guidelines for American, it is 3400 mg/d for male and 2300 mg/d for female.

2.3.6.2 Sodium (Na): Sodium is the primary cation found in the extracellular fluids. It performs the excitability of muscles and cell membrane permeability, activated the function of nerves and muscles, and plays a vital role in Na/K-ATPase, the preservation of membrane potential, and the transmission of nerve impulses (Babic and Kasprzyk, 2019). Additionally, sodium helps in the transportation of CO₂ while simultaneously preserving the osmotic pressure of bodily fluids. The regulation of sodium levels within the body is performed by the endocrine system. The necessity for sodium depends on factors such as physical action, environmental temperature, age and processes of growths (Jarosz, 2017). Growth retardation is observed in young animals if deficiency in sodium occurs (Soetan et al., 2010). The feed given to domesticated animals including pig, do not contain enough sodium to meet animal's dietary need, so there is a requirement of

common salt (sodium chloride) in their diet. When there is high amount of Na, it leads to the release of calcium causing adverse effect to bone mass (Teucher et al., 2008; Lanham-New et al., 2012). Sodium functions as a potassium antagonist and, when combined, generates a concentration gradient on the two sides of the cellular membrane. The variation in these concentrations enables the membrane potential, to perform certain functions, such as the transmission of nerve signals and the contraction and relaxation of muscle cells. The balance between sodium and potassium is of utmost important for maintenance of water holding capacity and pH in tissues (Guang-Zhi et al., 2008).

According to Dietary Guidelines for Americans (2020–2025), the daily recommended intake for sodium is 2300 mg/day for healthy adults and 1500 mg/day for people with hypertension and other health illness.

2.3.6.3. Magnesium (Mg): Magnesium is the second most abundant cation after potassium, and 4th most abundant element found in the human body. Magnesium ions present in the body are found in intracellular space, mainly stored in bone, which consist of more than 99 %. Mg maintains the blood pressure, prevents tooth decay, helps to keep bones healthy and repairs and improves the growth of human body. It is a basic component required for protein synthesis (Cygan-Szczegieliński et al., 2012). Magnesium is known to be the co-factor of more than 300 enzyme that perform various biochemical activities in the body, including nerve and muscle function. Furthermore, it plays an important role in transport of calcium and potassium ions across the cell. Magnesium along with potassium help to maintain the normal muscle tone. The presence of Mg ions can be altered or reduced due to high K ions, resulting in hypomagnesaemia (Cygan-Szczegieliński et al., 2012). Also, there exists antagonism between Mg and Ca ions, when there is low Mg ion content, it stimulates the secretion of parathyroid hormone and therefore Ca is released from bones and ultimately increasing its concentration in blood.

The Reference Dietary Intake (RDI) for males and females is considered to be 420 mg/day and 320 mg/day respectively (Barreca et al., 2023).

2.3.6.4. Calcium (Ca): Around 98 % of the Ca is stored in bones and the animal body uses the bone as the storage for calcium to maintain homeostasis of calcium (Institute of Medicine, 2011). Calcium helps in formation of bones and teeth, also helping in normal body functions by keeping the tissue strong, flexible and rigid. Other than that calcium

content found in circulatory system performs muscle function, blood clotting, hormonal secretion, nerve transmission, and contraction and dilation of blood vessel (Heaney, 2010). The bone undergoes remodelling, remodelling is needed to transform the bone size at the time of growth, to repair damage, maintain the serum calcium levels, offer a source for other minerals (Weaver, 2020). High content of calcium has positive effects on the bones and weight gain. Furthermore, calcium has an important role in enzyme activation. The requirement of calcium depends on the sex, age and is known to increase during growth and maturation of sex organs (Jarosz, 2017).

According to the National Institutes of Health (USA, 2019), the acceptable intake of calcium for adults is 1000–1300 mg/day depending on gender and age (Loska and Wiechuya, 2003), whereas when considered for male and female, it is established at 2000 mg/d by the same institute.

2.3.6.5. Iron (Fe): Iron is known as the most abundant element on Earth, serving as a crucial element in the composition of all organic organisms (Beard and Dawson, 1997; Quintero-Gutiérrez et al., 2008). Iron in human exists in many forms, like when bound to protein, it is called hemoprotein or to heme compounds (hemoglobin or myoglobin), or heme enzymes, or nonheme compounds (flavin-iron enzymes, transferrin, and ferritin) (McDowell, 2003), iron is involved in the synthesis of all of these compounds. Iron itself is recycled in the body and thus conserved, thus there is no loss. The iron absorption is reduced significantly, when gastric acid production in the stomach is impaired. Furthermore, the heme iron present in meat is known to get absorbed several-folds higher than that of non-heme iron found from other sources (Soetan et al., 2010; Nishito and Kambe, 2018). Studies have revealed that the absorption of iron in meat related products or meat itself is 20-30% more than that of vegetable products which is only 5 % (Krzecio-Nieczyporuk et al., 2013; Nikolic et al., 2015).

The daily recommended intake of iron is 16–18 mg/d for males and 12–16 mg/d for females (EFSA, 2017).

2.3.6.6. Zinc (Zn): Zinc is one of the important trace elements required by human body to perform various functions such as for growth, development and the immune system. The only metallic element that acts as a cofactor to more than 300 enzymes. Moreover, an estimated 3000 proteins rely on zinc for their proper functioning, encompassing not

only transcription factors but also signalling enzymes that play a vital role in cellular signal transduction processes (Gammoh and Rink, 2019). Zinc is responsible for regulating the pH of bodily fluids and plays a crucial role in the formation of collagen, which is essential for maintaining the health of the skin, hair, and nails. Moreover, it aids in improving cognitive function and memory. Furthermore, zinc has the ability to counteract oxidative stress, apoptosis, and the aging process, along with other immune responses (Prasad, 2014). There is a relation between zinc and calcium, when there is high intake of calcium, it requires the need of zinc to balance (Soetan et al., 2010). The red meat that includes the pork and more active muscles are known to have high concentration of zinc (Gerber et al., 2009).

The acceptable daily intake of zinc is 11 mg for men and 8 mg for women (Barreca et al., 2023).

2.3.6.7. Manganese (Mn): Manganese is an essential trace element, helps in the formation of structure of some enzymes (Tuzen, 2009). The role that manganese plays in the activity of enzymes are engaged in the process of urea formation, pyruvate metabolism, and the galactotransferase responsible for the biosynthesis of connective tissue, synthesis of acid mucopolysaccharides, such as chondroitin sulphate, to form the matrices of bones and egg shells (Chandra, 1990). When there is insufficient intake of manganese, it leads to deformities in skeletal system and shell quality (Gordon, 1977). Increased manganese concentration has been observed in sows (female pig that had one litter of piglets) during pregnancy (Kirchgessner et al., 1981). Manganese deficiency is seen various species including domesticated animals, with deformities like shortened legs, lameness, and enlarged hock joints in pigs (Soetan et al., 2010). There are many literature reviews, where the incorporation corn is used as feed in pig's diet, however corn is known to have low amount of manganese, therefore the pig may receive insufficient amounts if taken.

The daily recommended intake of manganese in adults is 3 mg/d by EFSA, (2017), however those of the US National Academy of Sciences (1980) recommends 2.5–5 mg/day and, the WHO (World Health Organization, 1994) recommends 2–9 mg/day for an adult. The Institute of Medicine recommends that intake of manganese from food,

water and dietary supplements should not exceed the tolerable daily upper limit of 11 mg per day (National Research Council Recommended Dietary Allowances, 1989).

2.3.6.8. Selenium (Se): It is an essential micronutrient in animal and humans. Selenium functions as a vital element of glutathione peroxidase. It represents an element that is a fundamental part of the defence system, protecting the living organism against the damaging effects caused by free radicals, including the heavy metal toxicity (Al-Saleh and Al-Doush, 1997). Organic and inorganic selenium compounds play a crucial role in the prevention of specific disease conditions that were previously linked to a deficiency in vitamin E. It also interrupts the normal reproductive process affecting the ovulation and fertilization process resulting in mortality of the young ones. Additionally, production of egg and conversion of feed is also affected (Merck, 1986). In pigs, the deficiency of selenium causes liver necrosis and hepatitis dietetica, that causes death in pigs. The mulberry heart disease in swine is also due selenium deficiency (Hays and Swenson, 1985).

According to Food and Nutrition Board, (2000), the daily dietary selenium intake ranges from 50 to 200 µg, with an average value of 55 µg for adult humans.

2.3.6.9. Copper (Cu): It is a micronutrient that is essential for the proper functioning of both the hematologic and neurologic systems (Tan et al., 2006). Copper is necessary for the development and growth of bone, establishment of myelin sheaths within the nervous systems, aids in the integration of iron into haemoglobin and facilitates the absorption of iron from the gastrointestinal tract (GIT) (Malhotra, 1998; Murray et al., 2000). Monogastric animals like pig, copper is absorbed in the upper part of the intestine, where pH is known to be acidic. Most of the copper appears in faeces due its poor absorption (Hays and Swenson, 1985). The high concentration of copper causes infections and chronic conditions such as cirrhosis, and rheumatoid arthritis, it is found to occur in malnutrition too (Malhotra, 1998). Even though copper is required for good health but its high intake can cause health problems such as liver and kidney damage (Ikem and Egiebor, 2005).

Several organisations suggest the intake of 1.2 mg copper daily (Commission of the European Communities, 1993), while that of WHO has referred an adequate intake

(AI) of 0.900 mg/d. Additionally, the recommended daily intake of copper by European Food Safety Authority (EFSA, 2017) is 1.6 mg/d.

2.3.6.10. Cobalt (Co): Cobalt is the component of vitamin B₁₂ and its metabolism is same as that of vitamin B₁₂. It is cofactor of many enzymes that are involved in amino acid metabolism and DNA synthesis (Arinola et al., 2008). Cobalt gets readily absorbed in the bloodstream and its excretion occurs primarily through urine. Deficiency of cobalt in ruminants can be alleviated by the use of cobalt oxide pellets, (Hays and Swenson, 1985). While in humans, its deficiency causes goitre, hypothyroidism and heart failure (Murray et al., 2000). Ruminants can synthesize cobalt from vitamin B₁₂ by microbial action in the digestive tract. However, the efficiency of this process is low and depends on cobalt intake. Non-ruminants, on the other hand, requires the intake of vitamin B₁₂ as they lack to synthesize the vitamin by microbial action in the digestive tract (EFSA, 2012). However, with the help of hindgut bacteria, the pigs and poultry are known to synthesize minor amount of vitamin B₁₂ (EFSA, 2012).

The sources for cobalt are same as that of vitamin B₁₂ such as animal foods or fermented foods where bacteria can produce the vitamin. Offal also called organ meats such as liver, kidney, heart, and pancreas are known as the best sources of vitamin B₁₂. The tolerable upper intake levels (UI) of cobalt are 3 µg/kg/day (Soetan et al., 2010).

2.3.6.11. Chromium (Cr): Chromium although is an essential element it is required in trace amount (Frieden, 1984). It maintains the figure of RNA molecule, as it known to be effective as a cross-linking agent for collagen (Eastmond et al., 2008). One of the dietary factors known as the glucose tolerant factor is required to maintain normal glucose tolerance in rat, where chromium is found to be the active ingredient of it (Brown, 2003). In humans, chromium poisoning is due to accidental intake of chromic acid or chromates, and in children protein-calorie malnutrition may lead to chromium deficiency (Mertz, 1974).

The recommended intake of chromium may vary with gender and age, i.e., for most adult females is 25 µg/d and 30 µg/d (Noel et al., 2012).

2.3.6.12. Nickel (Ni): Nickel is known to essential elements in animals including human beings, but when taken in excessive amount can cause deformities (Nielsen, 1976). Many

suggest that nickel help in the maintenance of structure of membrane, nucleic acid metabolism, control of prolactin, and act as a co-factor in enzymes (Soetan et al., 2010). When experimented with animals, e.g., chicks when not given nickel, tend to have short and thick legs, lower plasma cholesterol level and haematocrit and higher liver cholesterol level (Nielsen, 1974). In swine, there were abnormal hair coat, reproduction is impaired, and poor growth of the offspring (Anke et al., 1974; Tejada-Jimenez et al., 2009).

According to the World Health Organization, (1994), the daily recommended intake of nickel is 100–300 µg/d. And the tolerable daily intake amount is 2.8 µg/kg and 5.0 µg/kg body weight for adults by Agency (2015) and World Health Organization (1997).

2.3.6.13. Arsenic (As): The growth of tissue cultures is enhanced by the presence of arsenic, and there have been documented positive impacts on the health and productivity of pigs and poultry from the use of different organic arsenicals (Nandi et al., 2006). The mechanism of its action is similar to that of antibiotics and are associated with the control of microorganisms in the intestines (Hays and Swenson, 1985). There are reports of cancer to humans when exposed to arsenic (Kapaj et al., 2006).

The recommended intake for arsenic is not daily but on a weekly basis, according to Joint FAO/WHO (1998) Expert Committee on Food Additives, it is 0.015 mg/kg body mass/week. And the tolerable weekly intake by EFSA's Panel on Contaminants in the Food Chain (CONTAM, 2009) is 15 µg/kg/bw.

2.3.6.14. Cadmium (Cd): The long term and low-level exposure of Cd is regarded as harmful as it gets distributed in the tissues and tends to inhibit antioxidant enzymes (Asagba and Eriyamremu, 2007; Chater et al., 2009). The most damaged organs are the liver and kidney due to acute and chronic exposure to Cd (Asagba and Obi, 2000). Generally, Cd is known to be carcinogenic and nephrotoxic element and should be monitored in all food types (Shannon et al., 2007).

According to WHO, the provisional tolerable weekly intake (PTWI) of Cd is has also 0.007 mg/kg/b.w. (Shannon et al., 2007), while that of FAO (1983) and MAFF (1995) is 0.5 mg/kg and 0.2 mg/kg, respectively.

2.3.6.15. Mercury (Hg): Mercury, known for its toxicity exists in several forms. Its toxicity to humans depends on the form mercury, the dose and rate of exposure (Bernhoft, 2012). When mercury vapour is inhaled, it mainly affects the brain. The mercury salts primarily damage the kidney and gut lining while that of methyl mercury are distributed throughout the body (Berlin et al., 2007).

The weekly tolerable intake of mercury according to the Joint FAO-WHO Expert Committee on Food Additives (JECFA, 1986) is 4 µg/kg/b.w. (WHO, 2022).

2.3.6.16. Lead (Pb): Lead is a universal environmental and industrial contaminant that has been identified in all aspects of environmental and biological systems. Chronic lead poisoning is frequently observed in young children as a result of the licking of paint or toys, and also seen in individuals employed in the printing, paint, and petroleum sectors (Soetan, 2010). Lead is known to cause reduced cognitive development and intellectual performance in children, as well as elevated blood pressure and cardiovascular disease in adults (Commission of the European Communities, 2001).

According to EFSA, (2006) the maximum level of lead intake permitted in meat is 0.10 mg/kg, and offal is 0.50 mg/kg. And that of WHO (1996), and MAFF (1995) is 2.0 mg/kg, and 2.0 mg/kg.

2.4. Molecular marker for identification, genetic variation and phylogenetic studies

Taxonomic way of naming an organism at species level with the help of a molecular marker using DNA for identification is called DNA barcoding. Among the different types of molecular markers used for identification of species, DNA barcoding on the basis of mitochondrial DNA (mt-DNA) is regarded as one of the most applicable tools for identification at species level (Hubert et al., 2008). Moreover, molecular identification based on mt-DNA was found to have numerous benefits than conventional morphological system, a small tissue, skin, hair, teeth or blood is enough for DNA isolation. Furthermore, DNA exhibits greater stability than morphological traits and shows increased resistance to degradation (Endo et al., 2022). Mitochondrial DNA possess distinct characteristics that makes it a perfect molecular marker for assessing molecular evolution, classification, population genetic analysis, and identification (Nguyen et al., 2017). Mitochondrial DNA is a compact circular genetic material with a size of less than 20 kilobases (Bruford et al.,

2003), haploid, inherited from the mother, and lacking recombination (Giles et al., 1980), single base pair substitutions mutations is seen (Wolstenholme, 1992) and the rate of evolution is rapid and constant in comparison to nuclear DNA (Bruford et al., 2003; Brown, 1979). One region of mt-DNA used as a molecular marker for species identification is the *cytochrome b*. Because of its high discrimination power for identification of species and characterization at taxonomic level, it is regarded as the important marker (Kuwayama and Ozawa, 2000; Saif et al., 2012), to compare species of same genus or family, and are also used in the study of molecular evolution (Prusak et al., 2004).

Phylogeny is the study of the history or lineage of a species from their common ancestor that includes the branching order or the times of divergence. The term can also be utilized to describe the genetic lineage of genes that originate from a shared ancestral gene. In the context of molecular phylogeny, it generates the relationship between organisms or genes by comparing the DNA or protein sequences. The presence of dissimilarities between these sequences signifies a genetic divergence that arises as a consequence of molecular evolution over an extended period of time. Briefly, the classical phylogeny defines the morphological characters of an organism, while that of molecular phylogeny characterizes based on the nucleotide sequences of DNA and RNA or sequences of amino acids of a protein. Most of the previous studies have utilized mitochondrial DNA to find the insights of evolutionary history of pigs. Recent studies using mitochondrial DNA, provided important insights about the evolutionary history and migration patterns of *Sus scrofa* throughout Asia (Choi et al., 2020). Based on mt-DNA, Niedzialkowska et al. (2021) reported phylogeny study and phylogeography of wild boar in six countries of Central and Eastern Europe. Another study on *cytochrome b* by Long et al. (2014) compared sequences of Vietnamese pigs from the Vietnam Central Highlands with Asian and European wild boars and assessed genetic relationship. Wu et al. (2007) revealed multiple domestication events in East Asia via phylogenomic analysis of mitochondrial DNA in wild boars and domestic pigs. Again, Watanobe et al. (2001) in their report reveals the origin of *Sus scrofa* from Rebun Island, Japan through mitochondrial DNA. Ramayo et al. (2011) revealed diversity in wild boar from the Primorsky Krai region (East Russia) through mitochondrial DNA. Another work by Khederzadeh et al. (2019) emphasized on the uniqueness of East-Caucasian and Central

Italian populations of wild boar through Maternal genomic variability. While in India, very few and limited data are available regarding use of '*cytochrome b*' as a molecular marker to establish genetic diversity, identification and establishing phylogenetic relationship among the wild and indigenous pig breeds of India. Gupta et al. (2012) in his studies used '*cytochrome b*' as a marker to establish genetic difference of Indian wild pig (*Sus scrofa cristatus*) and domestic pig (*Sus scrofa domestica*) and its use in wildlife forensics. Another study using D-loop of mt-DNA established genetic difference and phylogenetic lineages among the five registered indigenous pig breeds of India (Laxmivandana et al., 2022). Sharma et al. (2023) in their findings highlighted possible sites of center of pig domestication in India and their dispersal to other continents (Asia, Africa, Europe and Oceania) based on mitochondrial D-loop fragment. In Assam only two studies were conducted regarding mitochondrial studies of pigs, where, Saikia et al. (2015) studied the molecular characterization of *cytochrome b* gene in indigenous pig. Another study determined the complete Mitochondrial Genome characterization using D-Loop gene of Indian Wild Pig (Das et al., 2022).

2.5. Study of meat quality on different muscles

There are many factors that determine the nutrition and composition of meat, one such important factor is the location of the muscle tissues or the type of cut (Rhee and others, 1982; Toldra, 2006; Toldra & Reig, 2004; Gil et al., 2003; Klosowska and Fiedler, 2003; Choi et al., 2007), and other factors include breed type, age, sex, rearing system, feeding type and its composition etc. (Toldra, 2006). The conversion of muscle to meat, post-mortem is a complex process where a multitude of biochemical reactions occur, which are influenced by the specific characteristics of the muscle fibre and its energy metabolism (Toldra, 2006). Muscle, as a vital tissue, plays a significant role in everyday activity and physiological metabolism. Furthermore, it serves as the primary reservoir of animal protein. About 40-50% of the overall dry weight of the body consists of skeletal muscle of mammals, and is a crucial part of the body. The development and growth of the skeletal muscle are closely related to the growth rate of animals in the livestock production. Additionally, lean meat is closely related to the development and growth of the skeletal muscle, which is an important economic trait and improvements in meat quality of pig (Lee et al., 2010; Zhao et al., 2011; Listrat et al. 2016). Therefore, it makes it obvious to study the biochemical determinants of the meat taken from different

cut/regions of the animal carcass, so that its nutritive value and the possibility of presence of bioactive compounds can be determined.

There are reports of good meat quality obtained from raw meat of native pig breeds called as autochthonous breeds or indigenous breeds (Maiorano et al., 2007; Szulc et al., 2012; Kasprzyk et al., 2013). Indigenous pig breeds are pig populations that are exclusive to a particular geographical region or native to a particular country. These breeds have well adapted to their environment, climate, feeding habits, and traditional farming practices of the respective regions. Additionally, they exhibit distinct genetic traits and physical characteristics that differentiate them from commercial or exotic pig breeds. Among the most prominent features of indigenous pig breeds, regardless of their origin, is their resilience, hardiness, and ability to thrive within the local climate and feed resources while being disease-resistant. These breeds possess unique genetic traits that can contribute to breeding programs and also add potential contribution to sustainable agriculture and the development of rural communities through increased food security, economic opportunities, and cultural identity.

According to Domestic Animal Diversity Information System (DAD-IS) founded by FAO, most of the Asian countries have high number of indigenous pig breeds, of which China has more than 50 indigenous pig breeds and most of them are non-descript. Some of the most extensively studied indigenous pig breeds around the world for their unique characters are Mangalica, also known as Mangalitsa pig originated from Hungary, Iberian pig originated from Iberian Peninsula, Meishan Pig (China), Suino Nero Lucano pigs (Italy), Liangshang pig (China), Celta pig (Spain), Tibetan pigs (China) and Prestice Black-Pied pig (Czech Republic).

2.5.1. Mangalica or Mangalitsa pig: Mangalica or Mangalitsa is one of the most popular European indigenous pig breeds (Parunovic et al., 2012). The fresh meat of the pig is of dark colour, also known to be softer and succulent than the meat of other pig breeds (Parunovic et al., 2013). The fat deposition around the muscle tissue is the reason for the cause of its distinct palatable flavor (Csapo et al., 2002). It is known to have strong and healthy body and slow growth rate with high amount of fat deposition and reduced lean meat than commercial pigs (Vranic et al., 2015). Mangalica pigs is considered one the fattest pigs in the world (Vranic et al., 2015). The carcass of Mangalitsa is composed of

65 -70% fats and 30 – 35% meat tissue (Egerszegi et al., 2003). Because of its high meat quality and tasty products, it has managed to attract increasing number of customers and long-term market opportunities can be assured (Parunovic et al., 2012; Parunovic et al., 2013).

Previous studies depicted the chemical composition, fatty acid profile and cholesterol in *longissimus dorsi* muscle of Mangalitsa pigs (Parunovic et al., 2012; Vranic et al., 2015; Parunovic et al., 2013).

2.5.2. Iberian Pig: Iberian pig is an important indigenous porcine breed of the Iberian Peninsula, both economically and in population size (Nieto et al., 2019; Diaz-Caro et al., 2019; Rauw et al., 2020). It is a medium size pig with skin colour from black to blond to reddish. The hair is scarce or absent. The legs are thin and the hooves are dark and uniformly colored (Nieto et al., 2019). Iberian pigs are known to have slow growth rate with high potential of accumulation of fat and low prolificacy (Vieira et al., 2021; Rauw et al., 2020). The dry-cured products obtained from prime cuts such as shoulder, ham and loin or cured sausages have high market prices and are valued high in international markets. The juiciness and fat content are highly accepted by the consumers (Rincker et al., 2008). The meat quality and products from Iberian pig that are highly accepted by the consumers are due to its high intramuscular fat and oleic acid contents (Vieira et al., 2021).

Earlier studies related to Iberian pigs determined the carcass, meat quality, essential fatty acids, physicochemical characteristics and sensory attributes in *longissimus dorsi*, *masseter*, and *serratus ventralis* muscles (Rauw et al., 2020; Vieira et al., 2021; Almeida et al., 2018; Serrano et al., 2008; Cava et al., 2003).

2.5.3. Meishan Pig: Among the Asian pigs, Meishan, an indigenous pig breed of China is popular around the world for its high fecundity (Zhao et al., 2018). Meishan belongs to the category of Chinese Taihu pig breeds, which are the most prolific indigenous pig breeds in the world. Other Taihu pigs include Erhualian, Fengjing, Jiaying Black, Shawutou, and Mizhu (China National Commission of Animal Genetics Resources, 2011). They are predominantly found in a mild sub-tropical climate situated in a narrow region of Taihu Lake in the lower region of Yangtze-Kiang River Valley of China (Wang et al., 2017b). Meishan pigs possess distinct traits such as, large drooping ears, early

maturity, and wrinkled black skin that differs them from other pig breeds (Zhao et al., 2018). Due to high reproductive rates of Meishan pigs, they are exploited for producing two-way or three-way commercial pigs (Jiang et al., 2012).

Zhao et al. (2018) and Sun et al. (2018) both studied the unique genetic and phenotypic characteristics of Meishan pigs.

2.5.4. Suino Nero Lucano pigs: Suino Nero Lucano (SNL) is an ancient native indigenous pig breed of Basilicata region of Southern Italy. This pig breed is the result of biological modifications that occurred over hundreds of years; therefore, the preservation of its genetic resources is a priority for contributing to the welfare of the region and preserving the local favours (Perna et al., 2015). It is a medium sized pig with bright black coarse hair bristles; long head with straight nose, legs are thin and long. Their prolificacy is low as compared to that of Meishan pigs of China (Valluzzi et al., 2021). Additionally, they are known to have slow growth rate and its fat content is high (Perna et al., 2015). They are known to have high rusticity and due to which they are reared outdoors provided with basic shelters (Valluzzi et al., 2021).

Perna et al. (2015) studied the fatty acids composition, cholesterol and vitamin E contents of *longissimus dorsi* and *semitendinosus* muscles of Suino Nero Lucano pigs.

2.5.5. Liangshang pig: The Liangshang pig is a small-sized indigenous pig breed of China. It is a mountain pig mainly found in Yi Autonomous Prefecture of Liangshang (Luo et al., 2015; Gan et al., 2020). Liangshang pig has a remarkable endurance against cold temperature and can feed on coarse diet (Luo et al., 2015; Gan et al., 2020). Like any other indigenous pig breeds, Liangshang pigs have slow growth rate, have the ability to thrive in harsh climatic conditions and has good meat quality (Gan et al., 2020). Primarily owing to its poor growth performance, its population is decreasing rapidly (Luo et al., 2015), therefore, Liangshang pig has been included in the National Preservation Program for Autochthonous Breeds of China. Gan et al. (2020) determined the meat quality, amino acid, and fatty acid composition of Liangshan pigs at different weights.

2.5.6. Celta pig: Celta is regarded as an important autochthonous pig of North Spain. Because it is well adapted to any environmental condition, its rearing is completely of extensive regime making the best use of natural resources. They have slow growth rate

(Dominguez et al., 2014). The raw-cured meat products produced by the Celta pig are of superior quality and are highly preferred by consumers (Franco and Lorenzo, 2013). At present, the Celta pig is a part of a project that aims to conserve the breed forwarded by the Autonomous Regional Government of Galicia (Carril et al., 2001).

Recent study by Dominguez et al. (2014) determined the fatty acid profile, cholesterol and retinol contents in *longissimus dorsi* and *psoas major*, and *biceps femoris* muscles of Celta pig breed.

2.5.7. Tibetan pigs: The Tibetan pig found in the high altitudes of the Qinghai-Tibetan Plateau about 3000 and 4300 meters above sea level are endemic to China, they hold a great importance for the sustenance of around 11 million inhabitants of the region. The ability of Tibetan pigs to acclimatize to the high-altitude environment is a distinctive trait that is absent in other pig breeds (Li et al., 2013). The thorough exploration of Tibetan pigs further promotes a comprehensive understanding of the hypoxic adaptation of species in the high altitudes Qinghai-Tibetan plateau (Kong et al., 2019). They have long and straight snout with skin covered with long and straight bristles. These bristles protect them from cold temperatures. The body size is small and an average weight of adults is about 35 kg. This breed has low prolificacy with an average of 5 litter per birth and has high fat content.

Gan et al. (2019) analysed the carcass and meat quality characteristics, including chemical composition in the *longissimus dorsi* muscle of Tibetan pigs. There are also reports of analyzing essential and non-essential/toxic elements in the edible viscera (heart, lung, stomach, liver, large intestine, small intestine and kidney) of Tibetan pigs (Mi et al., 2020).

2.5.8. Prestice Black-Pied pig: The Prestice Black-Pied is an indigenous pig breed of Czech Republic. Like any other indigenous pig breed, this breed too has good maternal attributes, has a robust physical structure, can adjust to climatic conditions and immune to diseases (Dostalova et al., 2020). The breed has low lean meat and high back-fat thickness mainly the intramuscular fat content which makes the meat tender and juiciness (Nevrkla et al., 2017). The breed is closely observed by the National Program on the Conservation and Utilization of Genetic Resources since 1992. Dostalova et al. (2020)

studied the meat quality characteristics in *longissimus dorsi* muscle of Prestice Black-Pied pig.

2.6. Study of meat quality on different muscles in Indian pigs

There are only few studies from India that determine the meat quality characteristics on muscles of pigs. Recent study analysed the carcass composition and meat quality parameters in *longissimus thoracis et lumborum* muscle of Ghungroo pigs (Thomas et al., 2016a). Another study by Thomas et al. (2018) determined the meat quality characters and nutritional attributes *longissimus thoracis et lumborum* muscle of Niang-Megha pigs. Parkunan et al. (2017) demonstrated the changes in expression of monocarboxylate transporters, heat shock proteins and meat quality of Large White Yorkshire and Ghungroo pigs during hot summer period in the *longissimus dorsi* (LD) muscle. Again, recent study determined the carcass and meat quality of triple cross (Ghungroo x Hampshire x Duroc) fattener pigs in *longissimus thoracis et lumborum* muscle (Thomas et al., 2017). *Longissimus thoracis et lumborum* or *longissimus dorsi* is the most common muscle where meat quality characters are studied not only in pigs (non-ruminant) but also studied ruminant domesticated animals as well. As mentioned earlier, it provides the whole carcass estimate (Konarska et al., 2017). However, in market the pork is sold in primal cuts that include ham, loin, bacon, spare ribs, picnic shoulder, boston butt and jowl (Thomas et al., 2016a; Kim et al., 2008) which is consist of different types of muscles. Each of these pork cuts differs from each other as they are formed of different muscle fibres, connective tissue and also its intramuscular fat content varies (Listrat, 2016). Therefore, it is important to study the different muscles of each of these standard pork cuts, so that its nutritive value and the possibility of presence of bioactive compounds can be determined. Recent study by Daimari et al. (2022a) determined the meat quality parameters such as proximate, mineral, fatty acid and cholesterol content in five muscles of Ghungroo pig breed.

2.7. Study of meat quality on viscera

Analysing the nutritional composition and other meat quality character on the viscera of pigs is a new topic in the field of science, there are very few studies that define the nutritional content of viscera. Even though few, most of the topics focused on the

element or heavy metal content, as they are the organs where there are high chances of accumulation of harmful elements that might occur through feed and their rooting behaviour, an act of searching food on the ground even though there is enough food (Beattie and O'Connell, 2002), which may lead them to exposure to soils that are contaminated.

Offal or viscera also known as organ meat are edible part of an animal that are not part of the skeletal muscle (Oloruntoba and Nathaniel, 2019). It is the non-carcass components of the animal's viscera that are not sold directly as consumable meat (de Queiroz et al., 2017). They are also called as the edible meat by-products, they make-up up to 52% of the live weight of the pigs (Liu, 2008). They include the organs such as liver, heart, kidney, spleen, large intestine, small intestine etc. These edible by-products are widely consumed in China, Japan, Germany, France and many other southeast Asian countries (Zeng et al., 2022). Not only the offal products of pigs are used, but also there are reports of usage of goat's and cattle's organs too (Seong et al., 2014). The proper use of these by-products will add a huge profit to the meat industry and will help to maintain hygienic environment overcoming the allegations of environmental pollution (Umaraw et al., 2015). One huge concerns of meat industry are the disposing of these meat by-products, as they cause pollution to the environment. Therefore, proper usage of these offal products can lead to a sustainable environment and profit to the meat industry (Jayathilakan et al., 2012).

Scientist and nutritionist recommend the consumption of viscera as they contain essential amino acids, minerals and vitamins (Kicinska et al., 2019; Toldra et al., 2016; Wu et al., 2018). They are regarded as cheap and nutrient-dense food. The protein and amino acid content supplied by them usually compensates for shortcomings of staple foods. They provide iron and aid in the absorption of iron and zinc from other food sources. Additionally, they contain large amount of vitamin B complex. Due to their nutritional richness, the consumption of offal can alleviate prevalent nutritional deficiencies (Umaraw et al., 2015). Remarkably high levels of indispensable fatty acids, particularly arachidonic acid, the omega-3 fats EPA and DHA, are present in visceral organs. In Africa, these visceral organs were a crucial component of the dietary habits providing a significant nutritional advantage to individuals with limited access to other nutritionally dense food sources. In fact, it can be argued that visceral organs are the most

nutritionally dense food on the planet, as they contain specific and readily absorbable forms of nutrient complexes (such as folate, choline, and B12) that are difficult to obtain from other sources (Oloruntoba and Nathaniel, 2019).

Even though intake of these edible by-products can supplement essential trace elements and vitamins, however there needs a careful vigilance towards the occurrence of potentially toxic minerals (Zeng et al., 2022). The levels of toxic elements in animal tissues exhibit specificity towards different organs. Meat and meat by-products are an important part in the dietary habits of humans. While consuming the offal care should be taken, as there are high chances of accumulation of toxic elements, and are organ specific. Toxic content in muscles is generally low, but that offal tends to contain high amount of metal concentration than other foods (Abou-Arab, 2001; Lazarus et al., 2005; Villar et al., 2005). Therefore, assessing the concentrations of toxic elements is vital for the safety and well-being of individuals. The assessment of health hazards posed by these food by-products can be conducted through a comparison of estimations regarding the intake of the recommended Provisional Tolerable Weekly Intakes (PTWIs) and Provisional Maximum Tolerable Daily Intakes (PMTDIs), which have been advised by the Joint Expert Committee on Food Additives (JECFA), the Food and Agriculture Organization (FAO) of the United Nations, as well as the World Health Organization International Programme on Chemical Safety and European Food Safety Authority (EFSA).

There are many factors that determine the accumulation of heavy metals in offal, primarily the feed, water and soil. Additionally, it has been well established that chemical elements cannot be synthesized in the animal body and can solely be obtained via ingestion of food and water (Długaszek, 2019). Soil serves as a reservoir for heavy metals over a long period of time, having distinct levels of movement and bioavailability (Nicholson et al., 2006). According to Sager (2007), heavy metals are not only in soil and water that might occur through anthropogenic activity, but are also added to the commercial feed artificially that contains elements such as Cu, Zn and As, known to promote growth rate and has antimicrobial properties.

Therefore, it is very necessary to monitor the concentration level of elements in feed, drinking water and soil, and to elucidate their relationship with concentration of element of the pig's viscera. These results will be useful for the local farms to maintain

hygiene environment and to improvise the feeding rations of the pigs. Not only that, it will depict the status of concentration level of potentially harmful metals and will serve as a baseline for other related animals in the selected study area.

Mi et al. (2020) analysed the essential and non-essential/toxic elements in the edible viscera (heart, lung, stomach, liver, large intestine, small intestine and kidney) of Tibetan pigs of China, including correlation of these edible viscera with drinking water, soils and feed. Other studies included the investigation of trace elements in pig's kidney and liver of southern China and their health risk assessment (Wu et al., 2016). Nikolic et al. (2017) studied the concentration macro- and micro-elements in pigs' liver and kidney that were reared extensively and intensively, and found that extensively reared pigs were more susceptible to contamination of harmful elements.

2.8. Study of meat quality on viscera in Indian pigs

At present, there are no such studies from India that analyses the nutritional attributes of viscera of pig breeds. However, recent findings analysed the toxic and non-toxic elements in edible offal and muscle of semi- extensively reared indigenous Doom pig breed of northeast India and was correlated with feed and environmental contaminants such as soil and water (Daimari et al., 2022b). Another study by same author examined the nutritional composition in six visceral organs of Doom pig (Daimari et al., 2022c).

These tissues (i.e. muscles and viscera) are known to bio-accumulate harmful toxic elements. When muscles and viscera contaminated with these elements are consumed, they pose significant health risks to humans, potentially causing inflammation and infection in the digestive system, as well as disorders of the nervous system (Bilandzic et al., 2009). It can also lead to anaemia by slow down the production of erythrocytes and increase their destruction (Halmo and Nappe, 2020). The accumulation of toxic elements such as arsenic (As), cadmium (Cd), lead (Pb) and nickel (Ni) can occur through feed and drinking water that is provided to the pigs including exposure to soil. Pigs' natural rooting behaviour, where they search for food even when sufficient feed is provided, can lead to ingestion of soil, further increasing the risk of toxic element accumulation (Beattie and O'Connell, 2002). Therefore, analysing the concentration of minerals (macro-, micro-, trace and potentially toxic trace elements) in feed, drinking water and soil is necessary to ensure food safety and protecting public health.