

Discussion

5.1. Pork Consumption: Nutritional, Cultural and Health Perspectives

Globally, pigs represent one of the most reared livestock animals for meat production. Accounting for over 40 % of the world's total meat production, pigs are a major source of meat for humans' consumption (Wang et al., 2017). Pork (34 %) is the second highest consumed meat worldwide, after poultry (40 %) (Kim et al., 2024; OECD, 2023). Compared to other meat types, pork is affordable providing essential micro-nutrients (Penkert et al., 2021) including iron, zinc, selenium, vitamin D, and B vitamins. It consists of 18 - 22 % protein content offering a balanced amino acid profile to meet human dietary needs (Higgs and Pratt, 1998). Pork also provides various health benefits when consumed in optimal amount, such as improving healthy skin, eyes, nervous and skeletal systems, as well as promoting mental well-being (Singh et al., 2003; Nistor et al., 2012).

When it comes to pork, one major concern among consumers is its high concentration of fat content, as diets high in saturated fatty acids have been linked to an increased risk of diseases such as coronary heart disease (CHD), colon cancer, and diabetes (Zhu et al., 2021; Roynette et al., 2004; Jiménez-Colmenero et al., 2001). However, fats in diet cannot be fully avoided as they regulate cell and tissue metabolism, and carry out various hormonal signals (Calder, 2015). Another important characteristic of dietary fatty acid is they alter the fluidity of the plasma membrane and influence the lipid mediators like eicosanoids and response to stimuli such as inflammation (Visioli and Poli, 2020).

Although, SFA's have been linked to adverse health outcomes, recent studies suggested that, the risk of coronary heart disease (CHD) cannot be solely blamed to intake of saturated fats, lifestyle habit of an individual and other confounding factors are also major factors that elevate the risk (Liu et al., 2017). Moreover, studies by Hu and Willett, (2002), Micha and Mozaffarian, (2010), Siri-Tarino et al. (2010) and Chowdhury et al. (2014) have consistently found that high risk of coronary heart disease are linked with high intake of trans-fat rather than saturated fat. Trans fatty acids are mainly found in hydrogenated oils that are available commercially, including dairy products. The hydrogenated oils (margarine, frying fats and baking shortenings) make-up to 40 to 50 %

of the trans fatty acids while fats from ruminant animals constitute about 1 to 8 % (Craig-Schmidt and Holzer, 2000). Therefore, it can be stated that majority of trans fat possessing high risk for CHD comes from commercially available oils than animal fats, like that of pork.

In India, pork consumption is highly influenced by culture and traditions and is often prohibited due to religious customs. Majority of pig meat production comes from northeast India and its consumption is also highly observed in northeastern states due to absence restriction of pork consumption (Abraham, 2014; Devi et al., 2014). A recent review on mortality due to CHD in India reported that states such as Punjab, Tamil Nadu, Andhra Pradesh and Goa had the highest cardiovascular-related death rates, while sub-Himalayan states like Meghalaya, Nagaland, Sikkim reported lower rates (Gupta et al., 2006). A positive correlation was seen between CHD mortality and intake of sugar, jaggery, milk and dairy by-products. Another factor, which seems more of individual lifestyle preferences is the smoking, was associated with increased rates of CHD diseases in India (Gupta et al., 2006). Interestingly, in most of the high-mortality states, pork consumption was minimal, except for Goa, suggesting that other dietary and lifestyle factors may play a more prominent role in cardiovascular health than pork fat itself.

In the present study, fatty acid composition was studied in two indigenous pig breeds, Doom and Ghungroo. The results revealed that saturated fatty acids (SFAs) were the most abundant across all muscles, followed by monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Among SFAs, palmitic acid (14.61 - 22.43%) was the dominant fatty acid, with the highest concentration found in the *tensor fasciae latae* muscle of the ham region. Moderate levels of stearic, lauric, and myristic acids were also recorded. Among MUFAs, oleic acid (12.59–30.63%) was most abundant, particularly in the *triceps brachii* of the shoulder region. In contrast, PUFAs such as alpha-linolenic acid (ALA) and arachidonic acid (ARA) were present in lower concentrations (1.23 - 3.85 % and 0.65 - 1.99%, respectively), with the *longissimus dorsi* of the loin region showing the highest total PUFA content.

When compared to previous findings, the SFA content observed in the present study was notably lower. For instance, SFA levels in crossbred pigs of Slovenia (Large White × Slovenian Landrace crossed with Pietrain, Duroc, or Pietrain × Slovenian

Landrace) ranged from 33 - 38% (Furman et al., 2010). Similarly, higher SFA concentrations were reported in Landrace × Yorkshire × Duroc (LYD) pigs (Choi et al., 2016), as well as in White Duroc × Erhualian and Large White × Meishan genotypes (Yang et al., 2010; Monziols et al., 2007). Among indigenous pig breeds, Mangalitsa, recognized as one of the fattest pig breeds globally, exhibited SFA contents significantly higher than Doom and Ghungroo pigs (Parunovic et al., 2012). Likewise, other native breeds such as Prestice Black-Pied, Pulawska, Suino Nero Lucano, and Tibetan pigs (Dostálová et al., 2020; Kasprzyk and Bogucka, 2020; Perna et al., 2005; Gan et al., 2019) also showed higher SFA levels, albeit still lower than those of most commercial crossbreds.

These findings from the present study demonstrate muscle-specific variations in fatty acid composition, suggesting that certain pork cuts may be nutritionally more favourable. The relatively higher presence of health-beneficial fatty acids, such as oleic acid, ALA, and ARA, particularly in muscles from the shoulder and loin regions, suggests potential health benefits. These essential fatty acids are well-documented for their roles in promoting cardiovascular health, enhancing immune function, and reducing inflammatory responses (Simopoulos, 2008; Visioli and Galli, 2002). Additionally, the overall fatty acid content of Doom and Ghungroo pigs was found to be comparatively lower than that of other crossbred and indigenous pig breeds. It is important to note that factors such as breed type, sex, muscle location, and diet can significantly influence fat composition (Delgado et al., 2002; Cordero et al., 2010).

In a country like India, where the majority of trans fat intake originates from commercial hydrogenated oils rather than ruminant or monogastric animal fats, it is reasonable to infer that health issues commonly associated with saturated or trans fats, such as colon cancer and cardiovascular disease are more strongly linked to the consumption of processed oils and foods than to pork itself. Even though monogastric animals like pigs typically have higher SFA content than ruminants, studies have shown that modification of dietary structure can positively influence the fatty acid composition of different muscles or fat deposits (Parunovic et al., 2012; Viera-Alcaide et al., 2007).

Beyond health considerations, fats also play a central role in determining the sensory quality of pork. Whether present in adipose or muscle tissues, fats contribute to the flavour, juiciness, texture, and overall palatability of the meat. These sensory and nutritional characteristics are largely governed by the types and proportions of fatty acids present (Vaclavkova and Beckova, 2007; Wood et al., 2004). Moreover, pork has been identified as a source of antioxidant compounds, which can provide protective effects against oxidative stress. These antioxidants are capable of neutralizing free radicals and reactive oxygen species (ROS) in cells, thereby reducing the risk of chronic conditions such as heart disease, cancer, aging, and inflammation (Abuajah et al., 2015; Vaibhav et al., 2011). Notably, research by Kęska et al. (2020) and Wojciak et al. (2021) highlighted the ham region as particularly rich in antioxidant peptides, supporting the recommendation of its regular inclusion in the human diet due to its functional health benefits.

The current study has analysed nutritional content of three muscles dissected from ham region of both Doom and Ghungroo pig breeds namely; *biceps femoris*, *gracilis* and *tensor fasciae latae*. Furthermore, *in-silico* studies have depicted that such meat (pork) resulting in the presence of peptides benefit in preventing diseases like hypertension, diabetes etc. (Keska et al., 2020).

5.2. Morphological authentication of the pig breeds

The morphological traits of Doom and Ghungroo pigs like, the body or coat colour, hair or bristles, external appearance, ears, snout, belly, teats and tail were recorded and analysed thoroughly.

Doom pig is the 6th registered breeds of India and first from Assam. The name, 'Doom' is referred to the local community that has been rearing the pig for decades. The Doom pig is characterized by a black coat and thick bristles extending up to the lumbar region. These features, along with its small head, erect ears, and short, concave snout, were observed in the present study and found to be consistent with earlier reports by Zaman et al. (2014), Rahman et al. (2019), Banik et al. 2022). The pig's wild-like appearance, compact belly, and short tail further corroborate with previous findings (Banik et al., 2022).

Unlike Ghungroo, the snout and ears of Doom pig is short and concave-shaped and erect. Furthermore, the belly of Doom pig is small, short and straight, while those of Nicobari pig from Nicobar Island are known to have long belly. Sows (female pig) of Doom pig of this study were found to have 5 pairs of teats. The tail of Doom pigs is straight and short. The meat of Doom is sold at higher market prices than other meats of commercial pigs. The meat of Doom is known to be highly palatable (ICAR-NBAGR).

Ghungroo is the first registered pig breed recognized by the ICAR-NBAGR. They have high prolificacy and able to survive in low-input systems. The Ghungroo pig observed in the present study exhibited a bulldog-like appearance, with folded skin around the neck and face. The ears were large and heart-shaped, while the belly appeared long and cylindrical in shape. Similar morphometric features have also been reported by Thomas et al. (2016). Hair line or bristles of Ghungroo is minimal or less visible, while pigs Agonda Goan, Doom and Mali pigs are known to have thick hairline. Both the sexes of Ghungroo, i.e., male and female are docile meaning they are not aggressive and are easy to handle. Ghungroo pig of this study was found to have 6 pairs of teats. Compared to the meat of Doom, meat of Ghungroo is less pricy. Their main purpose of rearing is to fatten producing more lean meat.

5.3. Molecular characterization of pig breeds

The molecular characterization of the Doom and Ghungroo pig breeds was performed based on molecular marker '*cytochrome b*' gene. *Cytochrome b* gene is utilized as a molecular marker for exploring the evolutionary lineage of various animal species (Bruford et al., 2003; Wang et al., 2014). Other than providing information on evolutionary lineage, characterizing at molecular level also assist in identification of the pig breeds and define their genetic diversity. The *cytochrome b* gene when compared with other phylogenetic markers was found to show greater compatibility than standard mammalian phylogeny marker, showing nucleotide variation at greater level in short sequences (Gupta et al., 2013; Tobe et al., 2010).

The results of the current study provide an outline of the partial fragment *cytochrome b* gene of Doom and Ghungroo pig. BLAST-N of partial fragment of *cytochrome b* gene of Doom and Ghungroo pig generated a total of 25 domestic pigs and 5 wild boar that showed 99 % similarity. Among 25 domestic pigs, 11 were selected based

on closest similarity scores. The 11 selected domestic pigs are from India, China, Japan, and European countries, and in addition to that, 5 wild boars from Asia and European countries. The samples of the present study (Doom and Ghungroo) including 11 domestic pigs and 5 wild boars were analyzed for nucleotide variation sites/positions, genetic distances and phylogenetic tree at MEGA 11 to ensure a comprehensive understanding of their genetic relationships.

Assessing the variable sites/positions showed that, Doom pig of the present study had identical nucleotides with Indian wild pig, Asian and European wild boars, and these nucleotide substitutions can be used for differentiating indigenous pigs and wild boars of Asian and European origin as suggested by previous reports (Gupta et al., 2013). The Doom pig sample of the current study showed six similar nucleotides with the wild boars, while Ghungroo pig showed only two similar nucleotides with the wild boars. While, the Ghungroo pig (retrieved from NCBI) did not show any nucleotides similarity. Furthermore, Ghungroo pig of the present study resulted in farthest distance from Doom pig and other wild boars. On the other hand, the phylogenetic tree showed that Ghungroo pig was found to originate from the Asian indigenous pig's clade, that included Satsuma and Ohmini pig of Japan, Ma Shen and Ya Chen of China, and northeast Indian pigs - Tenyi Vo, Niang Megha and Zovawk. In this clade (Asian indigenous pig's clade), the Ghungroo pig retrieved from NCBI is also included. The observed low genetic diversity in the Ghungroo pig may be attributed to the use of full-sibling individuals in this study. This reflects familial relatedness rather than the overall genetic diversity of the breed. Recent study showing low genetic diversity of Ghungroo pig population supports the findings of current in regard to Ghungroo pig (Sharma et al., 2023). The Doom pig having the nearest genetic distance to the wild boars' states that it is the most recently domesticated pig breed, as also revealed by Das et al. (2024).

The phylogenetic tree constructed depicted that Doom and Ghungroo pig including the Indian wild pig confined to one cluster, placed next to Asian indigenous pig breeds. Within this cluster, Doom pig was in close relationship with Indian wild pig. The results of the phylogenetic tree corroborate with the genetic distance i.e., the close distance between Indian wild pig and Doom breed pig suggests that, they are closely related to each other and Doom pig is the most primitive among the indigenous pig breeds

of India. Recent study too revealed through D-loop sequences of Indian pigs, that Doom pig has the least genetic distance with Indian wild pig (Das et al., 2024). Additionally, the inclusion of Doom pig (retrived from NCBI) in the European clade could suggest of common maternal haplotypes due to practice of crossbreeding carried out in India for better production (Sharma et al., 2023; Vashi et al., 2018).

5.4. Body weight and other growth parameters under a control and trial diet.

The body weight and other growth parameters of Doom and Ghungroo studied during the 8 months of rearing are body weight (B/W), chest girth (CG), height at withers (HAW), paunch girth (PG) and body length (BL). The measurement of body weight and other growth parameters was taken based on the three stages of growth (weaner, grower and finisher).

5.4.1. Pre-weaned period: The measurement of body weight and other growth parameters of pre-weaned pigs were taken at owners rearing site. The live body weight of 15 days old Doom piglets was in average 1.14 kg and that of Ghungroo piglets was 3.85 kg, resulting in 2 kg heavier than Doom piglets. The indigenous pig breeds of Tamil Nadu showed similar body weight with Doom but lower weight with Ghungroo (Kumar et al., 2018). Chest girth (CH) measurement of pre-weaned Doom pigs was about 25.83 cm on average and 34.29 cm for Ghungroo piglets. Ghungroo piglets had 9 cm larger chest girth than Doom. Chest girth of indigenous pig breeds of Tamil Nadu was smaller than both Doom and Ghungroo breeds (Kumar et al., 2018) at pre-weaned stage.

Height at wither measured from the ground to the highest point of its withers (shoulders) of pre-weaned Doom piglets was 14.73 cm and 23.44 cm for Ghungroo piglets, resulting in 9 cm higher than Doom. Height at withers for indigenous pig breeds of Tamil Nadu was high compared to Doom and low compared to Ghungroo (Kumar et al., 2018). Paunch girth is the circumference of pig taken just front of hind legs. Paunch girth of Doom piglets was about 27.03 cm, while Ghungroo piglets had 35.29 cm, which was about 8 cm larger than Doom. Paunch girth of indigenous pig breeds of Tamil Nadu when compared with both Doom and Ghungroo was found to be smaller (Kumar et al., 2018). Body length of Doom and Ghungroo was measured from the midpoint present between ears to the base of the tail. The body length of pre-weaned Doom piglets was

19.63 cm and that of Ghungroo was 32.62 cm. Ghungroo's length was 13 cm longer than Doom pig. Body length of indigenous pig breeds of Tamil Nadu recorded for males was similar with Doom (Kumar et al., 2018).

5.4.2. Growing stage: In the growing stage (62 to 153 days), the measurement of body weight and other growth parameters was taken two times. First measurement was taken at the 8th day, after acclimatizing the pigs for 7 days at grower stage. After acclimatizing, the pigs were fed with experimental diets consisting of control and trial diets. While, the second measurement of pigs was taken after completion of the grower stage at 154th day. The following difference in morphometric attributes are observed at grower stage. Body weight of Doom pigs was 6.88 kg on average, while Ghungroo pig showed 9.15 kg. Ghungroo pig was three kg heavier than Doom. Compared to pre-weaned stage, Doom's weight increased 5 kg and that of Ghungroo increased 6 kg more. Indigenous pigs of Tamil Nadu showed high body weight than Doom at the age of 60 days (Kumar et al., 2018). While local pig of Mizoram too showed slightly higher body weight than Doom (Kalita et al., 2018). The 154-day body weight measurement; for control diet of Doom pig was 32.36 kg on average, and for trial diet, 34.65 kg. The trial diet showed 2 kg more weight on average. For Ghungroo, the control diet showed 39.26 kg and for trial diet, 41.69 kg on average. The weight of trial diet fed was 2 kg heavier than control diet fed. Doom pig resulted in an increase of approximately 26 kg larger than the first weight taken at grower stage. While Ghungroo pig resulted in an increase of approximately 30 kg heavier than first weight taken at grower stage. Comparing with the indigenous pigs of Tamil Nadu and those local pigs of Mizoram, body weight of Doom and Ghungroo were heavier (Kumar et al., 2018; Kalita et al., 2018).

The 8-day measurement of chest girth for Doom pig was 32.98 cm and that of Ghungroo was 39.35 cm. The 154-day measurement for Doom pig was 75.48 cm (control diet) and 75.83 cm (trial diet). While for Ghungroo pig, 90.83 cm of chest girth for control diet and for trial diet chest girth was 91.68 cm. For Doom pig the chest girth increased approximately 43 times more than 8-day measurement taken at grower stage, while for Ghungroo pigs, the chest girth increased by 51 cm approximately. The indigenous pig breeds of Tamil Nadu showed smaller chest girth than Doom and Ghungroo (Kumar et al., 2018). The local pigs of Mizoram too showed smaller chest girth (Kalita et al., 2018). While the chest girth of Bareilly desi pigs was similar to Doom pig (Boro et al., 2018).

The 8-day measurement of height at withers Doom pig was 23.14 cm and that of Ghungroo was 26.89 cm. The 154-day measurement for Doom pig was 53.47 cm (control diet) and 53.76 cm (trial diet). While for Ghungroo pig, 59.67 cm for control diet and 59.00 cm for trial diet. There was an increase in height of withers approximately 30 cm for Doom between the first and second measurement and 33 cm for Ghungroo pigs. The height of withers observed for indigenous pig breeds of Tamil Nadu was similar to Ghungroo pig (Kumar et al., 2018). While the local pigs of Mizoram and Bareilly desi pigs showed smaller height (Kalita et al., 2018; Boro et al., 2018) than Doom and Ghungroo. The 8-day measurement of paunch girth for Doom pig was 33.90 cm on average and that of Ghungroo was 40.50 cm. The 154-day measurement for Doom pig was 76.86 cm (control diet) and 76.32 cm (trial diet). While for Ghungroo pig, 93.66 cm of paunch girth for control diet and for trial diet 94.99 cm was observed. There was increase of 43 cm for Doom and 60 cm for Ghungroo was observed. The paunch girth observed for male indigenous pigs of Tamil Nadu were larger than Doom and smaller than Ghungroo (Kumar et al., 2018). While that of Bareilly desi pigs, the paunch girth was smaller than both Doom and Ghungroo (Boro et al., 2018).

The 8-day measurement of length of body for Doom was 33.49 cm on average and that of Ghungroo was 34.00 cm. The 154-day measurement for Doom was 70.51 cm for control diet and 71.54 cm for trial diet. While for Ghungroo pig, 98.45 cm of body length for control diet and for trial diet 99.95 cm was observed. Approximately, 37 cm of increase in body length for Doom and about 65 cm for Ghungroo pig was observed. Compared to other indigenous pigs of Tamil Nadu, Mizoram and Bareilly (Kumar et al., 2018; Kalita et al., 2018; Boro et al., 2018), the body length of Doom and Ghungroo were larger.

5.4.3. Finisher stage: In the finisher stage (154 to 244.01 days), the body weight and other growth parameters measured were taken once the pigs has completed the rearing period which is 8 months before slaughtering. In this stage too, the pigs were fed the experimental diets (control and trial diet). The following differences in morphometric attributes were observed for finisher stage. The live body weight of Doom pig observed at the finisher stage was 49.27 kg for control diet and 49.78 kg for trial diet. While Ghungroo was 61.25 kg for control diet and 63.23 kg for trial diet was observed. Ghungroo pig was found to be 12 times heavier than Doom pig. Comparing with the body

weight observed at grower, an increase 17 kg weight was seen for Doom pigs and for Ghungroo, about 22 kg increase in weight was recorded. The indigenous pigs of Tamil Nadu showed similar body weight with Ghungroo pigs (Kumar et al., 2018). Body weight of Bareilly desi was less than both Doom and Ghungroo pigs at 10 months old age (Boro et al., 2018).

The chest girth of Doom pig was 83.20 cm for control diet and 84.27 cm for trial diet and that of Ghungroo was 107.28 cm for control and 108.76 cm for trial diet. Among the breeds, a difference of 24 cm was observed. Compared with 154-day measurement of pigs at grower, the size increased about 8 cm at the end of finisher stage for Doom pig. While for Ghungroo pig, the size of chest girth increased about 16 cm. The chest girth of indigenous pigs of Tamil Nadu was similar with Doom pig (Kumar et al., 2018). While comparing with local pigs of Mizoram, the size of chest girth was larger than Doom but smaller than Ghungroo (Kalita et al., 2018).

The height of the wither of Doom pig measured from the ground was 57.52 cm for control and 57.99 cm for trial diet. While Ghungroo pig resulted in 63.77 cm for control and 63.76 cm for trial diet. Among both the breeds, a difference of 6 cm is observed for height of wither. Compared with the earlier height taken at grower stage after the completion of 153 days, for Doom there was an increase in height of about 4 cm and for Ghungroo too, the height increased about 4 cm. The height at withers of local pigs of Bareilly had similar withers with that of Doom pig (Boro et al., 2018). The height at withers of indigenous pigs of Tamil Nadu are similar to Ghungroo pigs (Kumar et al., 2018).

The difference observed for paunch girth of Doom were 84.75 cm for control diet and 85.66 cm for trial diet. And for Ghungroo pigs, the paunch girth observed was 119.77 cm for control diet and 120.32 cm for trial diet. An 85 cm of difference was observed among the pig breeds. Compared with the second measurement taken at grower stage, there was an increase of 8 cm at finisher stage for Doom pig. And for Ghungroo, paunch girth increased about 25 cm at finisher stage. Similar size of paunch girth was observed for indigenous pigs of Tamil Nadu and Bareilly (Kumar et al., 2018; Boro et al., 2018) when compared with Doom pigs.

The body length of Doom pig at finisher stage was 77.09 cm for control diet and 77.32 cm for trial diet 121.39 cm. And for Ghungroo pig, the body length observed was 121.39 cm and 121.87 cm. Among the breeds, there was difference of 44 cm. Compared with the second measurement taken at grower stage, there was an increase of 7 cm at finisher stage for body length for Doom pig. And for Ghungroo, body length increased about 20 cm at finisher stage. The body length of indigenous pigs of Tamil Nadu and Mizoram was similar to Doom pigs (Kumar et al., 2018; Boro et al., 2018).

5.4.4. Highest attained growth observed among the pig breeds

The highest attained growth rate observed for Doom and Ghungroo was recorded at grower stage, with Doom pig achieving 25.48 kg and Ghungroo pig 30.11 kg. As mentioned earlier, at the grower stage two measurements were taken, the 154-day measurement taken after administration of experimental diets resulted in highest growth rate attained by the pigs.

5.5. Nutritional analysis of kitchen waste

5.5.1. Proximate composition and alcohol content of kitchen waste

Among the proximate compositions dry matter (91.02 %), available carbohydrate (83.72 %) and calorie (370.57 k/cal) content were the highest in kitchen waste. Moisture and protein content were recorded for 8.67 % and 5.21 % respectively, while fat (1.65 %) and ash (0.75 %) were the lowest. While, kitchen waste is primarily composed of rice and a limited variety of herbs, resulting in comparatively lower proximate values. This distinction underscores the impact of ingredient diversity on the nutritional profile of these by-products.

5.5.2. Amino acid content of kitchen waste

The essential amino acids (EAAs) analyzed in the kitchen waste showed the highest concentration for isoleucine (2.27 g/100g), while the lowest was observed for phenylalanine (0.20 g/100g). Valine (1.88 g/100g) and methionine (1.51 g/100g) were moderate. Among the non-essential amino acids (NEAA's), glutamic acid (3.47 g/100g) and aspartic acid (3.24 g/100g) were the highest. Alanine (0.12 g/100g) and tryptophan (0.26 g/100g) were the lowest.

For grower pigs (62 to 153 days of age), the daily amino acid requirements (g/day) are as follows: methionine – 0.21 g, cysteine – 0.16 g, methionine plus cysteine – 0.44 g, threonine – 0.38 g, arginine – 0.57 g, leucine – 0.98 g, isoleucine – 0.46 g, and valine – 0.59 g. While for finisher pigs (63 to 244 days of age), the daily amino acid requirements (g/day) are as follows: methionine – 0.18 g, cysteine – 0.12 g, methionine plus cysteine – 0.40 g, threonine - 0.49 g, arginine – 0.67 g, leucine – 0.96 g, isoleucine – 0.46 g, and valine – 0.563 g.

When compared to commercial rations (grower and finisher), the daily amino acid requirements of grower pigs, the kitchen waste seems to provide an adequate supply of several essential amino acids. For instance, the isoleucine content (2.27 g/100g) significantly exceeds the daily requirement of 0.46 g/day, and similarly, valine (1.88 g/100g) and methionine (1.51 g/100g) too surpass the respective daily needs of 0.59 g/day and 0.21 g/day. This indicates that, quantitatively, kitchen waste has the potential to fulfil a substantial portion of the amino acid requirements for pigs during the growing stage.

A similar comparison for finisher pigs (153 to 244 days of age) reveals that the dietary amino acid needs are slightly reduced; for example, isoleucine and valine requirements are 0.46 g/day and 0.563 g/day respectively, and methionine is needed at 0.18 g/day. Though high amino acid concentrations observed in the kitchen waste, it may serve as a useful protein supplement during both grower and finisher stages. Previous studies have suggested that incorporating high concentration of isoleucine in pig's diet, help to achieve better growth rate at growing and finishing stages (Figueroa et al., 2003; Zhao et al., 2019). Valine concentration in a pig's diet is particularly important, as its deficiency, especially in the presence of excess leucine, can significantly hinder the growth of pigs (Gloaguen et al., 2012).

5.5.3. Fatty acid content of kitchen waste

The fatty acid composition of kitchen waste was found highest for MUFA (14.92 %), followed by PUFA (8.70 %) and lowest concentration recorded was for SFA's (4.93 %). Saturated fatty acids such as palmitic acid was low for kitchen waste but was high for rice. Oleic acid content of kitchen waste was high (Yasumatsu and Moritaka, 1964). Concentration of PUFA's were higher than SFA's in kitchen waste. PUFAs are

essential fatty acids that play a crucial role in the development of organs during the early stages of life in vertebrate animals.

5.5.4. Mineral content of kitchen waste

The following minerals namely; macro- and micro-, trace both non-toxic and potentially toxic were analysed in kitchen waste. Kitchen waste resulted in high concentration of potassium (K - 23.47 mg/kg) and calcium (Ca – 21.84 mg/kg) among the macro-elements. High amount of K in feed contributes to the optimum health and growth of the pigs. While Ca helps in the growth and development of bones, blood clotting mechanism, and contraction of muscles and transmission of nerve (Koolman and Roehm, 2005). Concentration of magnesium (Mg) was the lowest among macro-elements. Even though it is in low amount, Mg content in feed helps in the better digestion, in sows it has also shortened the reproduction period (Gaál et al., 2004).

Among the micro-elements iron (Fe – 2.33 mg/kg) and manganese (Mn – 2.24 mg/kg) concentrations were observed the highest in kitchen waste. Iron is an essential element for the lactating piglets, as mother's milk do not contain enough iron. While Mn, along with calcium is important for formation of bone, immune response etc. (Li and Yang, 2018). Among the trace elements, highest concentration was noted for Cr (0.005 mg/kg) in kitchen waste. Chromium content was higher in kitchen waste (Malu et al., 2014).

Among the potentially toxic elements, lead (Pb - 0.057 mg/kg) was found highest in kitchen waste. Lead concentration was higher in kitchen waste (Malu et al., 2014). Lead which is a potentially toxic elements cause various carcinogenic illnesses and analysis of their maximum allowed concentration in animal feed is important for the safety of the animal. The recommended allowance concentration, according to national standards of China in animal feed are 4 mg/kg, 1.25 mg/kg and 10 mg/kg for As, Cd and Pb respectively. Comparing with the concentration and allowed concentration recommended in animal feeds, these potentially toxic elements were considered safe and did not exceed the suggested levels.

5.6. Meat quality analysis in muscles and viscera of pigs

5.6.1 pH (pH₄₅ and pH_u)

To estimate the meat quality characteristics, the measurement of pH is used in the meat industry (Kasprzyk et al., 2013). The pH levels measure at 45-minute (pH_{45}) and 24 hours (pH_u) post-mortem, help in the identification of defects observed in meat quality traits which are PSE (pale, soft, exudative) and DFD (dark, firm, dry) (Brewer et al., 2001). Muscle tissue acidification, measured by pH, plays a crucial role in regulating glycolytic processes (Choi and Kim, 2009). Additionally, pH is a key factor in evaluating shelf life, as acidity inhibits bacterial growth and prevents raw meat spoilage (Sieczkowska et al., 2009). The pH_{45} and pH_u results of the present study in muscles and viscera of Doom and Ghungroo pig breeds depicted good quality in terms of acidity of a meat. The declining of pH level post 24 hours indicates normal meat maturation (Kasprzyk and Bogucka, 2020). The results, which were similar with the muscles of Złotnicka pig breed fatteners observed by Grzeskowiak et al. (2009). Studies by Babicz et al. (2009) too found similar results in the *longissimus muscle* of Pulawska breed. The pH level studied in *longissimus thoracis et lumborum* muscle of Ghungroo pigs by Thomas et al. (2016) too found similar data with the current study.

5.6.2. Nutritional composition in muscles and viscera of pigs

Pork, a red meat, constitutes a major part of the global meat consumption, particularly in European countries and Asia (Lin-Schilstra et al., 2022), with China accounting for highest production of pig meat (46 %) (FAO, 2022). Red meat supplies an excellent source of essential nutrients, amino acid providing high value protein than other available food sources (Geiker et al., 2021; Strazdina et al., 2014). The protein gained from animal meat has high energy ratios and better digestibility (Greco et al., 2017). Red meat also provides essential minerals especially Fe, Mg and Zn required for human. Furthermore, minerals found in meat are easily absorbed than that of plants (de-Castro Cardoso Pereira et al., 2013). Besides meat, the consumption of viscera of pig by the locals in Assam including other northeastern states is very popular. The viscera of pig are considered as edible food and is recommended by nutritionist as they contain essential amino acids, vitamins and minerals (Kicinska et al., 2019; Toldra et al., 2016; Wu et al., 2016). Therefore, it becomes important to analysed the nutritional content of viscera as they consumed by local consumer's at large amount.

Analysis of proximate content helps one to understand, the basic nutritional compositions and quality of the food samples in the form of standardized amount. In the modern-day marketing, the proximate characteristics will determine its success and consumer acceptance.

5.6.2.1. Moisture content

Moisture content in foods samples is one the most important parameters that determine the quality and stability of a sample. High moisture content will shorten the shelf-life of a food and there are high chances of microbial contamination affecting its enzyme function. The muscles of Doom pig of control diet (overall average, 72.66 %) resulted in lower moisture content than trial diet (overall average, 78.77 %). While muscles of Ghungroo pig showed similar moisture content in control (overall average, 79.33 %) and trial (overall average, 79.50 %) diets. Previous studies on moisture content of indigenous pig breeds showed 72.91 to 73.21 % in commercial feed and 73.37 to 74.25 % on a mixed diet analysed in *longissimus dorsi* and *semimembranosus* muscles of Celta pig (Temperan et al., 2014). Provided with four different diets with oleic acid as main ingredient, the moisture content ranged from 66.57 to 68.63 % analysed in muscle *longissimus thoracis* of Iberian pigs (Vieira et al., 2021). The Pulawska, an indigenous pig breed of Poland showed a moisture content of 73.15 % determined in *longissimus lumborum* muscle (Kasprzyk and Bogucka, 2020). In the present study, muscles from the ham region showed high moisture content and muscle of loin region showed the lowest. When compared these indigenous breeds with the crossbred pig, analysed in same muscles, it showed an overall moisture content of 74.22 % (Kim et al., 2008). In spite of the differences, the distribution of moisture content was similar across the countries with 90 % of the samples having 65-79 % of moisture. The moisture content of Doom and Ghungroo pig were higher when compared with other red meats (goat, sheep and beef).

The moisture content of viscera of Doom pig for control diet (overall average, 60.85 %) and trial diet (overall average, 60.63 %) fed were similar and that of Ghungroo were found to be similar too for control (overall average, 60.37 %) diet and trial (overall average, 61.45 %) diet. Recent study performed in the crossbred pigs presented moisture content ranging from 82.48 to 69.73% in small intestine and large intestine, respectively (Seong et al., 2014). It can be concluded that viscera of Doom and Ghungroo pig had low

moisture content when compared with crossbred pigs. Spleen and liver of the both the pig breeds resulted in high and low moisture content, respectively. Comparing with the red meats that included goat, sheep and beef, the viscera of Doom and Ghungroo of current study depicted low moisture content.

5.6.2.2. Protein content

A meat is said to have good nutritional value when it is rich in protein content depicting high amount of essential amino acids (Listrat et al., 2016). The protein content from plant is considered incomplete in human diets due to lack of essential amino acids, and those of protein content from meat is considered complete as it contains essential amino acids (Klurfeld, 1994). Protein content in muscles of Doom pig of control diet ranged from 18.72 to 21.57 % and trial diet range from 18.53 to 22.30 %. Ghungroo pig's protein content in muscles ranged from 20.10 to 22.62 % for control diet and for trial diet ranged from 19.33 to 22.17 %. The current study depicted highest protein content in *longissimus dorsi* muscle of both the pig breeds dissected from the loin region. Loin region is considered one of the most important pork cuts. Muscles from this region are valued by most of the consumers due to their high nutritional content (Blicharski et al. 2013; Babicz et al., 2018).

Studies by Kim et al. (2008) in muscles of crossbred pig showed high protein content in *longissimus dorsi* (21.79 %) muscle and lowest was found for *tensor fasciae latae* (19.48 %) muscle. Another work on native Croatian pig and crossbred pig of Croatia in *longissimus thoracis et lumborum* muscle depicted a protein content of 21.75 % and 24.57 %, respectively (Kusec et al., 2022). Similar study in Ghungroo pig of Assam showed 21.25 % of protein in barrows of *longissimus thoracis et lumborum* muscle (Thomas et al., 2016). The protein content across the muscles viz., *triceps brachii* and *latissimus dorsi* dissected from the shoulder region of both Doom and Ghungroo pig and that shoulder region of red meats (goat, sheep and beef) was found same.

On the other hand, the protein content in the six viscera of Doom pig ranged from 6.85 to 22.36 % for control diet and for trial diet the content ranged from 5.95 to 22.03 %. While that of Ghungroo pig ranged from 5.51 to 23.29 % for control diet and for trial diet protein content ranged from 4.17 to 23.83 %. Earlier studies on viscera of crossbred pigs showed a protein content ranging from 8.45 to 22.05 %, with lowest for large

intestine and highest for liver (Seong et al., 2014). Like muscles, the protein content in viscera of Doom and Ghungroo pig was similar with that of viscera of goat, sheep and beef ranging from 16.24 to 18.23 %.

5.6.2.3. Fat content

One of the main meat quality attributes is the intramuscular fat content. The higher the intramuscular fat content, the better is the meat quality. The organoleptic features of the meat such as flavor, aroma, tenderness, juiciness, and the dietary attributes are contributed by intramuscular fat content and composition (Mourot and Hermier, 2001). Fat content of Doom pig muscles ranged from 2.79 to 3.55 %, where highest was shown by *tensor fasciae latae* muscle for control diet fed. Muscles of Doom pig for trial diet ranged from 1.79 to 3.09 % where highest amount was found for *gracilis* muscle. Ghungroo pig's fat content for muscles ranged from 3.03 to 4.25 % for control diet and was found to be higher than that of fat concentration of Doom pig's muscle content for control diet. For trial diet, Ghungroo pig's muscle ranged from 2.47 to 3.22 %. The higher concentration of fat content in muscles of Ghungroo than Doom pig may be due to the fact that most of the Ghungroo pigs are reared for fattening yielding more pork production.

Earlier findings showed an overall intramuscular fat content of 2.27 % in *longissimus dorsi* and 2.26 % in *semimembranosus* muscles of Celta pig fed with different diets (Temperan et al., 2014). Their findings were similar with the present data obtained for both the pig breeds. Another study analyzed in *longissimus dorsi* muscle of Iberian pigs fed with four different diets showed fat content ranging from 9.66 to 11.53 % (Vieira et al., 2021), compared to the present data, our findings had low intramuscular fat content. Gan et al. (2020) determined fat content at different growth stages of Liangshan pig in *longissimus dorsi* muscle and found that with increase of growth rate the intramuscular fat content also increased, with final growth stage at 91.5 kg, fat content was 5.02 %, whereas the breeds of our study, had final weights of 49.40 kg and 58.40 kg and fat content were low. Pulawska pig breeds showed fat content of 2.73 % in *longissimus lumborum* muscle (Kasprzyk and Bogucka, 2020), that was similar with our present findings. On the other hand, the Tibetan pigs (male and female) depicted low fat content of 1.88 % in *longissimus dorsi* muscle (Gan et al., 2019) when compared to our results. Therefore, it

can be concluded that the fat content depends on the diet that is provided including sex and breeds types. Doom and Ghungroo pig resulted in low fat content compared to that of goat, sheep and beef.

Viscera's fat content of Doom pig ranged from 0.47 to 9.85 %, for control diet and trial diet ranged from 0.50 to 8.45 % showing similar fat content in both the diets. Ghungroo pig's viscera fat content ranged from 0.41 to 10.05 % for control diet and for trial diet it ranged from 0.74 to 8.58 %. The present study showed high fat content in large intestine of both the pig breeds. There is limited data available on the viscera of pigs. However, a study by Seong et al. (2014) on crossbred pigs (Landrace × Yorkshire × Duroc) reported that fat content ranged from 0.28% to 19.54%, with the lowest observed in the uterus and the highest in the large intestine. The fat content in viscera of Doom and Ghungroo pig of current data are found to be similar with that of goat, sheep and beef.

5.6.2.4. Ash content

Ash represents the total content of minerals present in an animal meat, following its removal of water and organic matter by heating. This provides insights into the mineral composition of the meat, which is important for both animal health and human nutrition. Ash content in muscles of Doom pigs fed with control diet ranged from 0.63 to 0.77 % showing lowest in *gracilis* and highest in *tensor fasciae latae* muscles. Trial diet (0.70 to 0.83 %), showed lowest in *longissimus dorsi* and highest in *tensor fasciae latae* muscles. In both the diets highest ash content was found in *tensor fasciae latae* muscle, found in the ham region of pig.

For Ghungroo pigs, the ash content is relatively similar in both the control (0.75% to 0.84%) and trial diet (0.73% to 0.82%). The ash content in the muscles of Doom and Ghungroo pigs is lower than the range reported by Kim et al. (2008), which was 0.91% to 1.00%, showing lowest in *gracilis* and highest in *triceps brachii* muscles. This might be due to differences in pig breeds, sex of the pigs and compositions of diet. Another finding for Ghungroo pigs of Assam is similar to Thomas et al. (2016), which reported a mean ash content of 0.82% in the *longissimus thoracis et lumborum* muscle, indicating that the current results for Ghungroo pigs are within the expected range for this breed and muscle type. The ash content was slightly higher in the shoulder region of goat, sheep and beef than that of shoulder region of Doom and Ghungroo pigs.

Ash content for viscera of Doom pig ranged from 0.16 to 1.19 % (for control diet) with the lowest value observed for large intestine and highest for liver. Trial diet ranged from 0.17 to 1.42 %, showing lowest value in large intestine and highest for liver. Ash content in edible viscera of Ghungroo pig ranged from 0.22 to 1.27 %, showing lowest value for large intestine and highest for spleen for control diet. While trial diet ranged from 0.17 to 1.45 %, observing lowest for large intestine and highest for liver. Earlier studies showed ash content in viscera ranging from 0.15 to 1.24 %, showing lowest value for large intestine and highest for liver, which was also observed in Doom and Ghungroo pig. Ash content in viscera of Doom and Ghungroo pigs were slightly lower than ash content of other red meats (goat, sheep and beef).

5.6.2.5. Carbohydrate content

Carbohydrates is one of the important constituents of foods, it is stored in the form of glycogen in the liver and muscles of animal body, playing a critical role in determining the meat quality. They contribute to the sweetness, appearance and textural and water holding characteristics of the meat (Ahmad et al., 2018). Carbohydrate content in muscles of Doom pig for control diet fed ranged from 1.28 to 6.21 % showing lowest in *gracilis* and highest in *triceps brachii* muscles. In trial diet, it ranged from 1.57 to 3.20 %, observed lowest in *biceps femoris* and highest in *longissimus dorsi* muscles. For Ghungroo pig, carbohydrate content ranged from 0.44 to 5.43 % showing lowest for *triceps brachii* and highest for *tensor fasciae latae* muscles for control diet. And for trial diet, it ranged from 2.50 to 4.72 %, showing lowest for *biceps femoris* and highest for *longissimus dorsi* muscles.

Triceps brachii and *tensor fasciae latae* muscle consistently shows the lowest and highest carbohydrate content, respectively, in both pig breeds for control diet. While *biceps femoris* and *longissimus dorsi* muscle consistently shows the lowest and highest carbohydrate content in both pig breeds for trial diet.

Carbohydrate content in viscera of Doom pig for control diet ranges from 10.18 to 24.65 %, lowest value observed for spleen and highest for liver . Trial diet ranges from 10.03 to 25.65 %, observing lowest for spleen and highest for small intestine. Carbohydrate content in viscera of Ghungroo pig ranged from 11.92 to 26.10 %, showing lowest value for kidney and highest for heart for control diet. Trial diet ranged from 12.59

to 27.36 %, observing lowest value for kidney and highest for small intestine. The carbohydrate content of other red meats (goat, sheep and beef) for shoulder region as well as viscera were not available. So, comparison for carbohydrate content of Doom and Ghungroo pig could not be done.

5.6.2.6. Calorie content

Calorie content is an essential part of nutrition, indicating the amount of energy the body can derive from consumed food. Calorie content of Doom pig ranged from 116.82 to 144.38 kcal/100g found lowest in *triceps brachii* and highest in *tensor fasciae latae* muscles for control diet. Trial diet ranged from 107.29 to 123.49 kcal/100g, lowest observed in *biceps femoris* and highest in *triceps brachii* muscle. Like Doom pig, calorie content of Ghungroo pig was also found highest in *tensor fasciae latae* (144.38 kcal/100g) muscle and lowest in *triceps brachii* (116.82 kcal/100g) muscle for control diet. For trial diet highest was observed in *tensor fasciae latae* (133.24 kcal/100g) muscle and lowest value was observed for *biceps femoris* (113.04 kcal/100g) muscle. The muscle-specific variations noted in calorie content could be due to muscle fibre type, metabolic activity, and the impact of different nutrients on muscle energy storage. Calorie content when compared with other red meats showed low amount in shoulder region of Doom and Ghungroo pigs.

Calorie content in six viscera of Doom pig was found lowest in spleen (124.22 kcal/100g) and highest in large intestine (189.65 kcal/100g) for control diet. Trial diet was observed lowest in spleen (125.30 kcal/100g) and highest in large intestine (196.77 kcal/100g). For Ghungroo pig lowest was observed in spleen (136.43 kcal/100g) and highest for liver (200.41 kcal/100g) for control diet and for trial diet lowest was noted for spleen (150.31 kcal/100g) and highest for liver (178.13 kcal/100g). The study of calorie content in six viscera of Doom and Ghungroo pigs under different dietary conditions provides valuable insights into the nutritional energy distribution among internal organs. Calorie content in the viscera of Doom and Ghungroo pigs resulted in low amount when compared to viscera of goat, sheep and beef.

5.6.3. Statistical ‘T-test analysis’ for experimental diets

To determine whether both the diets (control and trial) were statistically different from each other, experimented in Doom and Ghungroo pig's muscles and edible viscera, an independent samples T-test was performed. The T-test resulted with no significant differences in the proximate composition (moisture, protein, fat, ash, carbohydrate and calorie) between the two experimental diets, meaning that both the diets resulted similar nutritional outcomes for the pigs in terms of basic nutritional data. As there were no significant difference, using one diet for further biochemical analyses i.e., for amino acid, fatty acid and mineral composition analysis, is justified.

After performing the statistical analysis, the data proximate composition of trial diet of Doom and Ghungroo pigs were compared with the proximate contents of other red meats that included goat, sheep and beef. The moisture content in the muscles of Doom and Ghungroo pigs was higher than goat, sheep, and beef. High moisture content may suggest that the meat from these pigs might be juicier and potentially more tender compared to the other red meats evaluated. While the viscera of Doom and Ghungroo pigs was lower than goat, sheep, and beef, indicating higher concentrations of other nutrients and solids. In-case of protein content, Doom and Ghungroo pigs had similar protein concentration with goat, sheep, and beef. The protein content for viscera is relatively similar among Doom and Ghungroo pigs and goat, sheep, and beef, indicating these meats as good sources of protein. Ash content, which reflects the mineral content of the meat, is lower in Doom and Ghungroo pigs compared to goat, sheep, and beef. For viscera too, the mineral content, is slightly lower in Doom and Ghungroo pigs compared to goat, sheep, and beef. This implies that lower mineral concentration is found in muscles and viscera of Doom and Ghungroo pigs. The calorie content in muscles and viscera of Doom and Ghungroo pig was significantly lower than goat, sheep, and beef. It may indicate that Doom and Ghungroo pig meats are leaner, which can be a healthier option for calorie-conscious consumers.

5.6.4. Amino acid composition

Cooked meat is significantly more appetizing than raw meat, as the process of heating enhances its taste, aroma, and flavour. The amino acid content in meat greatly affects its quality and is therefore known to enhance the flavour of pork as well as other types of meat (Ma et al., 2020). Pork, a red meat provides high biological proteins, made-

up by amino acids determining the quality of protein in the muscle (Vieillevoye et al., 2019). Essential amino acids (EAAs) preserve body's nitrogen equilibrium and overall well-being (Wang et al., 2017). The amino acids i.e., aspartate, glycine, alanine are known to bring flavour to the food; they react with glucose and fructose to form flavouring substances, and directly affect the freshness of the meat (Xu et al., 2019; Zhang et al., 2010). Culioli et al. (2003) stated that the amino acid profile in muscle tissue remains relatively consistent across different species, highlighting a fundamental biochemical similarity in meat composition.

Even though amino acid concentration is relatively constant among the same species, but when studied among different muscles of pig, it shows great variation. In the muscles of Doom pig, the essential amino acids (EAA's), methionine (1.28 to 3.83 g/100g) and phenylalanine (2.64 to 5.36 g/100g) were the highest among others, these amino acids were observed high in *longissimus dorsi* muscle. Similar results were observed in muscles of Ghungroo pig too, with high concentration of methionine (1.59 to 3.41 g/100g) and phenylalanine (2.87 to 8.72 g/100g), both amino acids observed highest in *longissimus dorsi* muscle. Previous studies in *semimembranosus* muscle of Yorkshire and Duroc breeds showed low methionine content of 0.53 g/100 in raw meat and 0.87 g/100g in cooked meat (Jensen et al., 2014). Studies by Thomas et al. (2016) depicted low concentration of methionine (0.24 g/100g) and phenylalanine (0.47 g/100g) in *longissimus thoracis et lumborum* muscle of Ghungroo pig of Assam. Another study too resulted in low amount of methionine (0.15 g/100g) and phenylalanine (0.28 g/100g) in *longissimus dorsi* muscle of native Liangshan pig of China (Gan et al., 2020). Reports by Zhang et al. (2022) in *longissimus dorsi* muscle of five native pigs of China too showed low methionine content ranging from 0.59 to 0.68 g/100g and low phenylalanine ranged from 0.88 to 1 g/100g compared to Doom and Ghungroo pig breeds. Methionine and phenylalanine are essential amino acids as they cannot be synthesized or produced by animal body and should be gain from dietary sources. Including humans and mammals, methionine is necessary for growth and development (Courtney-Martin and Pencharz, 2016). Methionine is a sulfur-containing amino acid and serves as a precursor for other sulfur-containing amino acids and their derivatives (Parkhitko et al., 2019). When the amount of tyrosine (non-essential amino acid) in the diet is low, it is produced by hydroxylation of phenylalanine occurring in the liver. Half of the phenylalanine is

converted to tyrosine and this tyrosine further breakdown to important substances e.g., catecholamines, melanin, etc. thus defining the importance of phenylalanine amino acid (Litwack, 2018). On the other hand, as mentioned earlier that amino acids contribute taste to the meat, the methionine and phenylalanine are known for imparting bitter taste, including valine, isoleucine and tryptophan (Lee et al., 2016). The muscles *triceps brachii* and *latissimus dorsi* belong to the shoulder region of pig and when comparing the essential amino acid content with the shoulder region of other red meats i.e., goat, sheep and beef, methionine content was found to have similar values. However, another EAA, phenylalanine, in shoulder region of Doom and Ghungroo pig showed higher data than that of goat, sheep and beef.

The recommended daily intakes for adults for valine is 10 mg/kg, for isoleucine is 10 mg/kg, for methionine is 13 mg/kg, for phenylalanine is 14 mg/kg and for tryptophan is 3.5 mg/kg. The values of EAA's of muscles of Doom and Ghungroo were converted to chemical score from gram, so that it can be compared with recommended intakes. Chemical score for amino acid helps in identifying quality of a protein quality for dietary purposes. It measures the quality of a protein based on the composition of amino compared to a reference protein. It was observed the EAA's methionine and phenylalanine had the highest amino acid chemical score. However, all the muscles dissected from three different regions showed high amino acid chemical score. All the muscles were found to meet the requirements as recommended for adults. (Note; the results of methionine and phenylalanine were shown without cysteine and tyrosine).

Among non-essential amino acids (NEAA), concentration of alanine was the highest of Doom (1.10 to 1.75 g/100g) and Ghungroo pig (1.51 to 3.25 g/100g). Previous studies reported similar alanine content in raw meat (1.2 g/100g) and cooked pork meat (1.9 g/100g) of *semimembranosus* muscle of Yorkshire and Duroc breeds (Jensen et al., 2014). Another study by Zhang et al. (2022) in *longissimus dorsi* muscle of five native pigs of China reported similar alanine concentration ranging from 1.16 to 1.32 g/100g. Low concentration of alanine (0.41 g/100g) was observed in the *longissimus thoracis et lumborum* muscle of barrows of Ghungroo pig of Assam (Thomas et al., 2016) when compare with the current results of Ghungroo pig. Liangshan native pig of China too showed similar concentration of alanine (1.91 g/100g) in *longissimus dorsi* (Gan et al., 2020). Current result showed *gracilis* muscles dissected from ham region showed the

highest concentration. Alanine, which is a non-essential amino acid, is produced in skeletal muscles by the process catabolism including various other reactions and at last taken up by liver. In the hepatocyte cells of liver, alanine transforms into pyruvate which is utilized in gluconeogenesis (formation of glucose) (Felig, 1973), thereby contributing energy, during exercise or fasting and when carbohydrates amount in the diets are low. The amino acid alanine is known to contribute sweet taste to the meat (Shahidi, 2001), other non-essential amino acids such as glycine also impart sweetness to the meat and that of aspartic acid and glutamic acid contribute sourness (Lee et al., 2016).

The highest essential amino acid (EAA) observed for viscera of Doom pig was isoleucine (6.21 g/100g) and phenylalanine (overall, 5.50 g/100g). While for Ghungroo pig too, similar results were observed with highest for isoleucine (6.21 g/100g) and phenylalanine (5.50 g/100g). Previous study in crossbred pigs (Landrace × Yorkshire × Duroc) on nine dissected viscera showed isoleucine content ranging from 0.33 to 1.02 g/100g with highest in liver and phenylalanine content ranged from 0.66 to 1.63 g/100g with highest in stomach (Seong et al., 2014). Studies by Seong et al. (2014) resulted in low EAAs content than that of Doom and Ghungroo pig breeds. Kidney and spleen of Doom and Ghungroo pig showed highest isoleucine and phenylalanine content. Isoleucine is a branched-chain amino acid and are known to be found in high concentrations in muscle tissues. Isoleucine plays various important roles and functions such as providing immunity, growth and development, metabolism of protein and fatty acid, transportation of glucose etc. It is also known to increase the expression of β -defensins to fight against pathogens (Gu et al., 2019). Comparing with other red meats (goat, sheep and beef) resulted in higher concentration of isoleucine content in the kidney of Doom and Ghungroo pigs.

The daily recommended intakes for adults for valine is 10 mg/kg, for isoleucine is 10 mg/kg, for methionine is 13 mg/kg, for phenylalanine is 14 mg/kg and for tryptophan is 3.5 mg/kg. Following is the chemical score for Doom pig's edible viscera. Chemical score for small intestine (250.4 %) is highest for valine, followed by heart (192.2 %), while kidney (6.4 %), spleen (2.4 %) and liver (7.6 %) showed low chemical score also did not meet the recommended intake for adults. For isoleucine, all viscera showed high chemical score meeting requirements for adults however for heart (2 %) it was the lowest. Most of the chemical score of viscera were low for methionine, except for spleen (56 %)

and liver (14.4 %). For phenylalanine, only score for heart (2 %) was the lowest, while others showed high score and met the recommended allowances for EAA's. For essential amino acid tryptophan, all the viscera met the required recommended intakes suggested for adults.

Chemical score for EAA's of viscera of Ghungroo pigs did differ much from that of Doom pig. Difference is observed among the viscera but among the breeds, the scores are quite similar. Chemical score for heart (146.8 %), liver (31.2 %) and kidney (50.8 %) were the highest for valine and met the recommended allowances. All the viscera for EAA isoleucine met the required intakes except for heart (4.8 %). For methionine, all the viscera meet up the recommended allowances, similar for phenylalanine too. Score of all the viscera for tryptophan too meet up the recommended intakes for adults.

Among the non-essential amino acids (NEAA), aspartic and glutamic acid were the highest for Doom as well as Ghungroo pig. Both aspartic acid (17.38 g/100g) and glutamic acid (25.19 g/100g) were seen highest in liver of Doom pig and for Ghungroo pig too aspartic acid (16.74 g/100g) and glutamic acid (22.73 g/100g) were seen highest in liver. While studies by Seong et al. (2014), the NEAAs i.e., aspartic acid (0.75 to 1.69 g/100g) and glutamic acid (0.57 to 2.11 g/100g) and low when compared to Doom and Ghungroo pig. Aspartic acid which is a naturally occurring non-essential amino acids plays an important role in urea as well as Krebs cycle (Emery, 2005). Aspartic acid also helps in the synthesis of purines and pyrimidines, required for DNA and RNA synthesis, contributing to cellular repair and growth. Glutamic acid is one of the most abundant NEAAs found in the central nervous system, it acts as an excitatory neurotransmitter helping in cognitive functions i.e., learning and memory (Greenberg, 2003). As mentioned earlier, isoleucine is known to impart bitter taste to the meat, while aspartic acid and glutamic acid are known to impart sour taste to the meat (Lee et al., 2016).

5.6.5. Fatty acid composition

The types of fatty acid and its composition determine the nutritional and physical features of fat. There are many reports stating that the fatty acid composition of muscle and adipose tissue of pig are affected by various factors. They are the breed type, feed composition, age, location of the muscle tissue in the carcass, sex (Gandemer et al., 1992; García-Olmo et al., 2002; Rentfrow et al., 2003; Bragagnolo and Rodríguez-

Amaya, 2002; Delgado et al., 2002; Cordero et al., 2010). The quality of meat is significantly influenced by its fatty acid composition, and scientists demonstrate considerable interest due to its correlation with human health.

Among the fatty acids studied in six muscles of Doom pig, highest concentration was accounted for saturated fatty acid (SFA's), followed by mono-unsaturated fatty acids (MUFA's) with a difference of 5% from SFA's and lowest concentration of fatty acid was observed for poly-unsaturated fatty acids (PUFA's). In the six muscles of Doom pig, for total SFA's, *tensor fasciae latae* (32.54 %) muscle noted the highest and lowest for *biceps femoris* (20.48 %). *Triceps brachii* (37.07 %) was found to have the highest concentration of total mono-unsaturated fatty acids (MUFA's) and lowest was for *biceps femoris* (14.17 %). Total poly-unsaturated fatty concentration was observed highest in *tensor fasciae latae* (5.54 %) muscle and lowest in *biceps femoris* (3.08 %).

While that of Ghungroo pig, total SFA's were found highest in *triceps brachii* (30.84 %), total MUFA's in *longissimus dorsi* (25.52 %) and total PUFA's in *tensor fasciae latae* (7.69 %). Previous studies resulted in high concentration of SFA's (37.03 to 88.11 %) in *longissimus dorsi* muscle of five native pigs of China (Zhang et al., 2022). Another study by Thomas et al. (2016) revealed high values of SFA's (37.69 %) in *longissimus thoracis et lumborum* muscle of barrows of Ghungroo pig of Assam. Another study by Kim et al. (2008) studied in 10 crossbred pigs of Korea revealed high SFA's (35.73 to 38.31 %) including high MUFA's (61.69 to 64.27 %) and PUFA's (10.32 to 12.94 %) than Doom and Ghungroo pig breeds.

Palmitic acid was the most abundant among the SFA's in both the Doom and Ghungroo breeds and among the MUFA's oleic acid was the most abundant, PUFA's were the lowest among fatty acids and are regarded as essential fatty acids. Many reports suggests that high SFA's content in the diet are linked to the development of coronary heart disease (CHD) and atherosclerosis (Liu et al., 2017; Palazzo et al., 2021), which is why, the WHO (2010) has limited the recommended intake of saturated fats to >10% for adults. Additionally, risk of CHD and other related diseases due intake of high SFA's cannot be solely blame to red meat such as pork, there are also individual-diet differences such as lifestyle habits including other confounding factors that might elevate the risk (Liu et al., 2017). The saturated fatty acids (SFA's) of other red meats were too found to

have similar values, however sheep and beef had slightly higher concentration of SFA's. The recommended intake for SFA's for an adult individual is less than 10 % put forwarded by WHO, (2010). All the muscles of both the pig breeds exceeds the recommended intake of SFA's.

MUFA and PUFA are together considered healthy fats. Oleic acid had the highest percentage among the detected MUFA's, while that of, palmitoleic acid was the lowest. Many reports suggest that, with the increasing body weight, fatty acid composition also increases (Palazzo et al., 2021; Pedrazzoli et al., 2017). From a nutritional perspective, oleic acid which was the most abundant MUFA, plays a significant role in the human diet by effectively lowering triglycerides and LDL cholesterol levels (Shramko et al., 2020). MUFA content of all the muscles of Doom and Ghungroo pig exceeds the recommended intake which is less than 20 % suggested by American Heart Association (2006).

PUFA's play an important and major role in the well-being of human health. PUFA contribute to a wide range of cellular activities such as maintaining cell fluidity and structural integrity, cell signalling, regulating blood pressure, nervous system, inflammatory reactions, glucose level and blood clotting (Kapoor et al., 2021). Compared to MUFA and SFA content of the present findings, the PUFA's were low, however, even low they have a huge impact on human health. The omega-3 fatty acids i.e., LA and ALA are important essential fatty acids as they help in the synthesis of other PUFA's. The concentration of LA depends on the type of diet taken, i.e., if the diet consists large amount of LA, there will be less conversion of ALA into omega-3 long chain PUFA. The MUFA content of goat, sheep and beef was found to have similar values with the samples of current findings. However, that of total polyunsaturated fatty acids (TPUFA) of goat, sheep and beef were slightly lower than Doom and Ghungroo pigs.

The omega-6 and omega-3 poly-unsaturated fatty acids (PUFA's) are the essential fatty acids. Pork provides good amount n-6 and n-3 fatty acids. The muscles dissected from various regions of Doom and Ghungroo pig are found to contain n-6 and n-3 fatty acids within the range of recommended intake put forward by FAO, (2010) which is 0.5 to 2 %.

In viscera of Doom and Ghungroo pig, SFA's were slightly higher than muscles with highest concentration observed in large intestine, but the content of MUFA and

PUFA were low. Earlier studies by Seong et al. (2014) in nine viscera of crossbred pigs (40.47 to 50.48 %) had higher SFA content than Doom and Ghungroo pigs with highest observed in large intestine and lowest in heart. The MUFA (15.73 to 38.61 %) and PUFA (14.92 to 40.40 %) were too high in crossbred pigs than Doom and Ghungroo pigs. The viscera of Doom and Ghungroo pig when compared with viscera of other red meats, it was observed that saturated fatty acids (SFA's) of Doom and Ghungroo pig were the highest than goat, sheep and beef excluding the values of small intestine and large intestine, as the data were not available. However, the mono-unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were low in viscera of Doom and Ghungroo pig than goat, sheep and beef.

Similar to muscles, the viscera of both the pig breeds exceeds the recommended intake of SFA's. However, the MUFA content of viscera of Doom and Ghungroo pig did not exceed the recommended intake, therefore its consumption can be recommended to gain fats that are healthy for human body. The n-3 fatty acids content of viscera of Doom and Ghungroo were slightly lower than the recommended intake. However, the n-6 fatty acid content is within the range of recommended intake and heart and liver data surplus the suggested intake of n-6 fatty acids put forwarded by FAO, (2010).

5.6.6. Mineral composition

Minerals are essential nutrients required for the proper growth, development, and maintenance of the body's overall health and vital functions. Pigs are regarded as the bio-indicators of environmental pollution, especially that of wild pigs due to their different habitat types like marshes, semi-arid environments, forests and grasslands (Palazzo et al., 2021). The scavenging pigs are also great indicators of heavy metal pollution of the human habitat. This bioaccumulation not only reflects the level of environmental contamination but also provides critical insights into the potential health risks posed to humans sharing the same ecosystem (Palazzo et al., 2021; Malmsten et al., 2021). Therefore, determining concentration of minerals in porcine muscles and viscera effectively assesses both their nutritional value and heavy metal contamination, providing key insights into environmental health and human exposure risks.

5.6.6.1. Muscles and viscera of Doom and Ghungroo breeds

Potassium (K) content was the highest among the macro-elements that were studied in the six muscles of Doom (152.88 – 215.14 mg/kg) and Ghungroo (165.87 – 226.00 mg/kg) pig, followed by sodium content. Sodium concentration in muscles of Doom (17.70 – 21.02 mg/kg) and Ghungroo (18.20 – 20.86 mg/kg) pig was not as high as that of potassium content. Muscle recorded with high concentration of K and Na was *triceps brachii*. Sodium is a vital element required by the human body. Along with potassium, it helps in the regulation of water and electrolyte metabolism, as well as the maintenance of acid-base equilibrium of an organism (Teucher et al., 2008; Lanham-New et al., 2012). Potassium content is said to increase with the increasing body growth and is known to be an important component of muscles (Mienkowska-Stepniewska et al., 2007). Studies by Nikolic et al. (2017) and Stasiak et al. (2017) in *longissimus thoracis et lumborum* of Landrace, Mangulica pigs and crossbred pigs of Poland depicted slightly higher sodium content but similar potassium content with the current findings of Doom and Ghungroo pigs. Another study by Babicz and Kasprzyk, (2019) too had similar results with slightly low sodium and similar potassium content. Adding all the K and Na values of the muscles provided 1161.87 mg/kg of K and 118.65 mg/kg of Na. K concentration found in muscles of Doom and Ghungroo pig are found within the range of daily recommended intake for adults. While Na concentration was quite low compared with the recommended intakes.

On the other hand, in viscera, potassium for Doom (32.15 – 101.45 mg/kg) and Ghungroo (33.82 – 106.46 mg/kg) and sodium for Doom (29.88 – 122.84 mg/kg) and for Ghungroo (29.63 – 120.51 mg/kg) were low compared to muscles. K was observed high in liver of both the pig breeds while sodium accounted highest in spleen. Liver plays many important roles in the mammalian body, like providing immunity, digestion, metabolism, detoxifying, storage of vitamin among many others (Kalra et al., 2021). While spleen is known for filtering out abnormal cells, pathogens found in blood facilitating immunological response to the body (Lewis et al., 2019). Earlier findings by Tomovic et al. (2016) and Stasiak et al. (2017) in Mangalica pigs and crossbred pigs of Poland depicted had high concentrations of K and Na content in spleen and kidney than current findings of Doom and Ghungroo pigs. The difference observed between K and Na content in both the pigs can be due to differences in age, sex and slaughter conditions (Kasprzyk 2015; Tomovic et al., 2016). Combining all the values of K content of viscera resulted in

359.32 mg/kg for Doom and 374.59 mg/kg for Ghungroo. And that of Na resulted in 390.91 mg/kg for Doom and 382.70 mg/kg for Ghungroo. K and Na concentration found in viscera of Doom and Ghungroo pig was lower than the daily recommended intake for adults.

Magnesium is crucial for the metabolism of proteins (Cygan-Szczegielniak *et al.* 2012). Compared to K and Na, the concentration of magnesium is low in muscles of Doom (9.06 – 17.32 mg/kg) and Ghungroo (10.94 – 15.18 mg/kg) pigs. Earlier studies showed similar concentration of Mg content in *longissimus thoracis et lumborum* muscle of Landrace and Mangulica pigs and crossbred pigs of Poland (Nikolic *et al.*, 2017; Stasiak *et al.*, 2017). Magnesium content in viscera of Doom and Ghungroo pigs ranged from 2 to 5 mg/kg, which were low compared to previous studies by Tomovic *et al.* (2015); Stasiak *et al.* (2017) and Nikolic *et al.* (2017). When analyzed with the recommended intakes of Mg which is 420 mg/d for males and 320 mg/d for males, the muscles including the viscera of both the pig breeds exceeds the recommended intakes, providing enough Mg.

Zinc, among the micro-element was recorded high in all the muscles of Doom (2.36 – 6.29 mg/kg) and Ghungroo (1.62 – 3.37 mg/kg) pig. Another micro-element observed high was iron (Fe), for Doom, it ranged from 2.88 – 4.02 mg/kg and for Ghungroo the range was between 1.54 – 2.02 mg/kg. Iron was observed high in *longissimus dorsi* muscle of both the breeds. The absorption of zinc content present in meat is better than those gained from other sources (Cabrera and Saadoun, 2014). Also reports suggest that, the iron concentration present in meat is absorbed 30 % more than that of iron present in vegetable which is only 5 % (Krzecio-Nieczyporuk *et al.*, 2013; Nikolic *et al.*, 2015). Another element that helps in the absorption of iron is the copper (Soetan *et al.*, 2010; Nishito and Kambe, 2018). Mn and Cu were the lowest among microelements in six muscles of Ghungroo and Doom pig. Manganese and copper help in the regulation and metabolism of a cell, they also act as enzyme co-factors and antioxidants, therefore Mn and Cu are the must need metals for maintaining the functions in the animal's body (Lenart *et al.*, 2023). Earlier findings in the muscle of indigenous Mangulica pigs depicted low amount of iron and zinc (Nikolic *et al.*, 2017), including the crossbred pigs of Poland (Stasiak *et al.*, 2017), however, iron and zinc concentration of that of domestic pig of Poland (Babicz and Kasprzyk, 2019) showed similar findings with

that of Doom and Ghungroo pigs. In the muscle (*longissimus thoracis et lumborum*) of Mangalica pigs and that of domestic pigs of Poland, low concentration of manganese and copper was observed than present data of Doom and Ghungroo pigs (Nikolic et al., 2017; Babicz and Kasprzyk, 2019).

Among the micro-elements in the viscera of Doom (0.89 – 11.70 mg/kg) and Ghungroo (0.97 – 10.59 mg/kg) pigs, iron content was the highest. Iron was low in the liver and kidney, but the zinc concentration was similar to present findings studied in crossbred pigs of Poland (Stasiak et al., 2017). Findings by Tomovic et al. (2016) in heart, liver, spleen and kidney of Mangalica pigs showed low concentration of iron and zinc than current data of Doom and Ghungroo pigs. Manganese and copper concentration in the heart, liver, spleen and kidney of Mangalica pigs and domestic pigs of Poland showed low level than current data of Doom and Ghungroo pigs.

The recommended intakes of Fe is 16-18 mg/d for males and 12-16 for females, and for Zn is 11 mg/d for males and 8 mg/d for females. The current data of muscles including the viscera of both the pig breeds exceeds the recommended intakes, providing enough Fe content. Mn and Cu in muscles and viscera of both the pig breeds too exceeds the recommended intakes. Again, comparing with other red meat, pork meat was found to contain high K, while other macro-elements (Na, Mg, Zn, Cu and Fe) were similar.

Among the trace elements Se was high in all the muscles of Doom (0.009 – 0.015 mg/kg) and Ghungroo (0.011 – 0.017 mg/kg) than Cr. Majority of the Se amount comes from the food. Reports state that individuals that consume low protein content are supplemented with low Se content, as selenium found in food is bound to proteins (Dumont et al., 2006). In the edible viscera of Doom and Ghungroo, the trace elements Se (0.011 – 0.056 mg/kg for Doom; 0.011 – 0.060 mg/kg for Ghungroo) and Cr (0.006 – 0.037 mg/kg for Doom; 0.007 – 0.037 mg/kg for Ghungroo) were observed high. Nikolic et al. (2017) too reported similar values of Se and Cr in the muscle and liver kidney of Mangalica pigs.

The recommended intakes for Se is 55 µg/d for adults and for Cr is 25 µg/d for females and 30 µg/d for males. Cr in all the muscles exhibited low concentration than the recommended daily intakes and for viscera kidney and small intestine exceeded the suggested intakes, while others were low. For Se all the muscles were low and did not

exceed the recommended intake, however for viscera like spleen, liver and large intestine exceeded the recommended intakes.

The potentially toxic elements investigated in the muscles are As (arsenic), Cd (cadmium), Pb (lead) and Ni (nickel). Lead was found to be high in all the muscles of Doom (0.013 – 0.026 mg/kg) and Ghungroo (0.013 – 0.035 mg/kg). In all the viscera too for Doom (0.016 – 0.140 mg/kg) and Ghungroo pig (0.016 – 0.144 mg/kg), the concentration of lead was found the highest and also exceeded the recommended intakes. The recommended intake for lead is 0.025 mg/kg on a weekly basis for adults. The two muscles of Doom and Ghungroo namely; *tensor fasciae latae* and *gracilis* both exceed the recommended intake, which is a concerning issue for the consumers. Water is the main source through which the intake of lead may occur and are accumulated in the skeleton (WHO, 1993). Exposure of Pb has the potential to cause various illness to humans such as, infection and inflammation to the digestive system and disorder of nervous system (Bilandzic et al., 2009). Lead can slow down the erythrocytes production and increase their destruction, leading to the development of anaemia (Halmo and Nappe, 2020). Another study in the viscera of Tibetan pigs stated that its consumption may pose risk due higher concentration of Ni, Pb and Cr exceeding the allowed intake (Mi et al., 2020).

The partial least squares - discriminant analysis (PLS-DA) plot score (figure 4.p. in result section) analysed in muscles and viscera of Doom pig and their element concentration. Muscles and viscera were clearly separated from each other. Porcine muscles showed minimal discrimination and clustered within a similar radius. However, the viscera demonstrated more distinct discrimination, particularly with the liver and spleen easily distinguished from the other samples. This can be due to the fact that accumulation of elements in Doom pig's liver and spleen was more significant than others. The gland, liver stores minerals and vitamins including absorption of toxic elements (Kicinska et al., 2019; Wu et al., 2018). While spleen as stated earlier is known for filtering out abnormal cells, pathogens found in blood facilitating immunological response to the body (Lewis et al., 2019).

Similarly with Ghungroo pig (fig. 4.r. in results section), the viscera's kidney and heart were discriminated from the other samples, while liver, spleen, small and large

intestine were found in same radius. This depicts contamination by heavy metals was found in higher amounts in the viscera than in the muscles, as organs such as the liver, spleen, and kidney are responsible for eliminating toxic and harmful substances from the body.

The hierarchical clustering (Fig. 4.q. and 4.s.) of Doom and Ghungroo pigs illustrates both similarities and variations among different tissues (muscles and viscera). In Doom pigs, the mineral composition of the *triceps brachii* and *longissimus dorsi* muscles exhibited greater similarity compared to other muscles. In contrast, the remaining muscles *latissimus dorsi*, *biceps femoris*, *tensor fasciae latae*, and *gracilis* formed another distinct group, indicating that the mineral content of the shoulder and loin muscles share similarities. Conversely, the viscera displayed a broader variation in mineral concentration. Among the viscera, the mineral content of the liver, kidney, and spleen were more closely related compared to the heart, small intestine, and large intestine. For Ghungroo pig, too same mineral concentrations were found among the muscles and viscera.

5.7. Correlation of mineral content between tissues (muscles and viscera) with feed, drinking water and soil.

Toxic elements in muscles of pig are low compared to those found in viscera. The viscera are known to accumulates higher metal concentrations than muscles (Lopez-Alonso et al., 2007). Earlier studies suggested that either present in extreme or deficit amount, the toxic elements such as Pb, Cd, Hg and Al, is known to cause many disorders to humans (Dlugaszek, 2019; Nikolic et al., 2017). Evidence from earlier studies suggested that chemical elements cannot be synthesized in animal body and can only be obtained through feed and drinking water (Dlugaszek, 2019; Mattei et al., 2015; Yipel et al., 2017). There is a probability of accumulation of chemical elements from soils, while searching for foods. Soil ingestion has a significant role in soil exposure as pigs are known to show rooting behaviour even though there is enough food (Beattie and O'Connell, 2002). Feed is regarded as one of the most important factors that influence the accumulation of elements in the tissues (Kicinska et al., 2019).

Recent study in muscle, liver and kidney of intensively and extensively reared pigs from Serbia and stated that extensively reared pigs are more susceptible to contamination of

toxic elements (Nikolic et al., 2017). Recent findings by Daimari et al. (2022), stated that the nickel concentration exceeded the recommended daily allowances in edible offal (heart, kidney, liver, small intestine, large intestine, and spleen) of Doom pig breed of northeast India.

Therefore, it becomes important to correlate the elements (macro- and micro-elements, trace-elements - both non-toxic and potentially toxic) concentration of tissues (muscles and viscera) with those of feed, drinking water and soil.

The mineral content (macro- and micro-, trace including both non-toxic and potentially toxic) in drinking water and soil was determined. High concentrations of K, Na, Mg, Fe and Cr was observed in soil sample where native and crossbred pigs of Serbia were reared as revealed by Nikolic et al. (2017) than our current data. In the case of drinking water, directly comparable quantitative data on mineral content are limited in the existing literature. Although Mi et al. (2020) conducted a study on drinking water, their findings were primarily presented in the form of correlations rather than absolute concentrations.

Among the potentially toxic elements, lead (Pb) was found highest in drinking water (0.051 mg/kg), while that of nickel (Ni) was found highest in soil (0.129 mg/kg). Lead too was found high in soil sample where native and crossbreds pigs of Serbia were reared and exceeds the intake allowance (Nikolic et al., 2017). These potentially toxic elements cause various carcinogenic illnesses and analysis of their maximum allowed concentration in drinking water and soil are important for the safety of the animal. According to World Health Organisation, 1993, the recommended guidelines for drinking water put forward jointly by various other institutes and organisation for potentially toxic elements are 2 µg/kg per body weight for daily intake for arsenic, 7 µg/kg per body weight for weekly intake for cadmium, 25 µg/kg per body weight for weekly intake for lead and for nickel is 5 µg/kg per body weight for daily intake. The drinking water sample that was supplemented to the pig breeds had high concentration of lead and nickel when analysed with daily and weekly suggested provisional intake meaning that the values exceed the recommended intake for drinking water.

Spearman's correlation coefficient (r) was applied to analyse relationship between the fourteen minerals determined in tissues (muscle and viscera) and feed, drinking water

and soil. The correlation seen between the muscles of Doom and Ghungroo pig and feed (kitchen waste), water and soil were positively significant. Feed had the highest correlation coefficient with the muscles of Doom and Ghungroo pig breeds. Muscle *gracilis* had the highest correlation coefficient of 0.9033 with the feed showing p-value of <0.0001. *Gracilis* muscle is located in the ham region of the pig carcass. It has been suggested that muscles found in ham region of pig carcass, has the highest antioxidant property (Wojciak et al., 2021; Kęska et al., 2020). Additionally, dry-cured ham is one of the most popular and highly demanded preserved pork meat and it is prepared from the ham part of the pig carcass. The reason of its demand is because of its specific taste and texture, which are determined by its organoleptic characters and its pH content (Petrova et al., 2015) meaning that this particular region has a good nutrient absorbing quality especially the minerals. Ramos et al. (2007) stated that leaner pig meat does not make a good dry-cured ham, which means that ham region is a non-lean meat. Ham region, also consists of muscles like *biceps femoris* and *semimembranosus*, which are highly studied for its meat quality.

Likewise, positive correlation is seen between the viscera and feed, drinking water and soil. It was observed that viscera have the highest correlation with the drinking water. Small intestine, of all the viscera, had the strongest correlations with drinking water. This might be due to the biological function of the small intestine which can absorb ~90% of nutrients and water from the food (Liao et al., 2018). Thus, it can be concluded that accumulation of elements in muscle meat and meat by-products is directly associated with environmental status of the living area which is in conformity with Kumaresan (2009).