MEAT QUALITY AND MOLECULAR CHARACTERIZATION OF DOMESTICATED SWINE BREEDS OF ASSAM, INDIA

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ABSTRACT

Introduction: Pigs are one of the most reared livestock species for production of red meat worldwide. In Northeast India, small-holder pig farming is a prevalent practice, with kitchen waste commonly used as feed. This approach is both cost-effective and environmentally sustainable, contributing significantly to meat production and income generation, particularly benefiting the ethnic communities of the region. Among the northeastern states of India, Assam is the highest pig meat producer. Rearing of both indigenous and crossbred pigs are observed in small-holder rearing system in Assam. However, due to high rate of crossbreeding, the population of indigenous pig breeds has declined. To address this issue, the ICAR-NBAGR emphasizes on conserving Indian indigenous pig breeds to preserve their genetic resources, ensure sustainable use and support livelihood. Despite declining numbers, the indigenous breeds such as Doom and Ghungroo pigs remain highly valued for their meat, by the local consumers. Additionally, the meat quality and molecular characterization of both the pig breeds are lowly documented. Furthermore, along with meat muscles, consumption of viscera is also highly preferred by the locals. However, they are prone to accumulating potentially toxic heavy metals, which can be acquired through feed, drinking water and soil. Therefore, the present study was undertaken with the following objectives:

1. Morphological and molecular characterization of Doom and Ghungroo pig breeds.
2. Nutritional analysis of kitchen waste as trial diet for animal feed
3. Observation of body weight and other growth parameters under control and trial diet.
4. Meat quality analysis of pork muscles and viscera after feeding period of control and trial diets.
5. Study the correlation between minerals composition between muscles and edible viscera of pig breeds with feed, drinking water and soil.

Methodology: Small-holder pig farms rearing Doom and Ghungroo pigs were visited and morphological characters such as body coat colour, hair, appearance, ears, snout, belly shape, teat numbers and tail feature were observed thoroughly and recorded. The morphological characters were further verified by Principal Scientist of ICAR-Centre of Pig, Guwahati. The molecular characterization of Doom and Ghungroo pig breeds was

conducted using the 'cytochrome b' gene as a marker. A total of 24 blood samples (12 Doom and 12 Ghungroo) were collected from male pigs for genomic DNA extraction. Gene amplification was done with universal primers as mentioned in Verma and Singh, (2003). For nucleotide variable positions/sites of cytochrome b gene GTR+G model using MEGA 11 was used. Genetic distances were computed using the Kimura 2-parameter model, and a phylogenetic tree was constructed using the Neighbour-Joining method with 1000 bootstrap replications.

Proximate analysis of kitchen waste, was performed by AOAC (2002). Carbohydrates and calorie content was conducted by James, (1995) and FAO, (2003). Alcohol content in kitchen waste was analysed by Caputi et al. (1986). pH level in tissues (muscles and viscera) was performed following Alonso et al. (2009). Proximate content in tissues (muscles and viscera) was analysed following AOAC (2005). Amino acid content in kitchen waste and tissues was analysed by UPLC system following Szkudzinska et al. (2017). Fatty acid content in kitchen waste and tissues was conducted by GC-MS using Bligh and Dyer (1959) and Joseph and Ackman (1992). Minerals in kitchen waste and tissues was digested following ASEAN Manual of Food Analysis (2011).

For this study, 24 male pigs (12 Doom and 12 Ghungroo) were reared. The period of experiment was undertaken from October 2019 to May 2020. Growth parameters, including chest girth, height at wither, paunch girth, and body length, were measured at three stages: weaner, grower, and finisher. At the end of the feeding period, pigs were slaughtered under veterinary supervision following standard procedures at a commercial abattoir in Tangla market, Assam, India. Post-slaughter, six muscles from the shoulder, loin, and ham regions, along with six viscera, were carefully dissected, transported to the laboratory, and stored at freezer of – 20 °C until for subsequent nutritional analysis.

Results: Morphological observations revealed that Doom pigs have an appearance similar to wild pigs, characterized by a small head, erect vertical ears, and a short-concave snout. In contrast, Ghungroo pigs have a bulldog-like appearance, marked by folded skin around the neck and face, large heart-shaped ears, an upward-curved snout. Molecular analysis of cytochrome b gene identified 18 nucleotide variable sites with no insertions or deletions observed by GTR+G model at MEGA 11. Doom pigs showed identical

nucleotide with Indian wild boar at six sites (15940, 16344, 16350, 16355, 16356, 16357), showing specific substitutions: A→C at 15940, C→T at 16344, 16350, and 16355, T→A at 16356, and A→G at 16357. Doom also showed similar site with Ryukyu wild boar at position 16350 (T→C). Ghungroo pigs shared identical nucleotides at two sites, with Indian wild boar at 16344 (T→C) and Ryukyu wild boar at 16350 (T→C).

Tamura-Nei model of genetic distance analysis showed Doom pigs were closest to Indian wild pigs (0.0238±0.0079), followed by Asian wild boar (0.0301±0.0014) and European wild boars (0.0119±0.0009). While Ghungroo pigs were closest to Asian indigenous pigs (Satsuma, 0.0044±0.0020; Ya Cha, 0.0021±0.0003), and indigenous Indian breeds (Zovawk, 0.0016±0.0003; Tenyi Vo, 0.0014±0.0003; Niang Megha, 0.0014±0.0003). Doom and Ghungroo pigs showed far genetic distance from each other. Phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with *Babyrousa babyrussa* as an outgroup. It revealed that Doom and Ghungroo pigs along with Indian wild pigs was confined to one cluster, placed next to Asian indigenous breeds. Within this cluster Doom pigs was closely related to Indian wild pigs, while Ghungroo pigs aligned with Asian indigenous clades, including Satsuma, Ohmini (Japan), Ma Shen, Ya Chen (China), and northeast Indian pigs—Tenyi Vo, Niang Megha, and Zovawk.

Nutritional analysis of kitchen waste revealed high protein content and essential elements such as K, Na, Fe, and Zn. Growth parameter analysis during the grower stage, showed that Ghungroo pigs outperformed Doom pigs, weighing approximately 7 kg more and exhibiting larger measurements in chest girth (15 cm), height at withers (6 cm), paunch girth (17 cm), and body length (28 cm). Nutritional analysis based on proximate content in tissues (muscles and viscera) after feeding of control and trial diets did not show any significant difference in meat quality statistically. As a result of which the pH level, amino acid, fatty acid and mineral analysis were continued with only one feed which is trial diet. pH measured at 45 min post-slaughter were slightly high than pH at 24 hrs post-slaughter.

Muscle and viscera analysis showed high levels of essential amino acids (EAAs), particularly phenylalanine and methionine, with the *longissimus dorsi* muscle having the highest EAA content. In edible viscera, isoleucine and phenylalanine were most prominent, and all EAA levels in muscles met FAO/WHO (1973) recommendations.

Saturated fatty acids (SFAs) were most abundant, followed by monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). Essential fatty acids alpha-linolenic acid (ALA) and arachidonic acid (ARA) were highest in *tensor fasciae latae* and *longissimus dorsi* muscles. SFA and MUFA levels exceeded recommended limits by <10% and <20% (WHO, 2010; AHA, 2006), while PUFA levels remained within the recommended range.

Among minerals, potassium (K) and sodium (Na) were most abundant in muscles and viscera. Trace elements like As, Cd, Pb, and Ni in muscles were below recommended dietary allowances, but Pb in viscera exceeded safe intake levels. Spearman correlation matrix revealed a positive correlation between mineral concentrations in tissues (muscles and viscera) and those in feed, water, and soil, with feed showing the highest correlation coefficient at a 95% confidence level.

Conclusion: Morphological and molecular analyses (nucleotide variation, genetic distance, and phylogenetic tree), revealed that the Doom pig, with its small size and thick hair, closely resembles wild pigs. While the Ghungroo pig was found to have unique bull-dog type appearance, with larger body size and scanty hairs. The molecular analysis of Ghungroo pig resulted in more close relation with the Asian indigenous pigs. Furthermore, least genetic distance between Doom and Indian wild pig shows close maternal resemblance revealing Doom is a recently domesticated pig. While Ghungroo generating farthest distance from Doom and other wild pigs, shows low genetic diversity indicating the existence of inbreeding within the population. Thus, characterizing the breeds at molecular level, broaden the understanding of their genetic makeup and evolutionary history thereby, contributing in their conservation.

Meat quality analysis based on pH level suggests, muscles and viscera of Doom and Ghungroo pigs depicted good quality in terms of acidity of a meat. While nutritional analysis reveals that muscles, *longissimus dorsi* and *tensor fasciae latae* contain high concentration of protein, EAA's, essential fatty acids and minerals. These muscles are located in loin and ham region. Therefore, meat from these regions can be suggested for consumption to gain high quality nutritional benefits. On the other hand, feed resulting in high correlation with concentration of minerals in tissues (muscles and viscera) reveals that feed can influence the mineral deposition in these tissues. Meat quality analysis of

current study will provide an updated database, encouraging and promoting the rearing of indigenous/native pig breeds among farmers.

Keywords: Indigenous pig breeds, Assam, meat quality, molecular characterization, ‘cytochrome b’ gene.