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Proximate, Mineral, Fatty Acid and Cholesterol Composition of Five Muscles of an Indigenous Swine Breed of North East India

Rijumoni Daimari, Silistina Narzari, Jatin Sarmah, Manab Deka[1]

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ABSTRACT

Background: Ghungroo is the first registered indigenous pig breed of India bearing accession number INDIA_PIG_2100_ GHUNGROO_09001 reported from Western Assam and North Bengal. The present study determines nutritional content (proximate, mineral, fatty acid and cholesterol) of five muscles that are considered most valued cuts by customers.

Methods: Thirty muscles were dissected from six reared Ghungroo pigs, each from shoulder (*Triceps brachii*, *Latissimus dorsi*) and from ham region (*Biceps femoris, Gracilis, Tensor fasciae latae*). Two months old piglets were reared from October, 2019 to May, 2020 and slaughtered. They were administered commercial diet *i.e.* starter, grower and finisher feed during different stages of growth.

Result: The highest protein, fat and ash were found in *tensor fasciae latae*, *gracilis* and *triceps brachii* respectively (P<0.05). In 100 g of meat, potassium, sodium, magnesium and zinc was found highest in *Tensor fasciae latae, Latissimus dorsi* and *Biceps femoris* respectively (P<0.05). The total saturated (SFA's), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were found highest in *Tensor fasciae latae* and *Latissimus dorsi* respectively (P<0.05), while trans-fatty acid and cholesterol were both found highest in *Gracilis.* The data shall be valuable for muscle specific improvement of meat quality and value addition for global markets.

Key words: Cholesterol, Fatty acid, Ghungroo pig, Indigenous swine breed, Minerals, Porcine muscles, Proximate.

INTRODUCTION

In India, there are around 10.29 million pigs, of which 2 million pigs are found only in Assam, highest among all the states (Livestock census of India, 2019; Singh *et al.,* 2020). Ghungroo is the first registered indigenous pig breed of India with accession number INDIA_PIG_2100_GHUNGROO _09001 (NBAGR, 2008). Due to its high productivity in terms of litter size (9-12 nos.) and good mothering ability, they have gained high rearing popularity in Northeast India. Ghungroo is a medium size pig with large drooping ears and dark-black color with scanty hairs and bristles. Their appearance is that of atypical bull dog face (Zaman *et al.,* 2013).

Pork meat is sold in primal cuts and these primal cuts are formed by various individual muscles which have different characteristics (Kim *et al.,* 2008). It is important to evaluate the chemical composition of different muscles so that its nutritional and human health perspectives are known. A meat is considered to exhibit good nutritional quality when it is rich in protein with a high amount of polyunsaturated fatty acids (Listrat *et al.,* 2016).The amount of fat content in meat is of a great deal of interest due to its organoleptic properties (Essien *et al.,* 1998).

Earlier studies have highlighted the minerals, amino acid and fatty acid contents of *M. longissimus thoracis et lumborum* (LTL) of Ghungroo pig and crossbred pigs (Thomas *et al.,* 2016; Thomas *et al.,* 2018). The present study is aimed to provide detail information on proximate, mineral, fatty acid and cholesterol content of five muscles, namely *Triceps brachii, Latissimus dorsi, Biceps femoris, Tensor fasciae latae* and *Gracilis*.

Department of Biotechnology, Bodoland University, Deborgaon-783 370, Assam, India.
[1]Department of Statistics, Arya Vidyapeeth College, Guwahati-781 016, Assam, India.

Corresponding Author: Jatin Sarmah, Department of Biotechnology, Bodoland University, Deborgaon-783 370, Assam, India.
Email: jatinsarmahindia@gmail.com

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MATERIALS AND METHODS

The study was approved by the Institutional Animal Ethics Committee; Bodoland University vide letter no- IAEC/ BIOTECH/2019/3. The muscles selected for the study were *Triceps brachii, Latissimus dorsi, Biceps femoris, Tensor fasciae latae* and *Gracilis.* The experiment was conducted with six Ghungroo barrows. They were born in the month of August, 2019. The experiment was undertaken during October 2019 to May 2020 and slaughtered. The reared pig breeds were administered commercial diet-starter, grower and finisher feed during different stages of growth. Prior to slaughter, the pigs were fasted for 12-16 hour with *ad-libitum* supply of water. Pigs were slaughtered in a commercial abattoir for poultry and swine in Tangla market, Assam, with the slaughtering process adapted for pigs. Five muscles *viz. triceps brachii* (291.91±42.0 g) and *Latissimus dorsi* (313.3±9.46 g) were dissected from shoulder; *Biceps femoris*

(305.95±17.79 g), *gracilis* (306.73±19.73 g) and *Tensor fasciae latae* (300.7±10.42 g) were dissected from ham region. The dissected muscles were identified and transferred to Bodoland University laboratory and stored at -20°C. 24-hour post-mortem, the subcutaneous fats from all the muscles were trimmed off by knife and homogenized using a mechanical grinder. The experiments were performed at the Department of Biotechnology, Bodoland University.

The chemical contents *i.e.* moisture, protein, fat and ash content of all the five muscles were determined according to AOAC (2005). The carbohydrate content was calculated by the difference method (FAO, 2003). The nutritive value or calorific value in kcal/100g was calculated with the help of the equation given by James (1995).

The determination of potassium (K), magnesium (Mg), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) were detected by Atomic Absorption Spectrometer (AAS-ICE 3500), Thermo Scientific, UK, at respective wavelengths with Instrument mode – Flame, Gas used - air acetylene. The meat samples were grinded and dried at oven at 105°C. For the digestion, 1 g of the dried sample was taken, into which 5 ml of HNO_3 and 1 ml of $HClO_4$ was added. The samples were left overnight to pre-digest and then placed in an oven at 100°C for 5-8 hrs. The cooled sample was diluted with deionised water to final volume of 50 ml (ASEAN Manual of Food Analysis, 2011).

For fatty acid determination, the dried muscle samples were subjected to lipid extraction with chloroform/methanol (Folch *et al.,* 1957). The lipid extract was esterified with BF_3-methanol (Joseph *et al.,* 1992) for preparation of FAME's (Fatty Acid Methyl Esters). The fatty acid composition of each aliquot was estimated by Gas Chromatography. GC-MS analysis of sample extracts was carried out with Perkin Elmer (USA), Model: Clarus 680 GC and amp; Clarus600C MS comprising a liquid auto-sampler. The Software used in the system was TurboMass Ver.6.1.2. The peaks were analyzed using data analysis software NIST-2014. The capillary column used is 'Elite- 5MS' having dimensions- length- 60 m, ID- 0.25 mm and film thickness-0.25 µm and the stationary phase is 5% diphenyl 95% dimethylpolysiloxane. Helium gas (99.99%) was used as carrier gas (*i.e.* mobile phase) at flow rate of 1 ml/minute. An injection volume of 2 µl was employed in split less mode. Injector temperature was 280°C and ion-source temperature 180°C. The oven temperature was programmed at 60°C (for 1 minute), with an increase at the rate 7°C/minutes to 200°C (hold for 3 minutes) then again increased at rate of 10°C/min to 300°C (hold for 5 min).The total run time is ~39 minutes. Solvent delay was kept for 8 minutes. MS Protocol Mass Spectra was taken in Electron Impact positive (EI+) mode at 70 eV. For MS scan, a solvent delay of 8 minute was provided with m/z range 50-600 amu. For the analysis of total cholesterol content, 1 gm of muscle sample was taken and 0.5 ml of 5α-cholestane (internal standard, 1 mg/0.5 ml of cyclohexane) was added. To it 5 ml of saturated methanolic KOH was added in a capped vial. Then the solution was heated for 30 minutes at 80°C. After cooling at room temperature, 5ml of cyclohexane was added and vortexed for 1 minute (Naeemi *et al.,* 1995). An aliquot of the cyclohexane layer was injected into the Gas Chromatography (Perkin Elmer, Clarus 680, USA).

The mentioned data were statistically analyzed using SPSS, Version 26.0 and demonstrated as mean±SEM (standard error of mean). The differences of readings in respect of muscles are analyzed with the help of ANOVA technique. Tukey's test has been employed to check the pair-wise association for those attributes which have significant effect (P<0.05).

RESULTS AND DISCUSSION

Proximate composition

The moisture, protein, fat, ash, total carbohydrate and calorie contents of five muscles of Ghungroo pig are shown in Table 1. Muscle *biceps femoris* (79.33%) had the highest moisture and presented an overall mean of 76.86%, while that of protein, highest content was reported in *tensor fasciae latae* with a mean of 21.99% and *gracilis* showing lowest protein value with a mean of 20.10% having a significant value of less than 0.05 (P<0.05). The present findings were similar to the results of LTL muscle studied by Thomas (2016) but when compared with the crossbred pigs of Korea in 21 different muscles by Kim (2008), they showed 3.82% less protein content than our present findings. In spite of the differences, the distribution of moisture content was similar across the countries with 90% of the samples having 65-78% moisture.

Intramuscular fat present in meat has a significant effect on its taste and meat quality. Though different regions showed different results, yet there was no huge difference between muscles. Intramuscular fat content varied from a mean value of 3.13% in *triceps brachii* to 4.25% in *gracilis* (P<0.05). Higher values of fat content have been reported with a mean of 4.46% for 21 different muscles of crossbred pigs of Korea (Kim *et al.,* 2008) and LTL muscle (Thomas *et al.,* 2016). Ash content did not differ among muscles and were similar to the study of Kim (2008) and Thomas (2016) with overall mean of 0.80%. In any meat, the most abundant chemical composition is water, followed by protein and fat, carbohydrates occur in much smaller amount. *Triceps brachii* (0.44%) has shown lowest amount of total carbohydrate content, whereas *Tensor fasciae latae* (5.43%) has shown highest amount. In 100 g of raw meat, nutritive value or calorific value was found highest in *Triceps brachii* (166.82 kcal/100 g).

Minerals

Differences in the type of tissue, sampling process, production system and seasonal changes can also be the reasons for variations in mineral composition (Tajik *et al.,* 2010). Table 2, represents the results of minerals in five muscles of Ghungroo pig. *Tensor fasciae latae* (154.59 mg/

Table 1: Proximate composition in five muscles of Ghungroo pig.

Parameters	*Triceps brachii*	*Latissimus dorsi*	*Biceps femoris*	*Tensor fasciae latae*	*Gracilis*	Significance (P-value)
Moisture (%)	76.00±3.60	77.00±3.60	79.33±5.03	78.33±3.05	73.66±1.76	0.438
Protein (%)	20.47±0.44	21.58±0.27	20.13±0.22	21.99±0.38	20.10±0.45	*0.012
Fat (%)	3.13±0.08	3.40±0.24	3.99±0.07	3.42±0.23	4.25±0.22	*0.011
Ash (%)	0.84±0.02	0.81±0.02	0.81±0.02	0.84±0.02	0.75±0.00	0.083
Total Carbohydrate (%)	0.44±0.01	2.77±0.02	3.84±0.05	5.43±0.16	1.28±0.02	*0.000
Calorie (kcal/100g)	166.82±2.86	127.74±1.43	133.51±1.73	144.38±2.38	122.27±0.87	*0.000

The values are expressed in mean ± SEM. *P<0.05.

Table 2: Mineral composition (mg/100 g) in five muscles of Ghungroo pig.

Parameters	*Triceps brachii*	*Latissimus dorsi*	*Biceps femoris*	*Tensor fasciae latae*	*Gracilis*	Significance (P-value)
Major elements						
K	122.72±1.52	147.71±1.49	142.48±1.37	154.59±1.16	151.40±0.87	*0.000
Na	18.79±0.34	20.86±0.31	20.84±0.28	18.20±0.16	19.44±0.28	*0.000
Mg	10.94±0.66	15.18±0.10	14.09±3.35	14.74±0.38	12.46±0.28	*0.000
Minor elements						
Fe	1.63±0.30	1.53±0.29	1.68±0.26	1.72±0.38	1.54±0.33	0.990
Zn	1.62±0.30	2.56±0.15	3.37±0.23	1.96±0.07	1.65±0.27	*0.001
Mn	0.02±0.00	0.03±0.01	0.03±0.01	0.02±0.01	0.02±0.01	0.972
Cu	0.03±0.00	0.05±0.01	0.03±0.01	0.04±0.01	0.03±0.01	0.907

The values are expressed in mean±SEM. *P<0.05.

100 g) and *Latissimus dorsi* had the highest content of K and Na contents, while *triceps brachii* (122.72 mg/100 g) and *tensor fasciae latae* (18.20 mg/100g) had the lowest K and Na content, which were significantly different from each other (P<0.05). A study on LTL muscle the amount of K was found to be higher than present study with a mean of 328.28 mg/100 g (Thomas *et al.,* 2016; Thomas *et al.,* 2018). Na and K are important for water and electrolyte metabolism and acid-base equilibrium in the organism (Mienkowska-Stepniewska *et al.,* 2007).

Mg is a basic component for protein metabolism. Present study shows high amount of Mg in *latissimus dorsi* (15.18 mg/100g) and lowest in *Triceps brachii* (10.94 mg/100g). While that of LTL muscle, showed low amount of Mg (6.27 mg/100g) (Thomas *et al.,* 2016; Thomas *et al.,* 2018). Absorption of Fe obtained from meat is approximately 20-30%, while that of plants is only 5% (Nikolic *et al.,* 2015). *Tensor fasciae latae* (1.72 mg/100g) had highest Fe content, while that of Zn was found highest in *biceps femoris* (3.37 mg/100 g) in the present study. Data of Fe (2.72 mg/100 g) content were similar to that of LTL, but amount of Zn (0.79 mg/100 g) was quite low compared to the present study (Thomas *et al.,* 2016; Thomas *et al.,* 2018). In our study, Mn content ranged from 0.02-0.03 mg/100 g and Cu ranged from 0.03-0.05 mg/100 g, which were quite similar to the results of LTL muscle (Thomas *et al.,* 2016; Thomas *et al.,* 2018).

Fatty acids

The fatty acid composition (% of total FAME) is tabulated in Table 3. Of all the determined fatty acids SFAs had highest percentage with an average of 44.55% (range, 25.29% to 63.62%). MUFA had an average of 32.38% (14.64% to 59.74%), while PUFA accounted for 15.81 % (range, 5.97% to 34.47%) which were significantly different from each other with P- value less than 0.05.

PUFA along with MUFA are considered healthy fats, as they can reduce the risk of heart disease. In our study, omega-3 PUFA fatty acid detected is alpha-linolenic acid (ALA) and for omega-6 PUFA is linoleic acid (LA). ALA was found highest in *tensor fasciae latae* (7.10%), while LA (4.22%) was found highest in *triceps brachii* muscles. Compared to our study, ALA (0.46%) content was low in LTL (0.46%) muscle, but the LA (17.90%) content was high (Thomas *et al.,* 2016). While, LA content on same muscle of Duroc (13.28%), Landrace (12.93%) and Yorkshire (13.63%) was high than our study (Choi *et al.,* 2016). Linoleic acid and alpha-linolenic acid are required in our body in order to synthesize other PUFA (Bentsen, 2017). On the other hand, among MUFAs, oleic acid (range, 3.45% to 20.16%) was found to be most abundant and found highest in *gracilis* (20.16%) muscle. Muscle LTL showed high oleic acid content (32.54%) than the present study (Thomas *et al.,* 2016). Another study, on Duroc (45.33%), Landrace (46.78%) and Yorkshire (46.29%) breeds too showed high oleic acid (Choi *et al.,* 2016). Among SFA's, most abundant fatty acid was palmitic acid (range, 7.47% to11.84%) followed by stearic acid (range, 0.79% to 6.01%) and lauric acid (range, 1.30%to 7.11%), all these were found highest in *tensor fasciae latae* muscle. Compared to our study, stearic

Table 3: Fatty acid and cholesterol composition (% of FAME in 100g) in five muscles of Ghungroo pig.

Parameters	*Triceps brachii*	*Latissimus dorsi*	*Biceps femoris*	*Tensor fasciae latae*	*Gracilis*	Significance (P- value)
SFA	38.29±1.09	25.29±2.16	42.13±3.11	63.62±1.72	53.44±1.82	*0.000
MUFA	35.22±1.73	59.74±1.97	14.64±0.06	27.55±2.12	24.75±1.66	*0.000
PUFA	34.47±1.96	24.96±8.47	6.88±1.00	6.79±1.12	5.97±0.29	*0.001
Oleic acid (18:1 n-9)	12.34±0.49	17.98±0.28	3.45±0.44	13.36±0.33	20.16±0.16	*0.000
ALA (C18:3 n-3)	5.28±0.54	3.19±0.47	1.28±0.04	7.10±0.44	2.86±0.18	*0.000
LA (C18:2 n-6)	4.22±0.37	2.85±0.35	2.82±0.28	2.68±0.28	2.65±0.08	*0.017
Stearic acid (C18:0)	3.90±0.41	0.79±0.46	2.82±0.18	6.01±0.18	1.86±0.19	*0.000
Lauric acid (C12:0)	3.92±0.44	1.30±0.59	3.33±0.44	7.11±0.30	2.22±0.39	*0.000
Palmitic acid (C16:0)	7.47±2.01	11.29±2.34	7.83±1.70	11.84±2.14	10.69±2.24	0.483
Trans fatty acid	12.69±0.17	18.17±0.38	6.75±0.23	13.28±0.49	19.64±0.33	*0.000
PUFA n-6/n-3	0.72±0.07	0.87±0.05	2.54±0.12	0.47±0.06	1.13±0.04	*0.000
PUFA/SFA	0.62±0.12	1.69±0.09	0.33±0.15	0.06±0.02	0.13±0.08	*0.000
Cholesterol	75.92±5.16	63.91±4.70	63.35±4.35	70.06±6.04	70.07±9.32	0.608

The values are expressed in mean ± SEM, SFA= Saturated fatty acid, MUFA= Monounsaturated fatty acid, PUFA= Polyunsaturated fatty acid, ALA = Alpha-linolenic acid, LA= Linoleic acid. *P<0.05.

acid (10.75%) and palmitic acid (22.46%) contents were high on LTLmuscle (Thomas *et al.,* 2016). Similarly, palmitic acid (range, 22.91-23.34%) and stearic acid (range, 12.74-13.78%) content was high on Duroc, landrace and Yorkshire breeds (Choi *et al.,* 2016). Other than these fatty acids, the short chain fatty acid (SCFA) such as propionic acid, acetic acid and butyric acid were too detected in some muscles in small amounts (0.45 to 1.36%). SCFA play an important role in maintaining a healthy body and help in proliferation of microbes (Hussein *et al.,* 2021).

Trans-fatty acid (TFA) was found highest in *gracilis* (19.64%) muscle with an overall mean of 14.10%. Study on LTL (3.48%) muscle showed lower content of TFA than our result (Thomas *et al.,* 2016). The ratio between n-6 and n-3 PUFA is considered vital because of its influence on human health. The n-6 fatty acid *i.e.* LA and the n-3 fatty acids, ALA, EPA and DHA collectively protect against coronary heart disease (Wijendran *et al.,* 2004). In our study, high amount of n-6/ n-3 was found in *biceps femoris* (2.54%) muscle, while other studies showed high PUFA n-6/n-3 content with a mean of 15.98% on LTL muscle (Thomas *et al.,* 2016).

Cholesterol content in five muscles of Ghungroo pig is also shown in Table 3. Highest cholesterol content was found in *Triceps brachii* (75.92%) muscle and lowest in *biceps femoris* (63.35%) muscle. Cholesterol content of 55.9%, 53.1% and 59.7% for *Longissimus dorsi*, *Semi-membranosus* and *Semi-tendinosus* muscles respectively, were found in crossbred pigs of USA (Bohac *et al.,* 1998).These results were considerably lower than our result. Cholesterol is required to our body to synthesize hormones, vitamin-D and digestive fluids.

CONCLUSION

This study contributes to the proximate composition, mineral content, fatty acid and cholesterol content of Ghungroo pig obtained from five porcine muscles. Our results showed high amount of potassium, sodium and magnesium content which can provide adequate amount of recommended daily allowances. SFA's are known to cause various health diseases, mainly related to heart but these SFA fatty acids can be substituted with PUFA n-6 and PUFA n-3. Moreover; it is found that muscle *tensor faciae latae* has the highest content of SFA's. The study establishes information on nutrient composition on Ghungroo pig meat, which can be traded and will be invaluable for nutritionist and dieticians towards improvement of meat quality and value addition for human consumption.

Conflict of interest: None.

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