

Profiles of colour, minerals, amino acids and fatty acids in *Asha*, the triple cross (*Ghungroo x Hampshire x Duroc*) fattener pig variety

R. Thomas*, S. Banik, K. Barman, N.H. Mohan and D.K. Sarma

ICAR-National Research Centre on Pig,
Rani, Guwahati-781 131, Assam, India.

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ABSTRACT

Carcass composition and meat quality were evaluated for *Asha*, a triple cross pig (*Ghungroo x Hampshire x Duroc*, $G_{25}H_{25}D_{50}$), which was developed and released by ICAR-National Research Centre on Pig as a fattener pig variety. A total of 14 gilts and 20 barrows from triple cross pigs were slaughtered at the age of 10 months for evaluating the different parameters. Instrumental colour measurement indicated that the coordinates were in the following range: lightness (L^*), 38.91 – 53.75; redness (a^* , red \pm green), 6.83 – 11.96; and yellowness (b^* , yellow \pm blue), 11.38 – 18.49. The mineral contents in the *M. longissimus thoracis et lumborum* were in the following range: potassium, 268 - 334 mg/100g; zinc, 0.59 - 0.83 mg/100g; copper, 0.04 – 0.1 mg/100g; manganese, 0.01 - 0.06 mg/100g and magnesium, 4.53 - 7.74 mg/100g. Significant ($P>0.05$) differences were not observed in the concentration of any of the estimated amino acids between the sexes. Fatty acid profiling of *M. longissimus thoracis et lumborum* indicated that saturated and unsaturated fatty acids were in the range of 31.14% - 38.65% and 60.27% - 69.49%, respectively. Results further indicated a concentration of 0.91% – 1.70% omega-3 fatty acids; 15.87% - 22.13% omega-6 fatty acids and 15.66% - 24.17% essential fatty acids in the muscle tissues.

Key words: Amino acid profile, Fatty acid profile, Indigenous pig, Mineral contents.

INTRODUCTION

ICAR-National Research Centre on Pig has developed a triple cross pig variety, namely *Asha*, as a fattener pig for releasing into the fattener pig farmers. Pure parental lines of *Hampshire* and *Duroc* (male) and *Ghungroo* (female) pigs, maintained through selective breeding and maintained at the pig breeding farm of ICAR-National Research Centre on Pig were used as exotic and indigenous germplasm, respectively. *Hampshire* and *Ghungroo* pigs were mated to produce F_1 , followed by *inter-se* mating for five generations to stabilize the heterosis effect. Subsequently, selected population was crossed with *Duroc* males to develop a triple cross fattener pig variety (*Ghungroo x Hampshire x Duroc*, $G_{25}H_{25}D_{50}$). The quantity and chemical properties of proteins and lipids in meat are regarded as important factors affecting carcass quality and it has been generally accepted that many factors, such as breed and anatomical location, produce changes in their amount and composition (Lawrie, 1998). The present paper reports the profiles of colour, minerals, amino acids and fatty acids in the *M. longissimus thoracis et lumborum* of the developed triple cross pig variety. A clear understanding of the parameters evaluated in this study are very much important before initiating a mass scale production of this variety for releasing to the farmers filed as a fattener pig. Also, the knowledge obtained in this study had to be transferred to the stakeholders associated with fattener pig farming and pork processing.

MATERIALS AND METHODS

Animals, slaughter, dressing of the carcass, chilling regime and cutting scheme: The experiment was conducted with 34 numbers of *Ghungroo x Hampshire x Duroc* ($G_{25}H_{25}D_{50}$) triple cross pigs (14 gilts and 20 barrows) reared at the Research Farm of ICAR-National Research Centre on Pig. The piglets were creep-fed on a 20% crude protein diet and 13.53 MJ/kg digestible energy; the diet for the weaners contained 18% crude protein, 13.33 MJ/kg digestible energy while the growing pigs were fed on a 16% crude protein diet and 13.53 MJ/kg digestible energy. The pigs were taken for slaughter at their predetermined slaughter age of 10 months, as per the institute's slaughter policy for the indigenous breeds. Pigs were slaughtered in the R&D Pork Processing Plant of the institute (HACCP and ISO 9001:2008 certified, certificate number -1100263; Food Safety Standards Authority of India Licensed, license number - 10312001000151), located at approximately 50 m from the Research Farm. Pigs were electrically stunned (head-only) by low voltage current, shackled on the left leg and exsanguinated in the vertical position on the over head rail. Thereafter, the pigs were scalded at 65°C, followed by hair removal on an automatic dehairing machine. Following slaughter, carcasses were scraped, washed, split, eviscerated and chilled according to standard commercial practices. A block of loin comprising between ribs 8 and 11 was taken from the left side of each carcass, samples were identified

*Corresponding author's e-mail: thomasrlpt@gmail.com

and frozen at $-20 \pm 1^\circ\text{C}$ until analysis of other parameters. Before being measured, samples were thawed at room temperature overnight.

Instrumental colour values: The color of the meat was measured at the 8th and 9th thoracic vertebra at 24 h post mortem using the *Easy Match* software of Hunter Lab (Model-Color Flex, Reston, Virginia, USA) with a 30 mm aperture set for illumination D 65/10° standard observer angle, after exposing the surface to the air for 30 min at 4°C. The instrument was first standardized with black tile followed by white tile. The samples were placed over the viewing aperture and then covered with the lid. The following colour coordinates were determined: lightness (L^*), redness (a^* , red \pm green) and yellowness (b^* , yellow \pm blue). In addition, hue angle, which describes the hue as well as the saturation index or chroma (C^*), which describes the brightness or vividness of colour, were also measured. All the determinations were performed in triplicate.

Heme iron and non-heme iron: The concentration of heme iron was assayed from the total content of heme according to Hornsey (1956). Nonheme iron content was determined by the method of Schricker *et al.* (1982) as modified by Rhee and Ziprin (1987). All the determinations were performed in triplicate.

Mineral composition: Meat samples from *M. longissimus thoracis et lumborum* were trimmed of visible adipose and connective tissue, chopped and dried in oven at 105°C to obtain a constant weight. After that, the samples were ashed in a covered crucible at 550°C in a furnace for 16 h to obtain a white residual ash. The ashes were subjected to an acid digestion process in an Erlen-flask, covered with a micro glass-ball to avoid projections, with 1 M hydrochloric acid and 1 M nitric acid solution heated on a hot plate (AOAC, 2005). Determination of Cu, Zn, Mg, K and Mn were performed by flame atomic absorption spectrophotometer (Model: A 3846, GBC scientific equipments, Australia) following the analytical methods described by AOAC (2005) and Jorhem (2000). All the determinations were performed in duplicate.

Amino acid composition: *M. longissimus thoracis et lumborum* samples were minced using 3 mm hole plate of a meat mincer (Sirman, Italy) and the amino acid composition was measured as per Suzuki *et al.* (1991). For assessing the amino acid composition, 10 g of minced meat was suspended in 2.5 volumes (v/w) of deionized water by homogenizing for 60 s. This suspension was centrifuged at 15000 x g for 15 min, and the supernatant was collected through two layers of gauze. The soluble protein in the supernatant was sedimented by adding the same volume (v/v) of 3% sodium sulphosalicylate. Precipitated protein was removed through filter paper no. 5. The filtrate clarified through the membrane filter (pore size 0.45 μm) was subjected to amino acid analysis on a Shimadzu LC-6A high-performance liquid chromatography

(HPLC) with a system for amino acid analysis composed of a Shim-pack ISC-07/S1504 column (25cm x 4.6mm) and a RF-535 OPA detector (Shimadzu Co., Japan). All the determinations were performed in duplicate.

Fatty acid composition: *M. longissimus thoracis et lumborum* samples were homogenized and 1 g of the sample was extracted with chloroform methanol 2:1(v/v) according to Folch *et al.* (1957) as modified by Salvatori *et al.* (2008). Fatty acid methyl esters (FAMES) were prepared by esterification using methanol in the presence of sulphuric acid (5% of sulphuric acid in methanol). FAMES were analysed in a Hewlett-Packard model HP-5890A gas chromatograph, equipped with a flame ionization detector (FID). They were separated on a semi capillary column (Hewlett-Packard FFAP-TPA fused-silica column, 30 m length, 0.53 mm i.d., and 1.0 mm film thickness). The injector and detector temperatures were held at 230°C and the oven temperature, at 220°C. The carrier gas was nitrogen at a flow rate of 1.8 ml min⁻¹. Identification of FAMES was based on retention times of reference compounds (Sigma, St Louis). Fatty acid composition was expressed as a percentage of the major FAMES. To assess the nutritional implications, the SFA/unsaturated fatty acids (UFA) and the PUFA/SFA ratios were calculated. All the determinations were performed in duplicate.

Statistical analysis: The data collected for different carcass and meat quality parameters were subjected to statistical analysis using SPSS, version 14.0. Mean, standard error of mean (SEM), t-values, minimum (Min) and maximum (Max) values are reported.

RESULTS AND DISCUSSION

Instrumental colour values and mineral composition of *M. longissimus thoracis et lumborum* of triple cross pigs are mentioned in Table 1. Instrumental colour measurement indicated that the coordinates were in the following range: lightness (L^*), 38.91 – 53.75; redness (a^* , red \pm green), 6.83 – 11.96; and yellowness (b^* , yellow \pm blue), 11.38 – 18.49. The L , a^* , and b^* observed were slightly lower than that reported for Ghungroo breed (Thomas *et al.* 2016b). The hue angle, which describes the hue as well as the saturation index and chroma (C^*), which describes the brightness or vividness of colour, were in the range of 54.88 – 66.27 and 15.17 – 22.35, respectively. Among the sexes, gilts had significantly ($P < 0.01$) higher lightness values, while barrows had significantly ($P < 0.01$) higher a^* and chroma values. The differences in redness in the *M. longissimus thoracis et lumborum* show that barrows had a higher concentration of haem pigments. Further investigations on pH, water holding capacity and colour are needed to better understand the interactions between them in triple cross fatter pigs. The results corroborates with the findings of earlier reported works (Radovic *et al.* 2017; Borah *et al.* 2016; Tae Wan Kim *et al.* 2017).

Table 1: Instrumental colour values and mineral composition of *M. longissimus thoracis et lumborum* of triple cross (G₂₅ x H₂₅ x D₅₀) fattener pigs.

Parameter	Gilts (n ₁) Mean ±SEM	Barrows (n ₂) Mean ±SEM	t-value	Total (n ₃) Mean ±SEM	Min.	Max.
Hunter colour values						
L*	47.28±0.39	43.69±0.55	173.81**	44.14±0.46	38.91	53.75
a*	8.37±0.16	10.98±0.30	94.37**	9.19±0.24	6.83	11.96
b*	15.34±0.20	15.87±0.27	4.58	15.49±0.24	11.38	18.49
Chroma	16.54±0.17	18.97±0.23	217.27**	18.03±0.19	15.17	22.35
Hue	58.45±0.20	59.92±0.27	1.48	59.30±0.24	54.88	66.25
Iron and mineral composition						
Total iron (mg/100g)	2.29±0.21	2.93±0.36	61.43**	2.79±0.28	2.13	3.35
Haem iron (mg/100g)	1.37±0.14	1.98±0.29	143.88**	1.78±0.23	0.99	2.24
Non-haem iron (mg/100g)	0.97±0.11	1.10±0.16	8.08	1.01±0.14	0.61	1.47
Potassium (mg/100g)	311±0.65	328±0.78	6.82	320±0.74	268	334
Zinc (mg/100g)	0.73±0.19	0.79±0.28	1.59	0.77±0.25	0.59	0.83
Copper (mg/100g)	0.07±0.19	0.08±0.25	3.60	0.07±0.22	0.04	0.10
Manganese (mg/100g)	0.05±0.06	0.04±0.08	3.00	0.04±0.06	0.01	0.06
Magnesium (mg/100g)	6.20±0.21	6.27±0.17	4.82	6.25±0.19	4.53	7.74

**P<0.01, *P<0.05; n₁=14; n₂=20; n₃=34

The average iron content in the muscle was 2.79 mg/ 100g, of which the contribution of heme iron was 1.78 mg/100g while that of non-heme iron was 1.01 mg/ 100g (Table 1), which was higher than that reported for Ghungroo breed (Thomas *et al.* 2016a). It was also found that iron content was significantly (P<0.01) higher in barrows compared to gilts, and this difference was observed only for haem iron, while non-haem iron content was identical for both gilts and barrows (Table 1). However, Cross *et al.* (2012), who analyzed iron content in different muscles of pork, showed that neither nonhaem iron content nor total haem pigment concentration in muscle tissue was significantly affected by sex. The possible breed and sex differences between the early maturing triple cross fattener pigs used in this study and the Large White castrated male pigs used by those workers could have accounted for the difference in the iron content. Estimated mineral contents in the *M. longissimus thoracis et lumborum* among triple cross gilts and barrows did not differ significantly (P>0.05) (Table 1).

The details of amino acid composition in *M. longissimus thoracis et lumborum* of triple cross pigs are mentioned in Table 2. Significant (P>0.05) differences were not observed in the concentration of any of the estimated amino acids between the sexes, and the present findings were in accordance with the data given by Suzuki *et al.* (1991). Also, as it is the first study of this kind in case of triple cross fattener pig, detailed studies involving muscles other than *M. longissimus thoracis et lumborum* need to be carried out for further validation of the current findings.

Estimation of fatty acid profiles of *M. longissimus thoracis et lumborum* indicated that saturated fatty acids (SFA) and unsaturated fatty acids (UFA) were in the range of 31.14% - 38.65% and 60.27% - 69.49%, respectively, while

the UFA: SFA ratio varied from 1.78 to 1.90 (Table 3). Palmitic acid and stearic acid constituted the major part of saturated fatty acids. Similarly in case of unsaturated fatty acids, mono-unsaturated fatty acids (MUFA) constituted about 38.79% while poly-unsaturated fatty acids (PUFA) accounted for 26.43%. Among MUFA, oleic acid alone constituted for 32.68%, while the major contributors in case of PUFA were linoleic acid (20.18%) and arachidonic acid (4.45%). The quantity and chemical properties of lipids in meat are regarded as important factors affecting carcass quality and it has been generally accepted that many factors, such as sex and anatomical location, produce changes in the amount and composition of meat lipids. The intramuscular fat has some effect on the organoleptic qualities of meat (Kauffman *et al.* 1986; Maw *et al.* 2003). Knowledge of the fat content in the carcass musculature and its composition is important, both for a better understanding of the growth processes and for the nutritional value of meat and its associated quality. It was also observed that the fatty acid profile of the polar lipids was more polyunsaturated in gilts compared to barrows. Even though we did not develop evidence to explain these results, it is reasonable to speculate that the level of intramuscular fat depot could account for the majority of the variations in the percentage of different fatty acid components among the sexes examined in this study. Numerous authors (Fortin *et al.* 2005; Wood *et al.* 2008) have stated that sex may play an important role in meat fatty acid profiles.

The percentage of C 18:1, C 18:2, C 18:3 and C 20:4 were significantly (P<0.05) higher in gilts, while barrows had significantly (P<0.05) higher percentage of C 14:0 and C 18:0 (Table 3). It was also observed that gilts had significantly (P<0.01) higher content of unsaturated fatty

Table 2: Amino acid composition (g/100g) of *M. longissimus thoracis et lumborum* of triple cross (G₂₅ x H₂₅ x D₅₀) fattener pigs.

Parameter	Gilts (n ₁)	Barrows (n ₂)	t-value	Total (n ₃)	Min.	Max.
	Mean ±SEM	Mean ±SEM		Mean ±SEM		
Aspartic acid	1.81±0.11	1.73±0.13	6.53	1.76±0.11	1.07	1.88
Serine	0.77±0.04	0.84±0.02	0.59	0.81±0.03	0.39	1.14
Glutamic acid	3.26±0.13	2.98±0.21	3.65	3.10±0.16	1.77	3.57
Glycine	0.02±0.01	0.02±0.01	1.70	0.02±0.01	0.01	0.04
Histidine	1.72±0.04	1.78±0.05	0.68	1.77±0.05	0.93	2.29
Arginine	2.38±0.07	2.51±0.09	1.80	2.41±0.08	1.57	3.08
Threonine	0.85±0.05	0.88±0.08	4.27	0.87±0.05	0.33	1.07
Alanine	0.57±0.03	0.63±0.03	3.57	0.62±0.03	0.41	0.79
Proline	1.35±0.05	1.22±0.09	8.82	1.29±0.08	0.85	1.62
Cystine	0.19±0.01	0.22±0.01	0.96	0.21±0.01	0.11	0.34
Tyrosine	0.65±0.02	0.76±0.02	9.17	0.72±0.02	0.29	0.93
Valine	0.82±0.03	0.66±0.04	7.81	0.79±0.03	0.31	1.04
Methionine	0.47±0.02	0.54±0.02	5.64	0.51±0.02	0.19	0.86
Lysine	1.61±0.03	1.73±0.04	2.56	1.65±0.04	1.18	2.21
Isoleucine	0.68±0.04	0.72±0.08	4.49	0.71±0.06	0.29	0.93
Leucine	1.51±0.07	1.39±0.09	8.97	1.46±0.07	1.15	2.08
Phenylalanine	0.72±0.02	0.77±0.05	5.83	0.75±0.03	0.28	1.05
Tryptophan	0.32±0.01	0.28±0.01	1.47	0.29±0.01	0.13	0.47

n₁=14; n₂=20; n₃=34**Table 3:** Fatty acid profile (% of total fatty acids) of *M. longissimus thoracis et lumborum* of triple cross (G₂₅ x H₂₅ x D₅₀) fattener pigs.

Parameter	Gilts (n ₁)	Barrows (n ₂)	t-value	Total (n ₃)	Min.	Max.
	Mean ±SEM	Mean ±SEM		Mean ±SEM		
Myristic acid, C 14:0	1.11±0.10	1.46±0.06	19.82*	1.20±0.08	0.68	1.51
Palmitic acid, C 16:0	21.14±0.30	21.47±0.39	4.87	21.31±0.35	18.69	24.02
Palmitoleic acid, C 16:1	2.65±0.17	2.52±0.09	9.18	2.57±0.11	1.81	4.03
Heptadecanoic acid, C17:0	0.22±0.01	0.21±0.01	0.29	0.22±0.01	0.08	0.83
Stearic acid, C 18:0	11.13±0.62	12.36±0.37	17.32*	11.67±0.42	9.87	14.17
Oleic acid, C 18:1	33.79±0.57	31.15±0.63	127.29**	32.68±0.58	18.19	38.69
trans Vaccenic acid, C 18:1, t11	3.63±0.21	3.51±0.32	8.51	3.54±0.28	2.83	4.31
Linoleic acid, C 18:2	21.77±0.38	19.18±0.28	18.41*	20.18±0.36	14.01	26.13
Alpha-linolenic acid, C 18:3	0.50±0.01	0.41±0.01	15.92*	0.45±0.01	0.21	0.77
Gamma-linolenic acid, C 18:3	0.18±0.01	0.14±0.01	2.37	0.15±0.01	0.08	0.28
Arachidic acid, C 20:0	0.36±0.03	0.42±0.02	3.65	0.40±0.03	0.17	0.77
Arachidonic acid, C 20:4	4.78±0.21	4.07±0.28	17.54*	4.45±0.26	1.98	7.63
Eicosa pentanoic acid, C 20:5	0.59±0.04	0.46±0.05	4.38	0.52±0.05	0.13	1.15
Behenic acid, C 22:0	0.36±0.02	0.46±0.03	5.29	0.41±0.02	0.19	0.93
Docosa pentanoic acid, C 22:5	0.36±0.03	0.32±0.05	4.68	0.35±0.03	0.14	0.87
Docosa hexanoic acid, C 22:6	0.34±0.02	0.29±0.05	4.19	0.33±0.04	0.11	1.04
Saturated fatty acids, SFA (%)	33.87±0.33	36.04±0.38	17.98*	35.21±0.35	31.14	38.65
Unsaturated fatty acids, UFA (%)	66.38±0.73	63.47±0.51	57.28**	65.22±0.59	60.27	69.49
UFA/SFA	1.88±0.04	1.76±0.03	6.28	1.85±0.04	1.78	1.90
Mono-unsaturated fatty acids, MUFA (%)	40.09±0.38	38.28±0.59	19.17*	38.79±0.46	33.13	44.08
Poly-unsaturated fatty acids, PUFA (%)	26.97±0.29	25.18±0.22	6.55	26.43±0.26	22.99	29.51
MUFA/SFA	1.12±0.02	1.06±0.02	18.19*	1.10±0.02	1.04	1.13
PUFA/SFA	0.79±0.02	0.68±0.04	5.89	0.75±0.03	0.70	0.78
PUFA n-6	26.79±0.41	23.17±0.52	142.29**	24.78±0.47	19.26	28.83
PUFA n-3	1.35±0.08	1.29±0.05	7.67	1.30±0.07	0.91	1.70
PUFA n-6/ n-3	20.19±0.19	19.46±0.23	8.51	19.06±0.20	15.87	22.13
Essential fatty acids, EFA (%)	21.86±0.47	19.15±0.29	38.73**	20.63±0.36	15.66	24.17

**P<0.01, *P<0.05; n₁=14; n₂=20; n₃=34

acids including essential fatty acids, while barrows had significantly ($P < 0.05$) higher saturated fatty acid contents. The changes in the fatty acid composition of intramuscular lipids are related to the relative changes in concentration among the lipid classes and this is an important factor in the alterations in specific fatty acid concentrations. The higher concentrations of C 18:2 and lower concentrations of C 18:0 in gilts were expected, as several studies have reported similar results (Fortin *et al.* 2005; Wood *et al.* 2008). Similar results have also been reported in other studies in which the level of C18:2 was higher in the meat from female pigs (Leskanich, 1999). We must also consider that the amounts of 18:2 accumulated in the animal tissues depend on the diet because its synthesis in the mammalian body is not possible and, for this reason, the accumulation of 18:2 in the muscle might be related to the amount of dietary linoleic acid (Wood *et al.* 2008). In conclusion, sex influenced fatty acid composition of *M. longissimus thoracis et lumborum* in this study. However, more research is needed to establish whether this difference exists among the other muscles in triple cross fatter pigs. The MUFA: SFA ratio was in the range of 1.04 -1.13, while PUFA: SFA ratio varied from 0.70 – 0.78. Results further indicated that the concentration of omega-3 (n-3) fatty acids in the *M. longissimus thoracis et lumborum* of triple cross pig was in the range of 0.91% – 1.7% (average, 1.3%), while the omega-6 (n-6) fatty acids were accountable for about 24.78% (range, 19.26% - 28.83%). A slightly wider

MUFA: SFA and PUFA: SFA ratios were reported for Ghungroo pig breed (Thomas *et al.* 2016b). It was also found that *M. longissimus thoracis et lumborum* of Ghungroo x Hampshire x Duroc triple cross pigs contain 20.63% (range, 15.66% - 24.17%) essential fatty acids.

CONCLUSION

The results of this study help to understand the profiles of colour, minerals, amino acids and fatty acids with respect to gilts and barrows among *Asha*, the triple cross (Ghungroo x Hampshire x Duroc, $G_{25}H_{25}D_{50}$) pig variety. The extent of differences in some of the observed parameters among the sexes in $G_{25}H_{25}D_{50}$ pigs and its implications for physical characteristics and carcass composition merits further investigation. Further studies are in progress at the institute to evaluate the sensory properties of processed pork products produced from meat of triple cross ($G_{25}H_{25}D_{50}$) pig variety.

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