



## **Phenotype and Genetic Characterization of Four Indigenous Chicken Breeds in Rwanda**

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Kampala, Uganda**ABSTRACT**

This study aimed to enhance the genetic improvement of indigenous chickens in Rwanda through phenotypic and genetic characterization to identify superior populations and recommend best-bet options for their sustainable use. Phenotypic data and blood samples were collected from indigenous chickens domesticated in the four dominant agro-ecological zones of Rwanda. A factorial experimental design was used to examine management systems, phenotypic and genetic traits of production. We then assessed phenotypic traits of 1,080 indigenous chickens and took morphometric measurements and whole blood samples of 120 chickens from twelve districts, to cover all the four indigenous chicken types of Rwanda. Those are Inshenzi (Fast growing), Sekaganda/Inganda (Dwarf ), Imirangi /Umurangi (Naked neck) and Indayi (adapted). The findings showed 23 related genes from the morphometric measures head, wattle, and beak length by using Single Nucleotide Polymorphism markers and 22 nearest genes from blood samples based on the highest genetic potential in terms of indigenous chicken production. Four different genotypes were discriminated by low homozygosity (0.140088 for Imirangi and 0.15327 for Inshenzi) while a low similarity (0.022467) between Imirangi and Inshenzi genetically distanced them from the two others (Inganda and Indayi). Among the four indigenous chicken genotypes in Rwanda, the Imirangi breed (Naked Neck) and the Inshenzi breed (Fast Growing Indigenous Chicken) demonstrated the highest genetic potential concerning key production traits, including egg yield and body weight. In contrast, the Indayi breed (Adapted Indigenous Chicken) and the Inganda breed (Dwarf Indigenous Chicken) exhibited lower genetic potential in these areas. With these findings, the selection of indigenous chickens with high genetic potential for production traits becomes feasible, providing valuable insights that can inform a national breeding program for indigenous chickens in Rwanda and enhance the productivity and sustainability of indigenous chicken populations across the country.

**Keywords:** Native Chickens, Single Nucleotide Polymorphisms, Genes, Traits.

**INTRODUCTION**

Poultry development is one of the most important pathways to pro-poor livestock-induced growth (Shyam et al., 2018) and the industry is growing the fastest of all smallholder livestock systems (Bugchio et al, 2018), particularly in the developing world. The lead benefits to farmers

are in the provision of animal protein, income, and gainful employment throughout the developing world (Bugchio et al., 2018). Though indigenous chickens have several valuable characteristics that are not found in commercial production flocks, they exhibit relatively slow growth and lay fewer and smaller eggs. The IC production system is predominantly free-range, a typical feature of poultry rearing in East Africa and much of the developing world (Dolberg, 2016; Mujiyambere et al., 2022). However, Rwanda is characterized by the coexistence of two production systems: a rudimentary village poultry production system and a commercial poultry production system (Hirwa et al. 2019; Gaspard et al., 2022). However, the latter is still in infancy, the two systems face persistent scarcity of inputs and evidence shows that chickens reared under both do not fully exploit their genetic potential (MINAGRI, 2019). Meat and egg consumption in Rwanda are nevertheless on the rise and current national production volumes do not always meet the demand.

The Rwanda poultry is projected to increase from 6,047,215 MT in the base year to 10,643,098 MT in 2030, showing a 76 percent growth as presented in the 5th Strategic Plan for Agriculture Transformation (IFPRI, 2024). Interventions may include advancements in poultry farming practices, disease control, and market access improvement. The country will have developed the production capacity required to compete with its neighbouring countries such as Uganda, Tanzania, and Kenya, where the poultry industry is more advanced (Cocchini and Steeg, 2019). The priority of rural poultry farmers traditionally is to keep the birds that lay eggs of optimal size and those that grow to optimum body weight with the quality of the offspring satisfying special morphological features of the chickens (Ogah, 2011; Munyaneza et al., 2021). The supply of chicken meat in urban areas relies on the importation of exotic breeds but this solves the production challenges in the short and medium term. The use of such stock to drive genetic improvement on farms (FAO, 2019; Munyaneza et al., 2021) through breed substitution and crossbreeding is not harmonious with the Global Plan of Action for the sustainable utilization of Animal Genetic Resources (FAO, 2007). These endeavours such as breed substitution have proven to be unsustainable due to consequential overdependence on external genetic materials (Alemayehu and Solomon, 2018). Genetic improvement through selection is a long-term, tedious, and costly undertaking. However, the application of Marker-Assisted Selection (MAS) considerably reduces the duration and cost of genetic improvement (Bahmanimehr, 2012).

African livestock species and breeds will be endangered in time if not well-differentiated and conserved; and may even get lost eventually (Liyanage, 2015; Hirwa et al., 2019). In particular, avian breeds are at an extinction risk of up to 30% (Semik and Krawczyk, 2011; Munyaneza et al., 2021). As would be expected, species that have less genetic variation are indeed at greater risk (Laikre, 2010). ICs have distinct physical variations for both qualitative and quantitative traits, and under intensive management, the conditions require the development of good breeding programs for rearing IC chickens (Khawaja et al., 2013). This study IC contributes to the sustainable growth of the poultry sector in Rwanda by developing market-driven solutions to incentivize indigenous chicken producers, in line with the recommendations of the Global Plan for the Sustainable utilization of farm animal genetic resources of the Food and Agriculture Organization of the United Nations (FAO, 2007; FAO, 2022). Also, the genetic improvement of IC is expected to increase access to better chicken genotypes for eggs and meat, and will

positively contribute to the conservation of indigenous animal genetic resources through direct farmer utilization.

ICs in Rwanda have shown the lowest genetic diversity between the Northern-Western and Central-Northern area and the highest genetic diversity between Eastern and Southern-Western parts of the country (Habimana et al., 2020; Munyaneza et al., 2021). Farmers in Rwanda categorize indigenous chickens into four types: Inshenzi, Sekaganda/Inganda, Imirangi/Umurangi, and Indayi, although the phenotypic and molecular variation among them is unknown. This study, therefore, aimed to identify the characteristics of the four indigenous chicken types and the preferred traits by their farmers to enable long-term exploitation of their genetic potential.

## MATERIALS AND METHODS

### Ethical Approval

This study was conducted in line with the principles of the Declaration of Helsinki. Approval was granted by the Research Screening and Ethical Clearance Committee of the College of Agriculture, Animal Sciences and Veterinary Medicine, University of Rwanda (Ref: 031/19/DRI September 2, 2019).

### Study Area

The study was carried out in four agro-ecological zones of Rwanda, each zone corresponding to a province of the country, over the period of July 2019 to June 2022. From each zone three districts were randomly selected, considering as much distance from the big market and cities as possible to reduce the possibility of sampling birds non-indigenous to the area. We took a total of twelve districts from the four zones. These were Bugesera, Rwamagana, and Nyagatare in the Eastern zone; Kamonyi, Nyamagabe, and Nyanza in the Southern zone; Gakenke, Musanze, and Rulindo in the Northern zone; then Ngororero, Nyamasheke and Rubavu in the Western zone (Figure 1).

### Phenotypic Characterization of Chickens in Four Agro-Ecologies of Rwanda

#### Sample Size Determination:

To characterize the production system, a field survey was done in a total of 1,080 IC rearing households calculated using a simplified process of determining the sample size for a finite population (Krejcie and Morgan, 1970). Based on this method, the sample size was determined as follow:

$$N = \sum_{i=1}^{n=12} (2n) (3c)(3v)(5IC)$$

Where:

N: Sample size; i: Number of selected Districts;

n: Number of selected Sectors per District;

c: Number of selected Cells per Sector;

v: Number of selected villages per cell; and

IC: Number of indigenous chickens selected per village

**Figure 1: Districts sampled in four agro-ecological zones in Rwanda**

**DNA Isolation and Genotyping:**

This research endeavour took whole blood samples from 120 ICs sampled from twelve districts of the Republic of Rwanda. From each chicken, two millilitres of blood was drawn from the pectoral vein into a tube with EDTA 0.5M. The tubes with blood were kept in a portable, insulated cool box with dry ice and transported to the laboratory at the Rwanda Agriculture and Animal Resources Development Board (RAB) Rubirizi Station, where they were stored at  $-80^{\circ}\text{C}$  until DNA extraction and amplification took place. DNA was extracted from blood samples using a Promega genomic DNA extraction kit following the protocol of the ReliaPrep™ Blood gDNA Miniprep System. Nanodrop and gel electrophoresis were used for DNA quantification and quality control respectively. The 40  $\mu\text{l}$  of a 50 ng/ $\mu\text{l}$  DNA of each sample was sent to Diversity Arrays Technology (DArT) Pty Ltd, Australia (<http://www.diversityarrays.com/dart-map-sequences>) for a whole genome scan using Diversity Arrays Technology (DArT) markers to obtain the raw reads.

**Reads Alignment and SNP Calling:**

The raw reads underwent trimming using sickle (Joshi and Fass, 2011) and were aligned to Galgal6 Chicken Reference Genome using the Burrows-Wheeler Alignment tool (BWA v0.7.17; Li et al., 2008). Duplicated reads were removed using the Picard package and SNP calling was conducted using SAMtools v1.3.1 (Li et al., 2009). The resultant SNPs underwent filtering based on the following criteria in Plink v1.07 software (Purcell et al., 2007): minimum SNP quality of 20, 5% missing SNP genotypes, Hardy-Weinberg equilibrium ( $p < 10^{-6}$ ), call rate  $>95\%$ , heterozygosity  $>0.4$ , and minor allele frequency  $>0.05$ . Genotype imputation (Marchini and Howie, 2010) was carried out to enhance the power of genome-wide analysis. Missing genotypes were imputed using the LD KNNi imputation method in Tassel 5.2.60 (Bradbury et al., 2007). Pairwise linkage disequilibrium (LD) for each chromosome was estimated using Tassel 5.2.60 (Bradbury et al., 2007). Autosomal SNPs were pruned based on the independent pairwise parameters described by Wang et al. (2009), resulting in 65,945 independent SNP markers.

**Genetic Parameters:**

Genetic parameters such as genetic diversity, heterozygosity (observed and expected), genetic differentiation and genetic distance were computed using the R package snpReady (Granato et al., 2018). A phylogenetic relationship was made in R using dartR (Swafford, 2003 & Zhu, 2018).

**Genome-wide Association Assessment:**

GWAS was conducted using the statistical genetics package Genome Association and Prediction Integrated Tool (GAPIT). The SNPTEST and PLINK software for the statistical mixed linear model was used to perform GWAS. The family-wise error rate was controlled by using a Bonferroni correction. Based on the estimated number of independent SNP markers, the threshold p-value of the 5% Bonferroni genome-wide significance was computed.

**STATISTICAL ANALYSIS**

The qualitative parameters were analysed by physical observation, using descriptive statistics procedure of the Statistical Package for Social Sciences (SPSS, 2004). The genotypic frequency

and P-values were estimated and calculated. The following model was fitted for the association of each SNP genotype:

$$Y_{ij} = Xb + Za + Zc + e$$

Where:

- Y = represented the dependent variable (growth performance and egg traits);
- b = vector of fixed effects of sex and hatch number;
- a = vector of random direct genetic effects;
- c = vector of random common environmental effects;
- e = vector of residual effects; and,
- X, Za and Zc are incidence matrices relating records to fixed, direct genetic and common environmental effects, respectively (Gilmour et al. 2006). The tree plot was made in R using dartR (Swafford, 2003 & Zhu, 2028).

## RESULTS

Phenotypic characterisation of local chickens was done in all provinces of Rwanda. Four types of ICs were identified: Umurangi, Inshenzi, Sekaganda (Inganda), and Indayi (Plates 1, 2, 3, 4). There was a nonsignificant ( $p > 0.05$ ) difference in the number of eggs per clutch (13-18), number of egg clutches per year (3-4), age of females at maturity (6-9 months), annual egg production (38-72), and hatching rate (80-89%), between the four studied chicken ecotypes. Similarly, cocks did not differ in age at sexual maturity by four types.



**Plate 1: Umurangi type of local chicken**



**Plate 2: Inshenzi type of local chicken**





**Plate 3: Indayi type of local chicken**



**Plate 4: Sekaganda (Inganda) type of local chicken**

Plumage patterns and comb structures of indigenous chickens across four agroecological zones are shown in Table 1. The uniform and coronation plumage patterns were the most prominent (24.7%) of all the patterns, followed by silver penciled (12.9%), partridge (10.8%), speckled (8.6%), naked neck and mottled (7.5%), with rumpless (2.2%) and barred (1.1%) being the least common. Inshenzi had the most dominant (60.2%) of all plumage patterns followed by Sekaganda (19.4%), Indayi (15.1%), and Umurangi (4.3%). Erect comb structure was the most dominant in the studied chickens (88.9%) compared to droopy comb which was only recorded in 11.1%. Inshenzi had the most dominant comb in all comb structures (60.0%) followed by Sekaganda, Indayi, and Umurangi with 21.1%, 14.4%, and 4.4 respectively.

**Table 1: Plumage patterns and structure of comb.**

		Sekaganda / (Inganda) (%)	Indayi (%)	Umurangi (%)	Inshenzi (%)	
	Mottled	1.1	2.2	1.1	3.2	7.5
	Silver penciled	3.2	1.1	0	8.6	12.9
	Speckled	1.1	1.1	1.1	5.4	8.6
	Partridge	4.3	3.2	0	3.2	10.8
	Uniform	5.4	3.2	0	16.1	24.7
	Coronation	3.2	2.2	0	18.3	24.7
	Barred	1.1	0	0	0	1.1
	Rumpless	0	1.1	0	1.1	2.2
	Naked neck	0	1.1	2.2	4.3	7.5
	Total	19.4	15.1	4.3	60.2	100.0

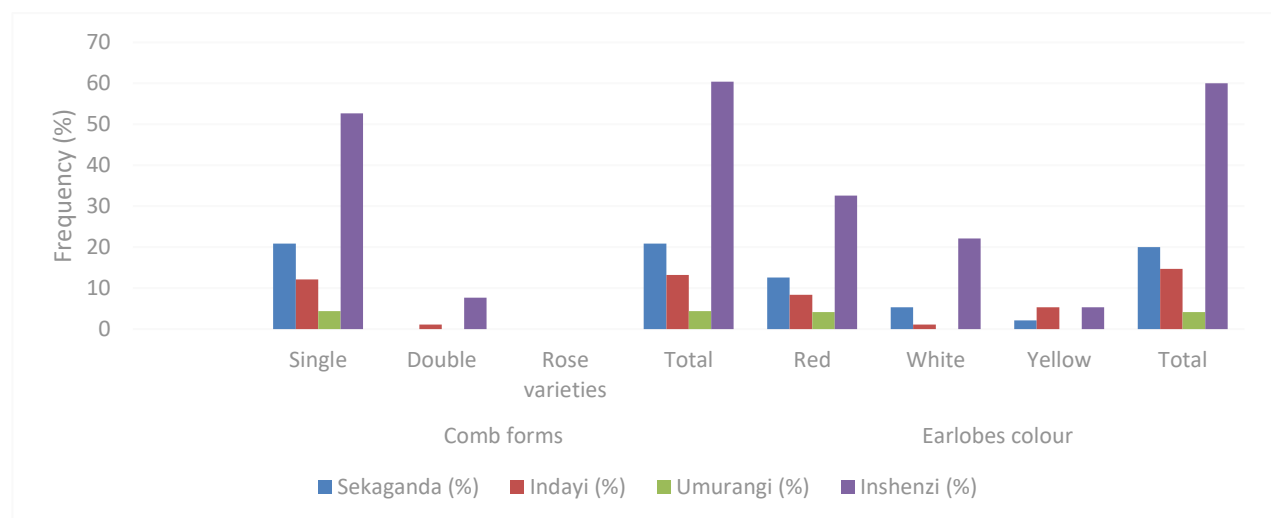


	Erect	20.0	14.4	3.3	51.1	88.9
	Droopy	1.1	0	1.1	8.9	11.1
	Total	21.1	14.4	4.4	60.0	100.0

Comb forms and earlobe colours of ICs in Rwanda are shown in Table 2.

**Table 2: Comb forms and earlobes colour.**

Traits	Levels	Type of local chicken kept				Total (%)
		Sekaganda (Inganda) (%)	Indayi (%)	Umurangi/Umurangi (%)	Inshenzi (%)	
Comb forms	Single	20.9	12.1	4.4	52.7	90.1
	Double	0.0	1.1	0.0	7.7	8.8
	Rose varieties	0.0	0.0	0.0	0.0	1.1
	Total	20.9	13.2	4.4	60.4	100.0
Earlobes colour	Red	12.6	8.4	4.2	32.6	58.9
	White	5.3	1.1	0.0	22.1	28.4
	Yellow	2.1	5.3	0.0	5.3	12.6
	Total	20.0	14.7	4.2	60.0	100.0



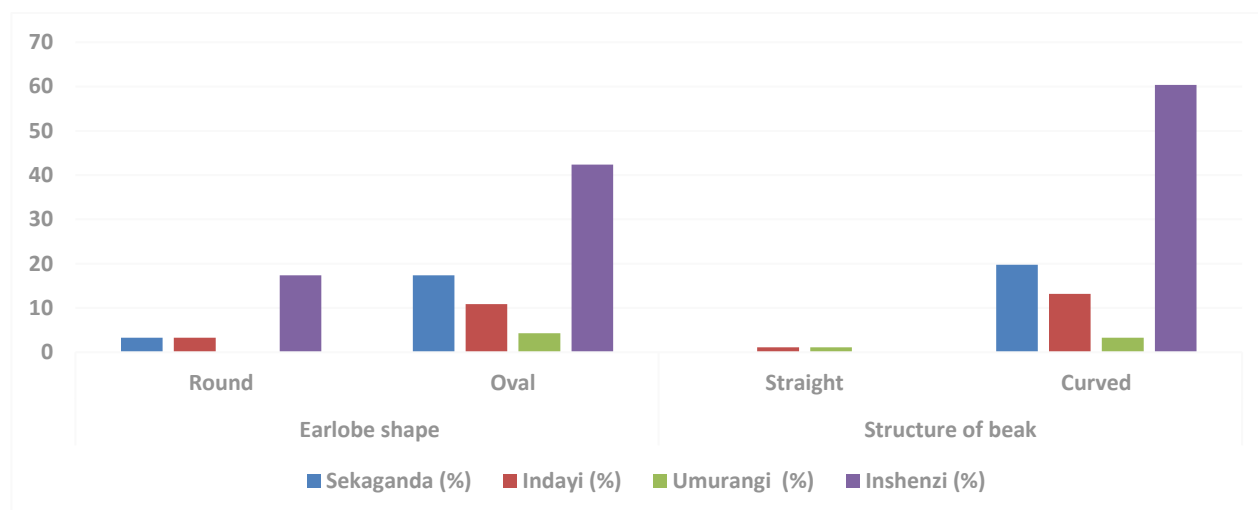
**Figure 2: Comb forms and Earlobe colour**

Earlobe shapes and beak structures presence in the local chickens in Rwanda are presented in Table 3. Inshenzi was the most observed in all earlobe shapes (59.8%), followed by Sekaganda, Indayi, and Umurangi with 14.7%, and 4.3% respectively. The curved beak was the most dominant (97.8%), compared to the straight beak (2.2%). Inshenzi had the highest beak structure observed in all beak structures (60.4%), followed by Inganda, Indayi, and Umurangi (19.8%, 14.3%, and 4.4% respectively).

**Table 3: Earlobe shape and beak structure**

Trait	Level	Type of the chickens kept				Total (%)
		Sekaganda (%)	Indayi (%)	Umurangi (%)	Inshenzi (%)	
Earlobe shape	Round	3.3	3.3	0.0	17.4	23.9

	Oval	17.4	10.9	4.3	42.4	76.1
	Total	20.7	14.1	4.3	59.8	100.0
Structure of beak	Straight	0.0	1.1	1.1	0.0	2.2
	Curve	19.8	13.2	3.3	60.4	97.8
	Total	19.8	14.3	4.4	60.4	100.0



**Figure 3: Earlobe shape and beak structure.**

Beak colour and feather structure of ICs across four agro-ecological zones are shown in Table 4. Brown beak had the highest number of observations (36.7%), followed by black, yellow and white (28.4%, 27.8% and 6.7% respectively). Inshenzi was the most prominent in all beak colours (61.1%), followed by Sekaganda, Indayi and Umurangi (18.9%, 14.4% and 4.4% respectively). Inshenzi had the most prominent feather in smooth feather structure (60.4%), followed by Inganda, Indayi, and Umurangi (19.8%, 14.3% and 4.4% respectively).

**Table 4: Beak colour and feather structure.**

Trait	Level	Type of local chicken kept				Total
		Sekaganda (%)	Indayi (%)	Umurangi (%)	Inshenzi (%)	
Color of beak	Yellow	4.4	3.3	2.2	16.7	27.8
	Black	7.8	3.3	1.1	16.7	28.9
	Brown	6.7	6.7	1.1	22.2	36.7
	White	0.0	1.1	0.0	5.6	6.7
	Total	18.9	14.4	4.4	61.1	100.0
Feather structure	Smooth	19.8	14.3	4.4	60.4	100.0
	Total	19.8	14.3	4.4	60.4	100.0

Eye and shank colours of IC in Rwanda are presented in Table 5. Pink eye was the most dominant (89.2%), followed by yellow (8.6%) and brown (2.2%). Inshenzi had the highest observed eye color in all eye colours (61.3%) followed by Sekaganda (18.3%), Indayi (15.1%), and Umurangi (4.3%). Yellow shank had the highest level of dominance (46.8%) followed by white, black, greenish, pink and still blue, 35.1%, 9.6%, 6.4%, and 1.1% respectively.

**Table 5: Eye and shank colours.**

Trait	Level	Type of local chicken kept				All
		Sekaganda (%)	Indayi (%)	Umurangi (%)	Inshenzi (%)	
Eye colour	Pink	16.1	14.0	4.3	53.8	89.2
	Yellow	2.2	1.1	0.0	5.4	8.6
	Brown	0.0	0	0.0	2.2	2.2
	Total	18.3	15.1	4.3	61.3	100.0
Shank colour	Pink	0.0	0.0	0.0	1.1	1.1
	White	5.3	5.3	1.1	23.4	35.1
	Yellow	8.5	8.5	3.2	25.5	46.8
	Still blue	0.0	0.0	0.0	1.1	1.1
	Greenish	2.1	0.0	0.0	4.3	6.4
	Black	3.2	1.1	0.0	5.3	9.6
	Total	19.1	14.9	4.3	60.6	100.0

The average of the different morphological and production parameters of indigenous chickens in Rwanda is shown in Table 6. The total body weight of the various IC types was not significantly ( $p > 0.05$ ) different and gave an overall mean of  $1.58 \pm 0.24$  kg. Umurangi has the highest body weight with  $1.771 \pm 0.11$  kg, followed by Indayi, Inshenzi and Sekaganda ( $1.66 \pm 0.45$  kg,  $1.59 \pm 0.005$  kg and  $1.44 \pm 0.39$  kg respectively). The average crest height of local chickens was not significantly ( $p > 0.05$ ) different with  $1.49 \pm 0.09$  cm. Indayi has the highest crest height, with  $1.59 \pm 0.06$  cm followed by Sekaganda, Inshenzi, and Umurangi ( $1.53 \pm 0.23$  cm,  $1.48 \pm 0.05$  cm and  $1.43 \pm 0.02$  cm respectively).

**Table 6: Average Means  $\pm$ SE of different morphological and production parameters in mature indigenous chickens of Rwanda.**

		Sekaganda (Inganda) (n = 30)	Indayi (n = 30)	Umurangi (n = 30)	Inshenzi (n = 30)	Total (n = 120)	P Value
Morphological parameters	Body Weight (kg)	$1.43 \pm 0.39$	$1.66 \pm 0.45$	$1.77 \pm 0.11$	$1.59 \pm 0.005$	$1.58 \pm 0.24$	0.752
	Crest Height (cm)	$1.53 \pm 0.23$	$1.59 \pm 0.06$	$1.45 \pm 0.02$	$1.48 \pm 0.05$	$1.49 \pm 0.09$	0.903
	Crest Length (cm)	$3.71 \pm 0.16$	$4.07 \pm 0.37$	$3.75 \pm 0.15$	$3.20 \pm 0.23$	$3.43 \pm 0.23$	0.178
	Head Length (cm)	$6.42 \pm 0.09$	$6.61 \pm 0.02$	$6.75 \pm 0.1$	$6.61 \pm 0.02$	$6.58 \pm 0.06$	0.911
	Beak Length	$2.86 \pm 0.02$	$2.88 \pm 0.01$	$3.13 \pm 0.14$	$2.89 \pm 0.00$	$2.89 \pm 0.07$	0.963
	Wattle Length (cm)	$1.92 \pm 0.02$	$2.03 \pm 0.08$	$2.13 \pm 0.14$	$1.83 \pm 0.03$	$1.89 \pm 0.07$	0.988
	Neck Length (cm)	$9.87 \pm 0.06$	$9.93 \pm 0.02$	$10.25 \pm 0.16$	$9.97 \pm 0.00$	$9.97 \pm 0.06$	0.979
	Diameter of Thorax (cm)	$9.71 \pm 0.22$	$10.25 \pm 0.09$	$7.75 \pm 1.35$	$10.35 \pm 0.15$	$10.09 \pm 0.45$	0.412
	Body Length (cm)	$19.18 \pm 0.65$	$21.37 \pm 0.61$	$18.63 \pm 0.97$	$20.56 \pm 0.14$	$20.31 \pm 0.59$	0.640
	Diameter of Tarsus (cm)	$3.19 \pm 0.15$	$3.50 \pm 0.02$	$4.38 \pm 0.53$	$3.49 \pm 0.02$	$3.46 \pm 0.18$	0.021
	Length of Tarsus (cm)	$11.37 \pm 0.12$	$10.00 \pm 0.67$	$11.50 \pm 0.19$	$11.37 \pm 0.12$	$11.16 \pm 0.27$	0.170
	Diameter of Shank (cm)	$1.24 \pm 0.07$	$1.48 \pm 0.64$	$1.13 \pm 0.13$	$1.38 \pm 0.01$	$1.36 \pm 0.21$	0.219
	Shank Length (cm)	$7.07 \pm 0.12$	$7.50 \pm 0.13$	$7.25 \pm 0.02$	$7.26 \pm 0.01$	$7.28 \pm 0.07$	0.434
	Wing Length (cm)	$13.25 \pm 0.33$	$14.60 \pm 0.45$	$13.25 \pm 0.33$	$13.90 \pm 0.04$	$13.82 \pm 0.29$	0.624
Production parameters	Wingspan (cm)	$52.05 \pm 0.18$	$54.64 \pm 1.331$	$34.75 \pm 10.17$	$53.03 \pm 0.38$	$52.37 \pm 3.01$	0.092
	Age at sexual maturity – females (months)	$6.25 \pm 0.18$	$6.00 \pm 0.33$	$9.00 \pm 1.40$	$6.64 \pm 0.04$	$6.57 \pm 0.48$	0.291
	Age at point of lay (months)	$7.00 \pm 0.34$	$6.00 \pm 0.92$	$18.00 \pm 6.00$	$7.29 \pm 0.18$	$7.60 \pm 1.86$	0.000

	Age at sexual maturity – males (months)	6.00±0.57	7.00±0.00	7.67±0.38	7.33±0.19	7.00±0.19	0.333
	Number of eggs laid/clutch	12.50±0.74	13.38±0.23	18.00±1.18	14.25±0.94	13.79±0.77	0.777
	Number of clutches per year	3.33±0.11	3.17±0.20	4.00±0.27	3.68±0.09	3.52±0.16	0.953
	Annual egg production	42.17±2.24	38.29±4.48	72.00±14.97	49.50±1.98	46.06±5.91	0.382
	Hatching rate (%)	82.00±0.17	88.71±3.7	99.00±9.64	79.81±2.06	82.30±3.89	0.135
	The hatchability under natural incubation	82.00±0.51	87.00±3.40	99.00±10.32	78.53±1.48	81.11±3.92	0.138

### Genetic Characterisation

The SNPs related to various beak lengths of ICs across four agroecological zones are presented in Table 7. The related gene STX18 was significantly different ( $p < 0.05$ ) from the rest, as it had a real position of 78612470 and was located on the 4th chromosome, with a relationship to 100044766.36.A.G marker, a related gene responsible for disease resistance.

Another related gene RFX6 was significantly different ( $p < 0.05$ ) from the rest, had a real position of 63678051 and was located on the 3rd chromosome with a relationship to 100039945.53.T.C marker. This related gene has a crucial role in egg production and reproductive performance.

**Table 7: Single Nucleotide Polymorphisms related to beak length (BL)**

SNP	Chr	Pos	P	Maf	nobs	R2 of Model Without SNP	R2 of Model With SNP	FDR Adjusted P-values	e	Related genes	Locus
100044766.36.A.G	4	78612470	4.57E-09	0.014851	101	0.145055	0.514037	7.09E-05	2.908033	STX18	within
100039945.53.T.C	3	63678051	2.86E-07	0.00495	101	0.145055	0.41568	0.001479	3.959038	RFX6	U31820
100041210.14.C.A	3	66408840	2.86E-07	0.00495	101	0.145055	0.41568	0.001479	3.959038	CDK19	within
100099665.64.T.C	3	56158908	1.31E-06	0.019802	101	0.145055	0.381648	0.002586	1.908161	SGK1	U46627
100045868.7.C.T	3	53903475	1.33E-06	0.00495	101	0.145055	0.38134	0.002586	3.865256	CITED2	U156192
100049572.56.A.G	2	73032184	1.33E-06	0.00495	101	0.145055	0.38134	0.002586	3.865256	ENSGALG00000012941	within
100080273.27.A.G	1	89772462	1.33E-06	0.00495	101	0.145055	0.38134	0.002586	3.865256	PHLDB2	within
100136093.24.G.C	3	2281	1.33E-06	0.00495	101	0.145055	0.38134	0.002586	-3.86526	ENSGALG000000049712	within
100081949.68.C.T	1	79874338	1.71E-06	0.029703	101	0.145055	0.375924	0.002945	1.608404	ENSGALG000000053660	within

Chr – Chromosome, Pos – Position, P – p Value, Maf – Minor allele frequency

The related gene ENSGALG000000053660 was significantly different ( $p < 0.05$ ) from the other studied genes. This had a real position of 79874338 and was located on the 1st chromosome with a relationship to 100081949.68.C.T marker that is known to intervene in egg-laying at the optimum level.

The SNP related to the wattle length of indigenous chickens across four agroecological zones are presented in Table 8. The wattle length related gene ADPRH was significantly different ( $p < 0.05$ ). It had a real position of 93661553 and was located on the 1st chromosome, with a relationship to 100045966.25.C.G markers.

**Table 8: SNP related to wattle length (WL)**

SNP	Chr	Pos	P	maf	no bs	R.withou t.SNP	R.with.S NP	FDR Adjusted P-values	effect	Related genes	Locus
100045966.25.C.G	1	93661553	4.59E-10	0.005	100	0.075962	0.541221	1.78E-06	11.95175	ADPRH	within
100054325.62.A.G	4	25655729	4.59E-10	0.005	100	0.075962	0.541221	1.78E-06	11.95175	ENSGALG000000050573	D13975
100095277.27.A.G	8	15870065	4.59E-10	0.005	100	0.075962	0.541221	1.78E-06	11.95175	ENSGALG000000050989	D313

100153470.22.T.C	5	57592228	4.59E-10	0.005	100	0.075962	0.541221	1.78E-06	11.95175	ENSGALG00000046839	within
100028819.26.G.C	18	4094235	5.53E-08	0.01	100	0.075962	0.411569	0.000172	7.541455	SEC14L1	within
100162872.64.C.T	5	48728971	2.97E-07	0.01	100	0.075962	0.36926	0.000769	6.75128	SLC25A29	U1270
100165698.21.G.T	1	2222	7.43E-07	0.01	100	0.075962	0.346841	0.001649	6.677714	ENSGALG00000054818	U3051
100050457.18.G.A	2	66369841	2.56E-06	0.01	100	0.075962	0.317332	0.004975	6.130294	NQ02	D4300

The SNPs related to the head length of indigenous chickens in Rwanda are presented in Table 9. The related gene EPS15 was significantly different ( $p < 0.05$ ) from the rest, similar to the previous ones reported. It had a real position of 24427568 and was located on the 8th chromosome, with a relationship to 100045814.61. C. A marker. This related gene played a significant role in growth and productivity.

Another related gene AUTS2 found was also significantly different ( $p < 0.05$ ) from the other genes underscored. It had a real position of 2115462 and was located on the chromosome 19, with a relationship to 100086967.33.C.T marker and is responsible for growth rate in chickens. In addition, related gene PRR5 was found to be significantly different ( $p < 0.05$ ) from others as well. It had a real position of 69759741 and was located on the chromosome 1, with a relationship to 100099368.67.T.C markers.

**Table 9: SNP related to head length (HL)**

SNP	Chr	Pos	P	maf	nobs	R.without .SNP	R.with.SN P	FDR_Adjust ed_P-values	Effect	Related genes	Locus
100045814.61.C.A	8	24427568	1.66E-06	0.004902	102	0.121884	0.359961	0.004295	-5.38639	EPS15	within
100086967.33.C.T	19	2115462	1.66E-06	0.004902	102	0.121884	0.359961	0.004295	-5.38639	AUTS2	within
100099368.67.T.C	1	69759741	1.66E-06	0.004902	102	0.121884	0.359961	0.004295	-5.38639	PRR5	within
100128859.59.C.A	4	53378062	1.66E-06	0.004902	102	0.121884	0.359961	0.004295	-5.38639	SPATA5	U3672
100097113.59.T.C	3	3082972	1.66E-06	0.004902	102	0.121884	0.359961	0.004295	5.386392	NPHP1	within

The nearest genes related to the identified SNP of indigenous chickens in Rwanda are presented in Table 10. The nearest gene LOC776067, CDC16 had a real position of 137692275 and was located at NC\_006088.4 on the 1st chromosome, with a relationship to T marker and is responsible for meat quality and quantity. The nearest gene DHRSX, CD99, LOC107051960 had a real position of 129958897 and was located at NC\_006088.4 on the 1st chromosome, with a relationship to the C marker, and this one has a role in meat quality. The nearest gene LOC101750813, MX1 had a real position of 101256943 and was located at NC\_00 6088.4 on the 1st chromosome, with a relationship to the T marker and plays a role in viral disease resistance.

**Table 10: The nearest genes related to identified SNPs.**

Locus	CHR	Real position	Nearest gene	Marker
NC_006088.4	1	137692275	LOC776067	T
			CDC16	
NC_006088.4	1	129958897	DHRSX, CD99, LOC107051960	C
NC_006088.4	1	101256943	LOC101750813, MX1	T
NC_006088.4	1	50383558	LOC107053692,	A
			GRAP2	
NC_006089.4	2	61140781	JARID2, PRL	C
			LOC10751657	
NC_006089.4	2	119443614	CALB1	T
			mRNA: XM_0049399117.3	
NC_006089.4	2	93149588	ADCYAP1, CDH2	C

NC_006091.4	4	51805394	ALB, IL8L2	A
NT_461106.1	7	8453	LOC10756603	G
NC_006095.4	8	4304485	C8H14ORF80	C
NC_006095.4	8	4304485	LOC10751673	A
			CDS:XP_015149132.1	
NC_006100.4	13	8272097	GABRB2	A
			CDS:XP_015149132.1	
NC_006100.4	13	16352162	LECT2	A
			CDS:XP_015149132.1	
NC_006104.4	17	8458226	NOTCH	C
			UBC1	
			CDS:XP_0153010.1	
NC_006106.4	19	3336281	SPNS3	A
			CDS: XP_015151456.1	
			mRNA: 015295970.2	
NC_006113.4	26	2497963	RASSF5	T
			XP_015151456.1	
			MRna:0155298926.2	
NC_006095.4	8	5672840	PBX1,	T
NC_006095.4	8	5672840	RXRG, FMO3, GLUL	T
NC_006098.4	11	10145725	LOC107054293,	T
			CEBPA,	
NC_006098.4	11	15831341	LOC107054316,	T
			BCO1, CDH13	
NC_006106.4	19	8356215	DHRS11	A
			LHX1	
			ACACA	
NC_006091.4	4	51805394	ALB, IL8L2, FGF2, ANXAS	A

The homozygosity ( $H_o$ ) of indigenous chickens in Rwanda is presented in Table 11. This current study showed that Umurangi was the lowest in homozygosity (0.140088), followed by Inshenzi, Inganda, and Indayi (0.15327, 0.154654, and 0.158944 respectively) as reflected in Table 11.

**Table 11: The homozygosity of indigenous chickens in Rwanda**

Generation	Inganda	Indayi	Inshenzi	Umurangi
0	189924	188564	570849	83931
1	38431	39497	114261	15230
2	20141	20435	60378	9556
$H_o$	0.154654	0.158944	0.15327	0.140088

Genetic distance of indigenous chickens in Rwanda is shown in Table 12 and Figure 2. This present study of the genetic structure based on phylogenetic tree from the breeds is the lowest homozygosity compared to each breed indicated that between Umurangi and Inshenzi was the lowest genetic distance (0.022467), followed by Umurangi and Inganda (0.02471 each) and

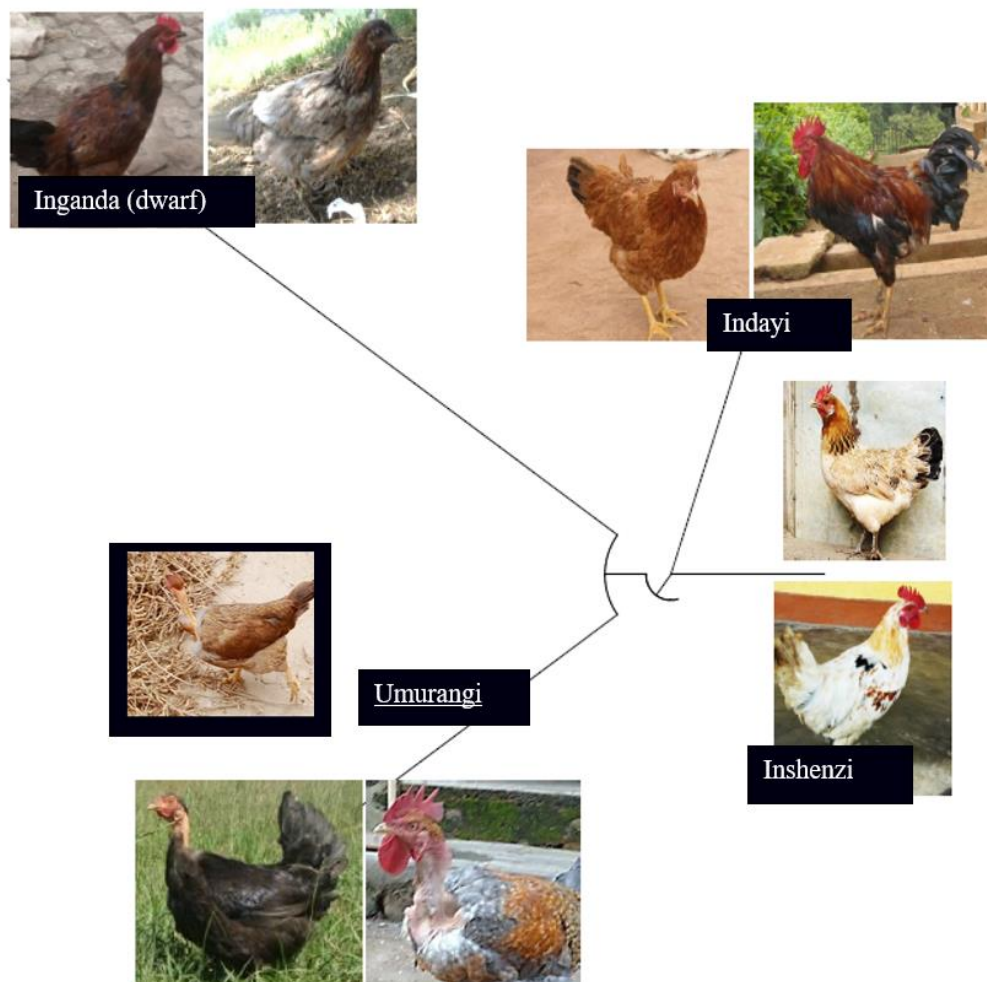


between Umurangi and Indayi (0.031528) as reflected in Table 12. So, the lowest genetic distance between breeds means minimal loss of genetic materials.

**Table 12: Genetic distance of indigenous chickens.**

Fst	Inganda	Indayi	Inshenzi	Umurangi
Inganda	1	-	-	-
Indayi	0.00684	1	-	-
Inshenzi	0.002248	0.009087	1	-
Umurangi	0.02471	0.031528	0.022467	1

Note: Empty space in this above table means not applicable for that chicken.



**Figure 2: Phylogenetic tree of indigenous chickens.**

## DISCUSSION

Characterisation of Indigenous Chickens (ICs) was done in all provinces of Rwanda. Four types of indigenous chicken were identified. These were Umurangi (Umurangi), Inshenzi, Sekaganda (Inganda) and Indayi. All types of ICs were found to have similar female age at sexual maturity (6.6 months); similarly, all males were found to reach sexual maturity at about 7 months. There

was variation in age at sexual maturity of the male and the female ICs (Isidahomen, 2012; Hirwa et al., 2019). There was no significant difference, however, in a number of eggs per clutch (13-18) for each type of IC. The same result was reported in Central Tigray where the average number of clutches per year was 3.15 to 3.2 and 3.2 in the lowland and midland agro-ecological zones respectively (Alem, 2014) while age at sexual maturity of female ICs was 6.9 months.

The overall mean neck length in the indigenous chickens kept in the various agro-ecologies of Rwanda was not significantly different ( $p > 0.05$ ). Results showed that overall mean neck length was  $8.9 \pm 0.50$  cm, similar to findings of Odah et al (2019). Umurangi chickens had the longest necks ( $10.25 \pm 0.16$  cm), followed by Inshenzi, Indayi and Sekaganda ( $9.97 \pm 0.00$  cm,  $9.93 \pm 0.02$  cm and  $9.87 \pm 0.06$  cm respectively). The total diameter of the thorax in ICs was not significantly different ( $p > 0.05$ ), with  $10.09 \pm 0.45$  cm. Inshenzi had the highest thorax diameter ( $10.35 \pm 0.15$  cm), followed by Indayi, Sekaganda and Umurangi ( $10.25 \pm 0.09$  cm,  $9.71 \pm 0.22$  cm and  $7.75 \pm 1.35$  cm respectively). The overall mean of body length across the IC kept was not significantly different ( $p > 0.05$ ), with  $20.31 \pm 0.59$  cm. These are the same findings as in Badubi (2006) where average body length was recorded as 18.00 cm and 20.00 cm for male and female ICs in Botswana. Indayi had the longest body ( $21.37 \pm 0.61$  cm), followed by Inshenzi, Sekaganda and Umurangi ( $20.56 \pm 0.14$  cm,  $19.18 \pm 0.65$  cm and  $18.63 \pm 0.97$  cm respectively).

The total tarsus diameter of ICs kept in the various Rwandan indigenous chicken ecotypes was significantly different ( $p < 0.05$ ) with an overall mean value of  $3.46 \pm 0.18$  cm. According to the report of Isidahomen et al.(2012), the result was differed here 1.5cm was measured for IC's tarsus diameter. Umurangi had the widest tarsus diameter ( $4.38 \pm 0.53$  cm), followed by Indayi, Inshenzi and Sekaganda ( $3.50 \pm 0.02$  cm,  $3.49 \pm 0.02$  cm and  $3.19 \pm 0.15$  cm respectively). The average tarsus length of IC was not significantly different ( $p > 0.05$ ) among the four IC genotypes, and had an overall mean of  $11.16 \pm 0.27$  cm. Similar to measurements on indigenous chickens of Ethiopia, (Hailu and Aberra, 2018), the maximum tarsus length of the chickens was  $10.9 \pm 1.5$  cm while the minimum was  $8.6 \pm 1.5$  cm. Umurangi chickens had the highest tarsus length ( $11.50 \pm 0.19$  cm), followed by Indayi and Sekaganda ( $11.37 \pm 0.12$  cm) and then Inshenzi had the shortest ( $10.00 \pm 0.67$  cm).

The total diameter of the shank of the various ICs ecotypes kept was not significantly different ( $p > 0.05$ ), and had an overall mean of  $1.36 \pm 0.21$  cm. In agreement with Demeure (2013) shank width (cm) had a maximum of  $1.73 \pm 0.05$  cm for one test and  $1.32 \pm 0.03$  cm for another test. Indayi was the most dominant in shank diameter ( $1.48 \pm 0.64$  cm), followed by Inshenzi, Sekaganda and Umurangi ( $1.38 \pm 0.01$  cm,  $1.24 \pm 0.07$  cm and  $1.13 \pm 0.13$  cm respectively). The overall mean of shank length among the IC types was not significantly different ( $p > 0.05$ ) and across all ecotypes, the mean was  $7.28 \pm 0.07$  cm.

These results are similar to Melesse and Negesse (2011) where 9.4 cm was the shank length for male adult ICs. Indayi has the longest shank ( $7.5 \pm 0.13$ cm), followed by Inshenzi, Umurangi, and Inganda ( $7.26 \pm 0.01$ cm,  $7.25 \pm 0.02$ cm and  $7.07 \pm 0.12$ cm respectively). The total wing length of the indigenous chickens kept was not significantly different ( $p > 0.05$ ) and had an overall length of  $13.82 \pm 0.29$  cm. Indayi had the longest wings ( $14.60 \pm 0.45$  cm), followed by Inshenzi and Sekaganda (both  $13.90 \pm 0.04$  cm) and then Umurangi ( $13.25 \pm 0.33$  cm).

The average wingspan across the IC kept was not significantly different ( $p > 0.05$ ), and had an overall mean $\pm$ SE of  $52.37 \pm 3.01$  cm. According to Guni et al. (2013), Sheka chicken populations had shorter wingspans (47.6 cm) almost similar to the chickens in our study, could point to close relatedness between the African chicken populations. In our study, Indayi chickens has the longest wingspan ( $54.64 \pm 1.331$  cm), followed by Inshenzi, Sekaganda and Umurangi ( $53.0 \pm 0.38$  cm,  $52.05 \pm 0.18$  cm and  $34.75 \pm 10.17$  cm respectively). The overall mean of female age sexual maturity across the IC was not significantly different ( $p > 0.05$ ). In agreement with Isidahomen et al. (2012), the mean age of sexual maturity for female ICs in Rwanda was at 6.67 months. Kamali et al. (2007) described the same result in different agro-ecological zones of the Amhara region of Ethiopia (6.6 months). Umurangi has the highest female age at sexual maturity ( $9.00 \pm 1.40$  months), followed by Inshenzi, Sekaganda and Indayi ( $6.64 \pm 0.04$  months,  $6.25 \pm 0.18$  months and  $6.00 \pm 0.33$  months respectively). The overall mean age of male at sexual maturity in IC kept was not significantly different ( $p > 0.05$ ) with 7 months. The same findings are reflected in the report of Isidahomen et al (2012), which showed that male ICs in Rwanda to reach age sexual maturity at 7.39 months. However, his findings differed from the reports of Yadessa et al. (2017) whose findings showed the mean age at sexual maturity of ICs in the benchi-Maji zone as 5.2 months. Umurangi has the highest age of male sexual maturity ( $7.67 \pm 0.38$  months), followed by Inshenzi, Indayi and Sekaganda ( $7.33 \pm 0.38$  months,  $7.00 \pm 0.00$  months and  $6.00 \pm 0.57$  months respectively). The age at the point of laying was significantly different ( $p < 0.001$ ) for all Indigenous chickens studied, with an overall average of  $7.60 \pm 1.86$  months. In line with Isidahomen et al. (2012), the local female chickens in Rwanda started to lay eggs at 7.31 months of age. The findings of Kamali et al. (2007) reflected similarities to the current study where the age at the first egg of scavenging chickens in different agro-ecological zones of the Amhara region was 6.6 months. In our current study, Umurangi has the highest age at the point of laying (18.0 months), followed by Inshenzi, Sekaganda, and Indayi (7.29 months, 7.00 months and 6.00 months respectively). However, the total number of eggs laid per clutch among the IC was not significantly different ( $p > 0.05$ ) with  $13.79 \pm 0.77$  eggs.

In accordance with Isidahomen et al. (2012) & Hirwa et al. (2019), an IC hen laid 9-15 eggs per clutch. Umurangi has the most observed in a great number of eggs laid per clutch ( $18.00 \pm 1.18$  eggs), followed by Inshenzi, Indayi, Sekaganda ( $14.25 \pm 0.94$  eggs,  $13.38 \pm 0.23$  eggs and  $12.50 \pm 0.74$  eggs respectively). The total number of clutches per year of IC kept was not significantly different ( $p > 0.05$ ), and had an overall mean of  $3.52 \pm 0.16$  clutches per hen per year. The same result was reported in Central Tigray where the average number of clutches per year was 3.15 to 3.2 and 3.2 at lowland and midland agro-ecologies zones, respectively (Isidahomen et al., 2012, Hirwa et al., 2019). Umurangi has the highest number of clutches per year ( $4.00 \pm 0.27$  clutches), followed by Inshenzi, Sekaganda and Indayi ( $3.68 \pm 0.09$ ,  $3.33 \pm 0.11$ ,  $3.173.17 \pm 0.20$  clutches respectively). The total annual eggs produced across the IC kept was not significantly different ( $p > 0.05$ ) between chicken types, but had an overall mean of  $46.06 \pm 5.91$  eggs.

According to CSA (2016), similar results were obtained in Ethiopia where indigenous chickens produce 48 small eggs per hen per year under farmers' management conditions. Furthermore, a similar result was reported by Alganesh et al. (2003) and Hirwa et al. (2019), where the egg production potential of local chicken is 30-60 eggs/year/hen under farmers' management

conditions. Umurangi has the highest annual egg production ( $72.00 \pm 14.97$  eggs) followed by Inshenzi, Sekaganda and Indayi ( $49.50 \pm 1.98$ ,  $42.17 \pm 2.24$  eggs and  $38.29 \pm 4.48$  eggs respectively).

The hatching rate among local chickens kept was not significantly different ( $p > 0.05$ ) and had an overall mean value of 82.30%. In agreement with Adeleke et al. (2012); the hatching traits between frizzled and normal feathered chickens, presented 90.5 and 84.8% fertility, respectively, and Anak Titan and Naked Neck presented 80.1 and 76.7% hatchability, respectively. Umurangi had the highest hatching rate (99.00%), followed by Indayi, Sekaganda and Inshenzi (88.71%, 82.00% and 79.81% respectively).

The hatchability rate under natural incubation for all ICs kept had an overall mean value of 81.11% but did not significantly differ ( $p > 0.05$ ) between ecotypes. In line with Ahmed et al (2012) the hatchability under natural incubation of Naked Neck and Full feathered was 87.40% and 86.98% respectively. The same findings were reported by Sumy et al. (2010), where hatchability was at 85%-89% (Naked Neck) and 80%-92% (Full feathered) (Dunya et al, 2014). Umurangi has the most hatchability rate under natural incubation (99.00%), followed by Indayi, Sekaganda and Inshenzi (87.00%, 82.00%, 78.53%, and 62% respectively).

The results showed that the related gene STX18 was significantly different ( $p < 0.05$ ) in all length of the beak for all ICs. This gene is responsible for disease resistance. A similar result was reported by Beilharz et al. (1993), where there was an intervention of this gene to disease resistance. The related gene RFX6 was also significantly different ( $p < 0.05$ ) in all length of the beak for all ICs. This gene had a crucial role in egg production and reproductive performance. The same outcome was reported by Tadelles et al. (2000) in southern Ethiopia, where the gene has played a role in hatching their eggs. The related gene CDK19 was significantly different ( $p < 0.05$ ) in all ICs beak length. This gene was reported to be responsible for egg and meat quality. Conformity results were reported by Moula et al. (2009), where it has an eggshell thickness which is slightly thinner compared to other shell zones and had meat quality regarded to the pH after 24 h of H'mong breed where 5.8 for pectoral muscles and between 5.8 for females and 5.9 for males for thighs meat muscles (Phuong et al. 2017). The related gene SGK1 was significantly different ( $p < 0.05$ ) in all ICs beak length. This gene was targeted for meat quality. In line with Isidahomen et al. (2012), this gene had a better carcass trait.

The related gene CITED2 was significantly different ( $p < 0.05$ ) in all ICs beak length. This gene was responsible for resistance to a harsh environment. The result was in conformity to the observation of Welc et al (2013) and it played role in response to stress. The related gene ENSGALG00000012941 was significantly different ( $p < 0.05$ ) in all ICS beak length. This gene intervened in egg production and growth. In agreement with Dessie et al. (2012), it had the growth and egg production traits that are responsible to pro-poor for small holder chicken farmers.

The related gene PHLDB2 was significantly different ( $p < 0.05$ ) in all ICS beak length. This related gene played important role in disease resistance. According to Axford (2000), a similar result was observed where the MHC alleles of this gene are worked with genetic resistance to

diseases like avian leucosis, fowl cholera, Salmonella and coccidiosis. The related gene ENSGALG00000049712 was significantly different ( $p < 0.05$ ) in all ICS beak length. This gene was responsible for disease resistance and stressful conditions.

A similar result was found by Walugembe (2019). It was important for IC to adapt to a variety of conditions such as high altitude, aridness, and stressful factors like disease resistance, poor nutrition, oxidative and heat stresses. The related gene ENSGALG00000053660 was significantly different ( $p < 0.05$ ) in all ICS beak length. This related gene intervened in egg-laying at optimum level. In accordance with Bridle et al. (2006), it was responsible for an immune response that should influence the genetics on laying hen welfare.

The findings indicated that the related gene ADPRH was significantly different ( $p < 0.05$ ) in all ICs wattle length. This gene was responsible for meat and egg quality. In line with Kranis et al. (2013); it was related to the level of tenderness with the age at slaughter. Eggshell quality is a crucial factor in egg production (Roberts, 2004). The related gene ENSGALG00000050573 was significantly different ( $p < 0.05$ ) all ICs wattle length. This gene played role in the growth and weight gain. According to Allais et al. (2019), the same result was obtained where it was responsible for body weight and body size which are the keys factors for the body development of chickens.

The related gene ENSGALG00000050989 was significantly different ( $p < 0.05$ ) all ICs wattle length. This related gene intervened in reproduction and growth. This was in conformity to the finding of (Moula et al., 2009). It played a crucial role in reproductive performance, growth and survival in harsh conditions. The related gene ENSGALG00000046839 was significantly different ( $p < 0.05$ ). This related gene intervened in egg production. The findings of Kranis et al. (2013) similarly reflect that the QTLs of this gene were associated with egg production traits.

The related gene SEC14L1 was significantly different ( $p < 0.05$ ) in all ICs wattle length. This related gene had high resistance to stressful conditions. In agreement with Apuno et al. (2011); it had the quality of thermo-regulation for indigenous chickens. The related gene SLC25A29 was significantly different ( $p < 0.05$ ) in all ICs wattle length. This gene was responsible for egg and meat quality. A similar finding was confirmed by Duc (2008) who showed that the gene played a role in egg production (55 to 66 eggs/year) and it has water retention quality which favours meat sweetness (Kranis et al., 2013).

The related gene ENSGALG00000054818 was significantly different ( $p < 0.05$ ) in all ICs wattle length. This related gene was responsible for egg and meat quality and quantity. In line with (Kranis et al. 2013), this gene was related to meat or egg production in indigenous chicken. The related gene NQO2 was significantly different ( $p < 0.05$ ) in all ICs wattle length. This related gene was responsible for egg production. In agreement with Tadelle (2003), the gene intervened in average egg production per year for ICs (43.4 to 54.3 eggs per year).

The results showed that the related gene EPS15 was significantly different ( $p < 0.05$ ) in all IC head length. This gene played a significant role in growth and productivity. Our result was per Alabi et al. (2013). This gene was associated with fed nutrients (protein, lysine, and energy) to

local chickens for optimal and maximum productivity. The related gene *AUTS2* was significantly different ( $p < 0.05$ ) in all IC head length. This related gene intervened in the growth rate. A similar finding was confirmed by Emmerson (2003), where this gene contributed to early growth selection traits among the broiler ICs at 40-50 days.

The related gene *PRR5* was significantly different ( $p < 0.05$ ) in all IC head length. This related gene played an important role in the growth rate. In agreement with (65), where feeding them a protein at a level of 16% aged 14-21 weeks was optimizing the feed intake and growth. The related gene *SPATA5* was significantly different ( $p < 0.05$ ) in all IC head length. This gene was responsible for growth. According to Kingori et al. (2007), it was a similar result for optimizing growth which was not depending on the highest level of protein.

The related gene *NPHP1* was significantly different ( $p < 0.05$ ) in all IC head length. This related gene was responsible for the chicken's resistance to the harsh environment. A similar finding was reported by Van Marle- Köster et al. (2009); it was responsible for better adaptation to stressful environmental conditions. The related gene *ENSGALG00000053126* was significantly different ( $p < 0.05$ ) in all IC head length. This related gene was responsible for egg quality. The same finding was observed by Alipanah et al. (2013), where it contributed to genetic and phenotypic correlation in terms of egg quality.

The finding confirmed that the nearest gene *LOC776067*, *CDC16* was responsible for meat quality and quantity. According to Wang et al (2006), similar findings were observed when it involved growth and meat quality. Furthermore, it intervened in muscle development with associated fatness traits (Dunislawska et al., 2020). It can be used in the improvement of growth performance (Habimana et al., 2020). The nearest gene *DHRX*, *CD99*, *LOC107051960* played a role in meat quality. In agreement with (70); it intervened in meat quality by controlling liver and whole-body fatness.

The nearest gene *LOC101750813*, *MX1* played a role in viral disease resistance. This conformed to the findings of (Jump et al., 2005). Mx proteins induced by an infection with avian influenza virus can block an early step of the viral replication cycle (Jump et al., 2005). In addition, the chicken Mx gene is highly polymorphic and it has antiviral activity (Rautenschlein et al., 2020). Furthermore, this nearest gene was responsible for influenza virus resistance (Habimana et al., 2020). The nearest gene *LOC107053692*, *GRAP2* was responsible for egg production. Kamali et al. (2007), found the same result and it contributed to the highest value of cumulative egg production for the first 12 weeks.

The nearest gene *JARID2*, *PRL*, *LOC10751657* had a great impact on local environment adaptation. According to Matsuzaki et al. (2017), a similar result was reported where it contributed to neuron proliferation that is responsible for acclimatization. The nearest gene *CALB1*, mRNA: XM\_0049399117.3 was responsible for egg quality. As described in the same result by Nys and Roy (2017), it played a crucial role in eggshell quality due to the accumulation of calcium carbonate. The nearest gene *ADCYAP1*, *CDH2* was responsible for eggshell quality. Following Jonchère et al. (2010), this nearest gene was associated with eggshell quality. Furthermore, the gene contributes to egg strength (Moreki et al., 2011).



The nearest gene LECT2, CDS: XP\_015149132.1 was responsible for increasing egg production. Higher values were reported by Kamali et al. (2007), where this nearest gene was responsible for egg production with a heritability of 0.49 for Iranian indigenous fowls in the first 12 weeks. The nearest gene NOTCH, UBC1, CDS: XP\_0153010.1 contributed to better adaptation to bad conditions in the local area. Confirming to Aberra et al. (2013), this nearest gene in Naked-neck has heat resistance in tropic zones. The nearest gene SPNS3, CDS: XP\_015151456.1, mRNA: 015295970.2 played a great role in increasing egg production. In agreement with Kamali et al. (2007), it was responsible for egg production in Iranian indigenous fowls.

The nearest gene RASSF5, XP\_015151456.1, mRNA: 0155298926.2 contributed to growth and meat quality. According to DLS (2015), a similar result was reported where it has the traits that were responsible for growth or fat accumulation. The nearest gene PBX1 intervened in growth and immunity. In conformity with Ohta et al. (1992), it has the pituitary-specific transcription factors which can bind to and transactivate promoters of growth hormone- and prolactin-encoding genes and has an important regulatory effect on animal growth and immunity.

The nearest gene RXRG, FMO3, GLUL was responsible for development and meat color quality. In accordance with Le Bihan-Duval et al. (2011), the gene has played role in meat quality appreciation. The nearest gene LOC107054293, CEBPA contributed to an increase in body weight. The same finding was reported by Gous et al (1999), where the gene was related to an increase in body weight. The nearest genes LOC107054316, BCO1, and CDH13 played a crucial role in meat quality and growth weight. According to AACMC (1984), it contributed to the body weight of indigenous chickens with a 1.5 kg live weight at 24 weeks of age.

The nearest genes DHRS11, LHX1, and ACACA were associated with an increase in egg production. In agreement with Gous et al. (1999), the gene was responsible for egg production for the first 12 weeks in Iranian indigenous fowls. The nearest gene ALB, IL8L2, FGF2, and ANXAS were responsible for the improvement of egg and body weight traits. According to Bahmanimehr (2012), this nearest gene intervened in an increase in egg production and body weight. Further research supported our study which showed that the gene contributed to an increased number of eggs per year (Woldegiorgiss, 2015).

## **CONCLUSION**

The study identified key genetic markers and potential production traits in indigenous chickens in Rwanda, highlighting Imirangi and Inshenzi as the genotypes with the highest genetic potential for traits such as egg production and body weight. The genetic diversity observed among the four genotypes, particularly the low similarity between Imirangi and Inshenzi, underscores the opportunity for targeted selection. These findings provide a robust foundation for developing a breeding program that optimizes the production potential of indigenous chickens, contributing to sustainable poultry farming and food security in Rwanda.

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