International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2022; 7(4): 26-35

www.veterinarypaper.com
Received: 07-05-2022
Accepted: 08-06-2022

E Mbakaya
Department of Animal Sciences, Egerton University, P.O. Box 536-20115, Nakuru, Kenya

T Muasya
Department of Animal Sciences, Egerton University, P.O. Box 536-20115, Nakuru, Kenya

K Ngeno
Department of Animal Sciences, Egerton University, P.O. Box 536-20115, Nakuru, Kenya

Corresponding Author:
E Mbakaya
Department of Animal Sciences, Egerton University, P.O. Box 536-20115, Nakuru, Kenya

Immunogenetics and genetic variations in indigenous chicken in the tropics using SNP data

E Mbakaya, T Muasya and K Ngeno

DOI: https://doi.org/10.22271/veterinary.2022.v7.i4a.428

Abstract
This study used data from 150 indigenous chicken from four agro-ecological zones in Rwanda to provide deep insight of the population structure and variation of the immunogenetics using several approaches based on phenotypic and SNP data. The population structure of indigenous chicken was analysed using Principal Component Analysis (PCA), ADMIXTURE analysis, and phylogenetic relationships for the whole genome and at chromosome 16. The study used 65,945 SNPs from the collected chicken. Phenotypic analysis was done for the Newcastle disease titer (ab) alongside bodyweight at 20 weeks with the highest having 1.6 kg. The genome analysis was done using the genotyping-by-sequencing approach. The grouped the indigenous chicken into two genetic clusters, which was confirmed by ADMIXTURE analysis that revealed that the lowest cross-validation (CV) error (0.51) was at K=2. The analysis of Population structure at chromosome 16 showed that the population had the lowest CV error (0.50) at K=1. The mean body weight and antibody titer were 1673.61±237.14 g and .5±55.35, 1311.34±121.9 g and 8832.5±55.36, respectively, depicting an inverse relationship between bodyweight and antibody titers. The cluster means for body weight and antibody titers were significantly different ($P<0.001$) for body weight and antibody titers. The indigenous chicken genetic clusters in Rwanda have variation in antibody titers which can be attributed to varied selection pressure. The observed genetic diversity of the indigenous chicken for disease resistance should be well-thought-out when scheming a selection programme to ensure that the ICs population is sustainable, flexible and simultaneous improvement of this trait. Based on this study's findings government should implement strategies that conserve and maintain the genomic diversity of Rwanda indigenous chicken.

Keywords: Chromosome 16, immune traits, flexibility, MHC, sustainability

Introduction
Indigenous Chicken farming has been gradually shifting from subsistence to commercial chicken farming due to increased demand for IC meat and eggs (Magothe *et al.*, 2019) [25]. The increase in IC demand makes farmers house birds in large flocks at high stocking density thus the increased risk of diseases, and disease spreading (Mujyambere *et al.*, 2022) [26]. Newcastle disease is one of the common diseases affecting IC farming (Kapczynski *et al.*, 2013; Walugembe *et al.*, 2019) [20, 43]. Identifying the genes that control disease resistance would make the selection of IC for improved productive performance and enhanced disease resistance possible. A useful spinoff would reduce cost of production due to decreased use of drugs, as well as better product quality due to lowered drug residues (Jie & Liu ,2011; Dar *et al.*, 2018) [19, 9].
A number of efforts have been practiced worldwide to appreciate and improve resistance to disease in livestock through the application of immunogenetics. In pigs improvement of resistance to disease was applied using gram–negative bacteria (Zhao & Chen, 2012) [47] and in ruminants it was done using gastrointestinal nematodes (Sweeney & Good, 2016) [39]. In bovine, immunogenetics was applied to improve resistance to mastitis (sodeland *et al.*, 2011) [38]. Information on both immunology and genetics of animal would well describe the disease phenotype (Bishop, 2014) [6]. Immune capability related to a particular disease used can be to show indirect selection for resistance to disease because these traits can be assessed and measured in breathing animals (Luo *et al.*, 2013) [22]. Santos-Argumedo, 2012 showed that antibody titers are immulogical traits which can be inheritated in poultry thus making it easy to determine loci or a particular gene associated to immune-related traits.

In chicken, selecting for growth and production traits has been related with decrease in immunity (Bayyari *et al.*, 1997; Wondmeneh *et al.*, 2015) [4, 46]. Selecting chicken with increased weight of the body is genetically related to a reduction in disease resistance. Genetic correlations between BW & AB in chicken (r_g) are medium to high (Mebratie *et al.*, 2019). Using microsatellites, Ngeno *et al.*, (2015) and Habimana *et al.* (2022) [18] showed genetic structuring in IC in Kenya and Rwanda. The studies reported that body weight was inversely related to Abs. Microsatellites are more variable but suffer from ascertainment bias, homoplasy and amplification variation of primers (Tian *et al.*, 2008) [41]. High-density single nucleotide polymorphism has made it possible in the investigation of the population's genetic structure through use of large numbers of markers and identify regions in the genome where events related to the traits we are interested with (Groenen *et al.*, 2008; Wollstein *et al.*, 2010) [17, 45]. SNPs are more abundant and evenly distributed across the genome hence more informative. Therefore, the study aimed to examine the population structure of ICs at both whole-genome and chromosome 16.

Materials and Methods

The study population and housing

A total 150 IC were sampled randomly from the northern, southern, central, and eastern agro-ecological zones of Rwanda. The indigenous chickens were kept at the University of Rwanda under the same environmental conditions under deep litter system. The standard density of 12birds/M^2. 22-23 hours of light was provided. Infrared heat lamps of 250 Watts were used to heat the house. Afterward, 23-25 °C of temperature was provided during the entire study period.

Collection of phenotypic data

The indigenous chickens were vaccinated with two commercial New castle Disease virus live vaccines. At 2days of age, AVI New castle Disease HB1 was put in water and the second shots of the vaccine (AVI ND Lasota) were given on their eye when they were at 28 days old. On the 35th day, collection of blood was done without anticoagulant for separation of serum for detection of antibody titers against New castle disease vaccine. The detection of antibody responses to New castle disease was using an indirect ELISA test. The Antibody levels were computed using the ID Soft TM data analysis programme. At the 20th week of age, the body weights of the IC were measured.

Extraction of genomic DNA and genotyping

Blood samples for DNA extraction were collected using 2.5ml EDTA tubes. Genomic DNA from blood was extracted by a DNA extraction kit. The concentration of extracted genomic DNA and the qualities were evaluated using a Nano Drop™ 2000 spectrophotometer (Thermo Scientific™ Nanodrop 2000) and gel electrophoresis (1% agarose) (Lu, *et al.*, 2016) [23]. Raw reads were obtained using the Genotyping-by-sequencing (GBS) approach (Jain *et al.*, 2016) [24].

Alignment of the reads and calling of the SNPs

Trimming of the raw reads was done using the sickle tool and then they were aligned to the Galgal4 chicken reference genome using Burrows- Alignment tool (BWA v0.7.17), afterward sorting of the reads was done. Removal of the duplicated reads was performed using SAM tools v1.3.1. The calling of SNPs was done using SAM tools v1.3.1.

Quality control of the SNPs

Trimming of the reads was done using a sickle and then alignment was done by aligning the raw reads to the Galgal6 (chicken reference genome) by the Burrows-Alignment tool (BWA v0.7.17). The removal of duplicated raw reads and calling of the reads was performed using the SAM tools v1.3.1 (Li *et al.*, 2008). The SNPs obtained were thereafter subjected to the standard filtering procedures using 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 > 0.03). Pairwise linkage disequilibrium (LD) measured by r^2 values for each chromosome (Bradbury *et al.*, 2007) [7] was calculated using Tassel 5.2.60

Statistical analysis

Phenotypic characterization

Data collected on antibody titers against Newcastle disease and BW of ICs populations were entered into a database using Microsoft Office Excel 2016. The phenotypes were USED to place the chicken in unique clusters based on either AB, BW and AB and BW using PROC FASTCLUS in SAS software v 9.4 (2008). The within-cluster variation was described using descriptive statistics. The PROC GLM of SAS was used to determine whether the clusters were differing significantly for AB and BW based on the different clustering approaches. The following linear model was used:

$$y_{ij} = \mu + t_i + e_{ij}$$

Where

y_{ij} is the total

IgY titer or body weight; μ is the overall mean;

$t_i$is the effects of the fixed factor and

e_{ij} is the residual term

Genotypic clustering of IC into genomic clusters

The SNPs genotypes of the IC were used to assess the genetic differentiation of the IC population. The study used PCA to minimize the dimensionality of the SNP data set with a large number of interrelated variables. This was achieved by converting the original variables into a new set of variables, the principal components (PCs), which were uncorrelated, and ordered so that the first few retain most of the variation present in all of the original variables. The genomic clusters were determined by plotting PC1 and PC2 in tassel software.

Admixture analysis

ADMIXTURE was to determine population relatedness and assigns populations to ancestral clusters. Population structure was examined investigation of the population structure was done using the model-based clustering algorithm that was run in ADMIXTURE (Alexander *et al.*, 2011) [2], from K = 2 to 4. The cross-validation method was used in estimation number of populations that are most likely to be found. The K value reduces the cross-validation prediction error was then assumed as the most likely (Evanno *et al.*, 2005) [12]. The ip ADMIXTURE R package was used to represent the results in a graph (Amornbunchornvej *et al.*, 2020) [3].

Estimation of genetic differentiation

The unbiased genetic differentiation estimate, F_{ST} (Weir & Cockerham, 1984) [44] was calculated using admixture software in Kenet vLAB (a virtual software) with the quality-controlled SNP dataset to estimate genetic differentiation between populations using the fixation index.

Phylogenetic analysis

The relationship among IC from the four agro ecological zones was determined by constructing a matrix distance, from the matrix distance a phylogenetic tree was drawn in Tassel software (Bradbury *et al*., 2007) [7]. The genetic relationship between the IC from the four zones was determined based on the neighbour-joining tree algorithm in TASSEL software v5.2.35 (Bradbury *et al*., 2007) [7]. The neighbour-joining tree cladogram generated by TASSEL was visualized in the archaeopteryx tree (Bradbury *et al*., 2007) [7].

Genetic diversity at chromosome 16

The study extracted SNPs from chromosome 16 (position) using 90 SNPs that covered from LOC425771 through CD1A1 (210.000 bp) (Fulton *et al.*, 2016) [14]. The extraction was done using VCF tools v0.1.14 (Danecek *et al*., 2011) [10]. Diversity was done following steps described above that is i) PCA ii) admixture analysis and iii) Phylogenetic relationship that is the neighbour-joining analysis. The SNP allele frequencies for chromosome 16, expected (He) and observed (Ho) heterozygosity and hardy Weinberg equilibrium (HWE) were computed using PLINK (v1.90b) software (Slifer, 2018) [40].

Results

Phenotypic clustering: Phenotypic clustering indicated that the Rwanda indigenous chicken were clustered into two populations. Cluster one mean body weight of 1673.61 ±237.14g and antibody titer were 4912.5±55.35. Cluster 2 had mean body weight of 1311.34±121.9g and mean antibody titer of 8832.5±55.36. The clusters differed significantly (P<0.001) for body weight and antibody titer. The cluster with high mean in bodyweight and low mean in titer and vice versa

The analysis of Population structure

This was done using the principal component analysis (PCA), admixture and neighbour-joining tree analyses. This analysis was done at the whole-genome and chromosome 16.

Population structure analysis at whole genome

At the whole genome level, PCA showed that principal component one (PC1) amounted to 38% and principal component two (PC2) amounted to 26% of the total variability (Figure 1 and 2).

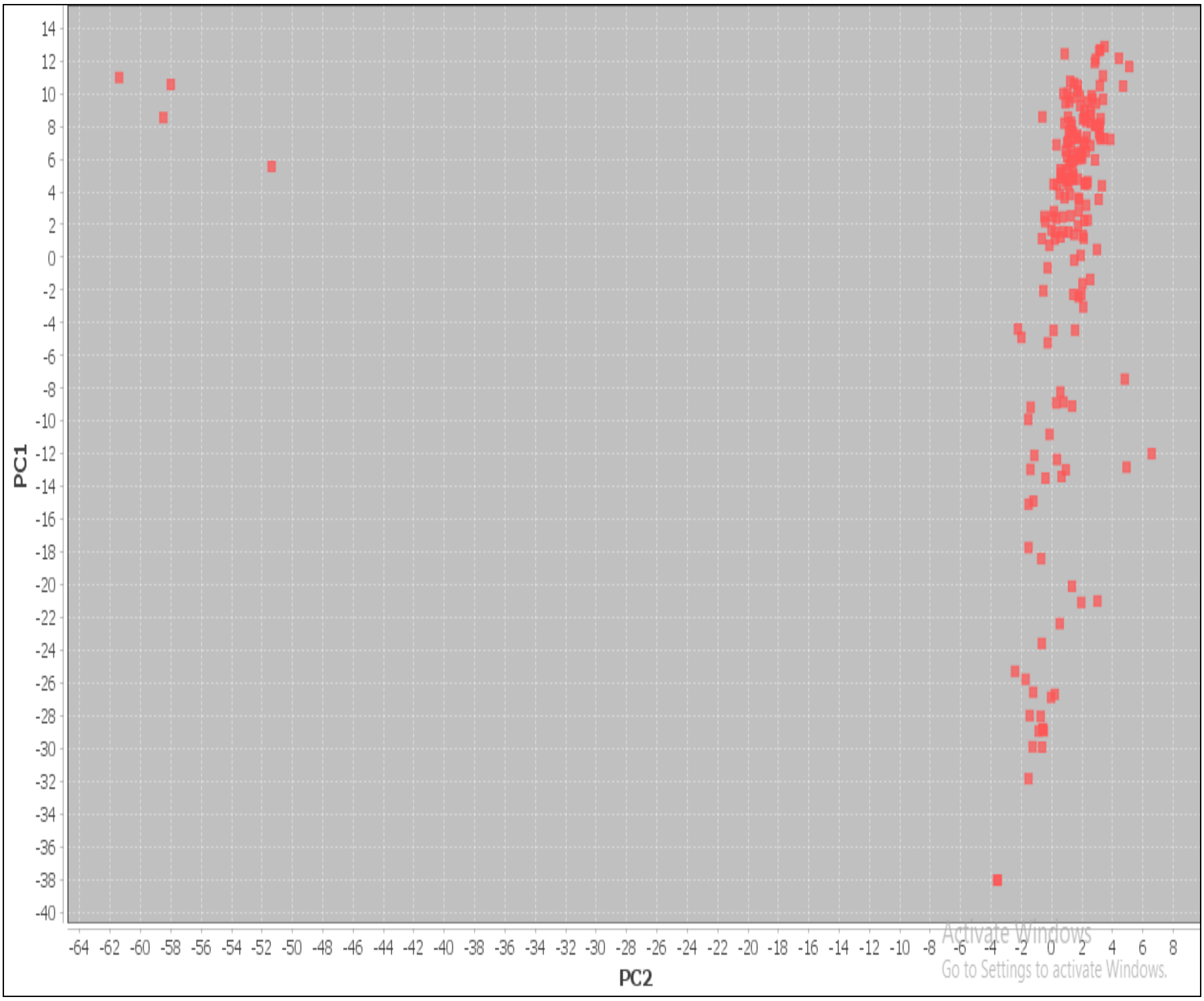

Fig 1: Plot of principal component analysis in tassel software showing the two genetic clusters of indigenous chicken from the four ecological zones.

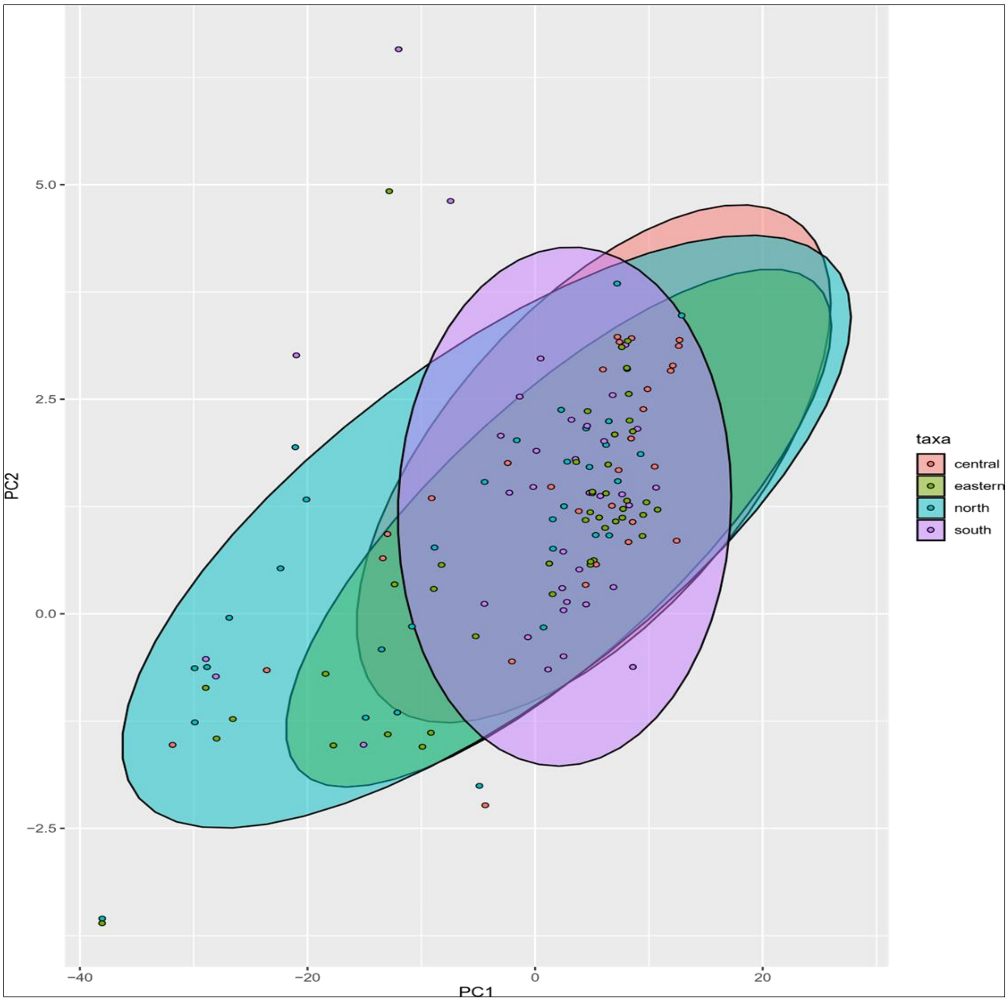

Fig 2: Principal component analysis of the whole genome showing two clusters, one cluster seemed to be the outliers form of the other cluster.

Admixture analysis

Admixture analysis performs maximum probability approximation of individual ancestries from multilocus SNP genotype data. A R package, ipADMIXTURE (Amornbunchornvej *et al.*, 2020) [3] was then used to plot the ancestry of the indigenous chicken from the four zones. Admixture plots arranged the individuals according to the portion of origin they shared with other individuals. The Bayesian clustering analysis of ADMIXTURE resulted to K values from K2 to K4 as in Figure 4, and K2 was found to be with the lowest cross-validation error (Fig. 3). When K was 2 the indigenous chicken from the four agro-ecological zones were totally admixed with the majority being almost pure. When K was 3, IC from northern were pure. When the K value was 4, central and S (southern) showed that the IC were pure breed.

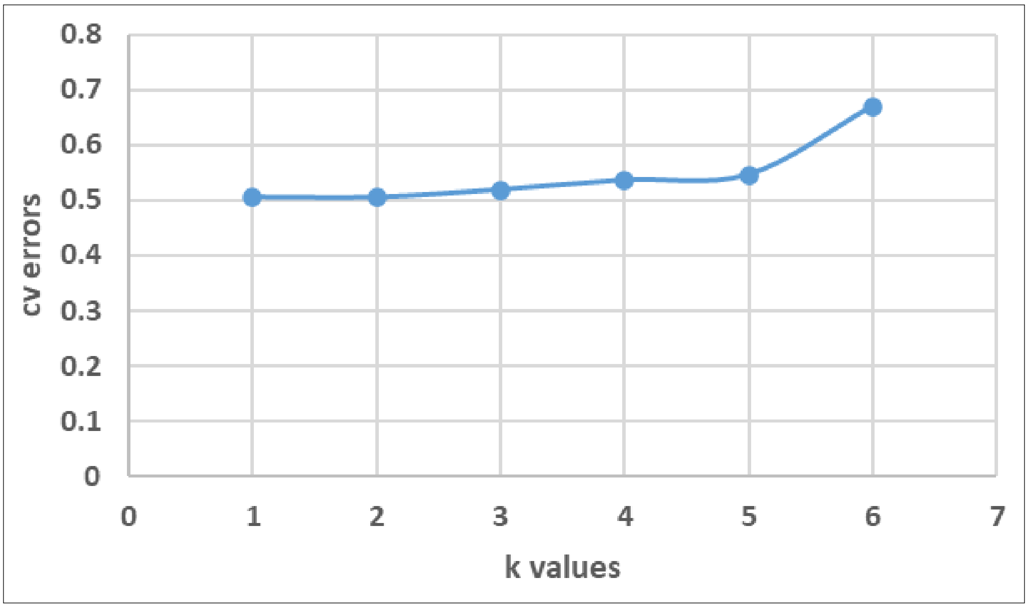

Fig 3: The cross-validation errors of the k values

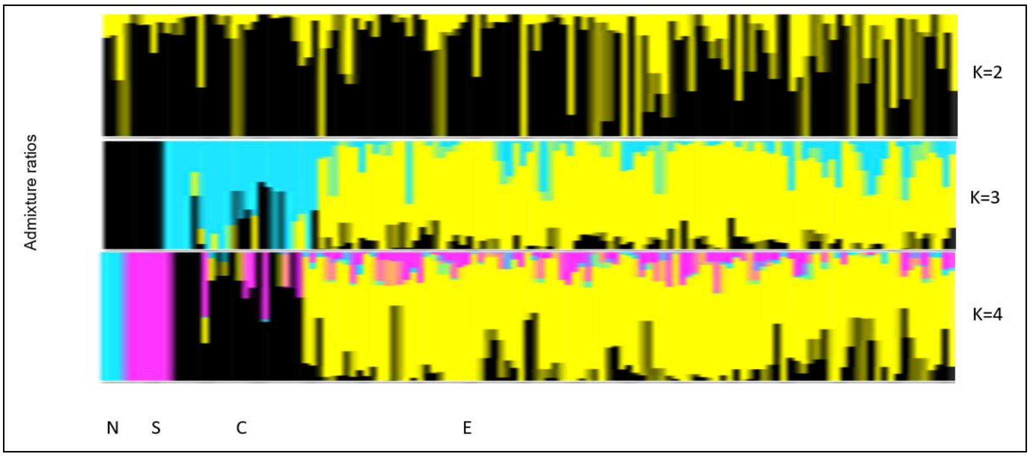

Fig 4: The ADMIXTURE analysis from the four agro ecological zones showing from K-values of 2 to 4. The color of the vertical bar on the x-axis signifies the quantity of membership of each accession in each cluster. The geographic regions are indicated on the x-axis as follows N (northern), S (southern), C (central) and E (eastern).

The analysis Population differentiation

The magnitude of population differentiation between different IC from the four agro-ecological zones was investigated by using F_{ST} values which were calculated using genotype data that was filtered (Table 1). For the whole population F_{ST} values ranged from 0.071 to 0.218, indicating genetic differentiation appeared between IC from the four regions. However, these F_{ST} values seem to increase. For instance, the F_{ST} value was 0.092 between northern and southern and increased to 0.186 between northern and central zones. The opposite was observed between southern vs central (0.176) and southern vs eastern (0.071).

Table 1: Fixation index (Fst) of the population from the four agro-ecological zones

	Northern	Southern	Central	Eastern
	Northern			
southern	0.092			
central	0.186	0.176		
eastern	0.140	0.071	0.218	

Population structure analysis at chromosome 16

Twenty variants were extracted from chromosome 16. The variants were retained for downstream analysis that is, PCA, admixture analysis and neighbor joining analysis. The PCA and admixture analysis placed the indigenous chicken as one population as in (figures 5, 6, 7). Admixture analysis revealed lowest cross-validation error when K was 1.

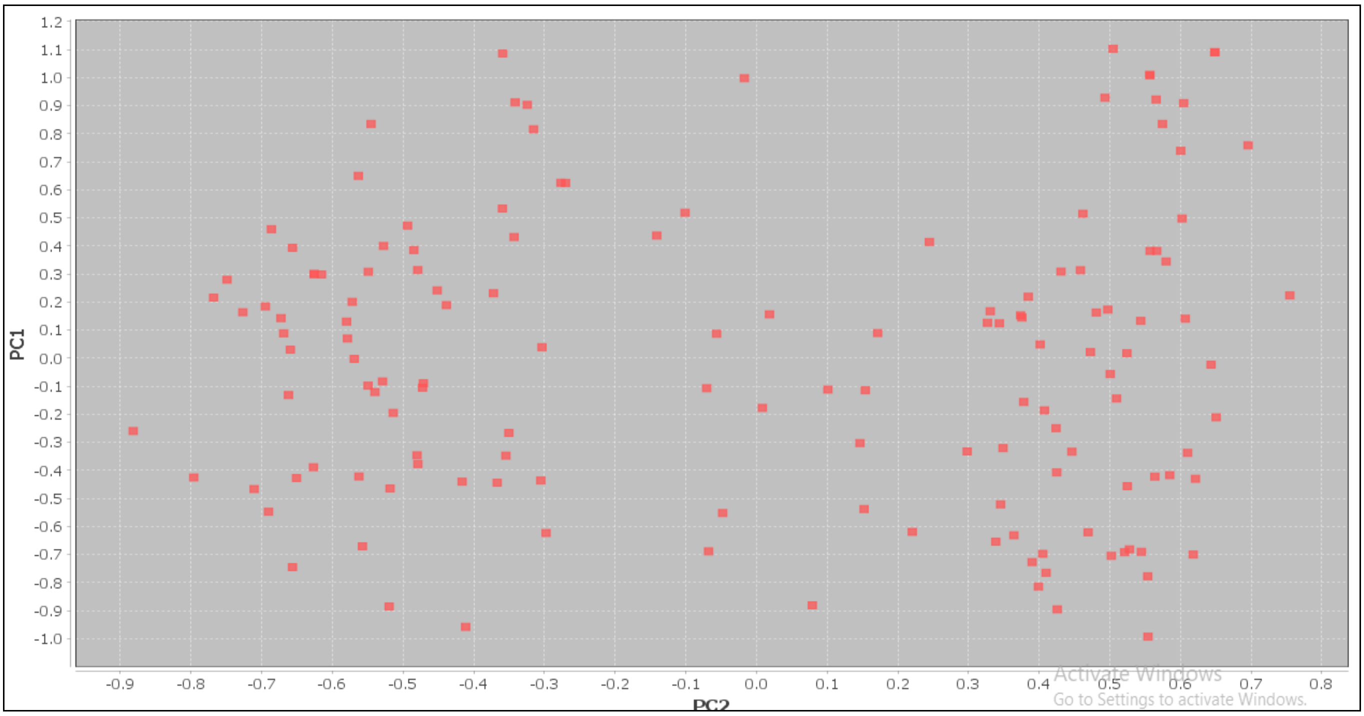

Fig 5: Plot of principal component analysis in tassel software indigenous chicken from the four ecological zones as one population.

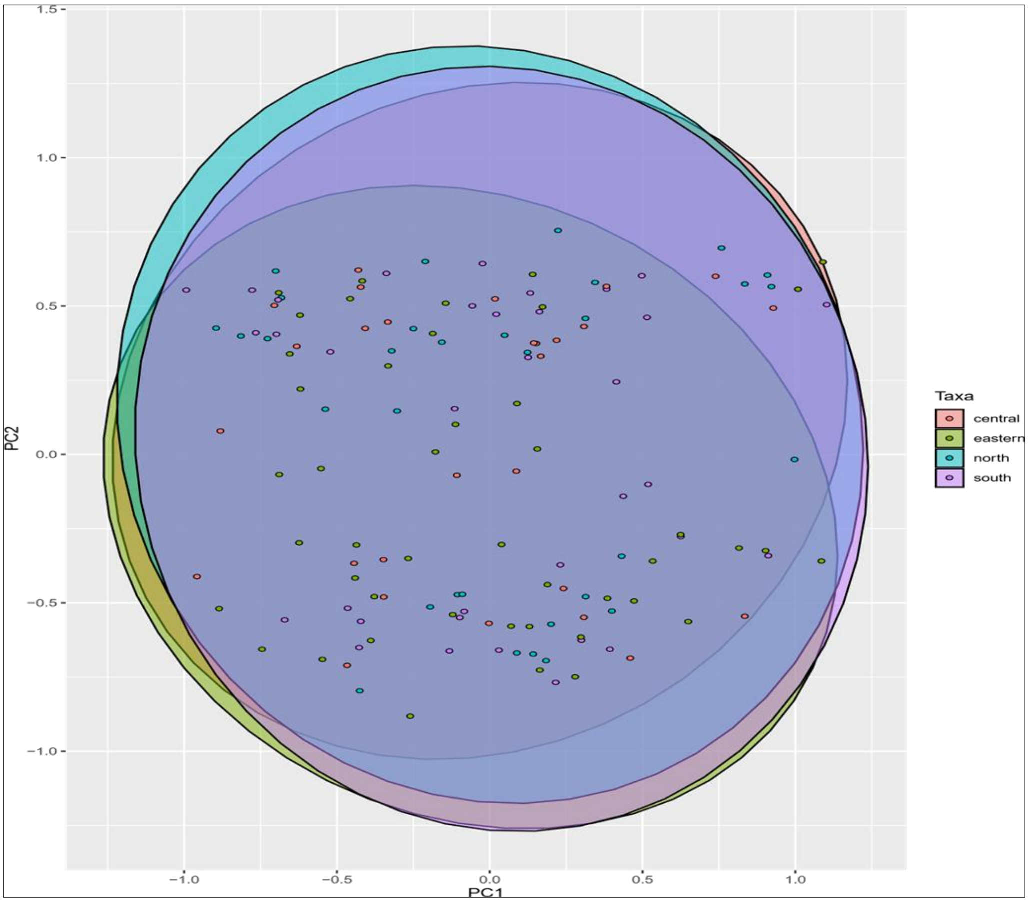

Fig 6: Principal component analysis of chromosome 16 showing one cluster of indigenous chicken from the four agro-ecological zones

Phylogenetic relationship

The chromosome 16-based SNP phylogenetic analysis was used to deduce the relationships between indigenous chicken collected from four agro-ecological zones in Rwanda. The phylogenetic tree, together with the details on each indigenous chicken is shown as in Figure 7. The neighbour-joining tree reveals all the indigenous collected from the four zones had one common origin thus grouping them as one population.

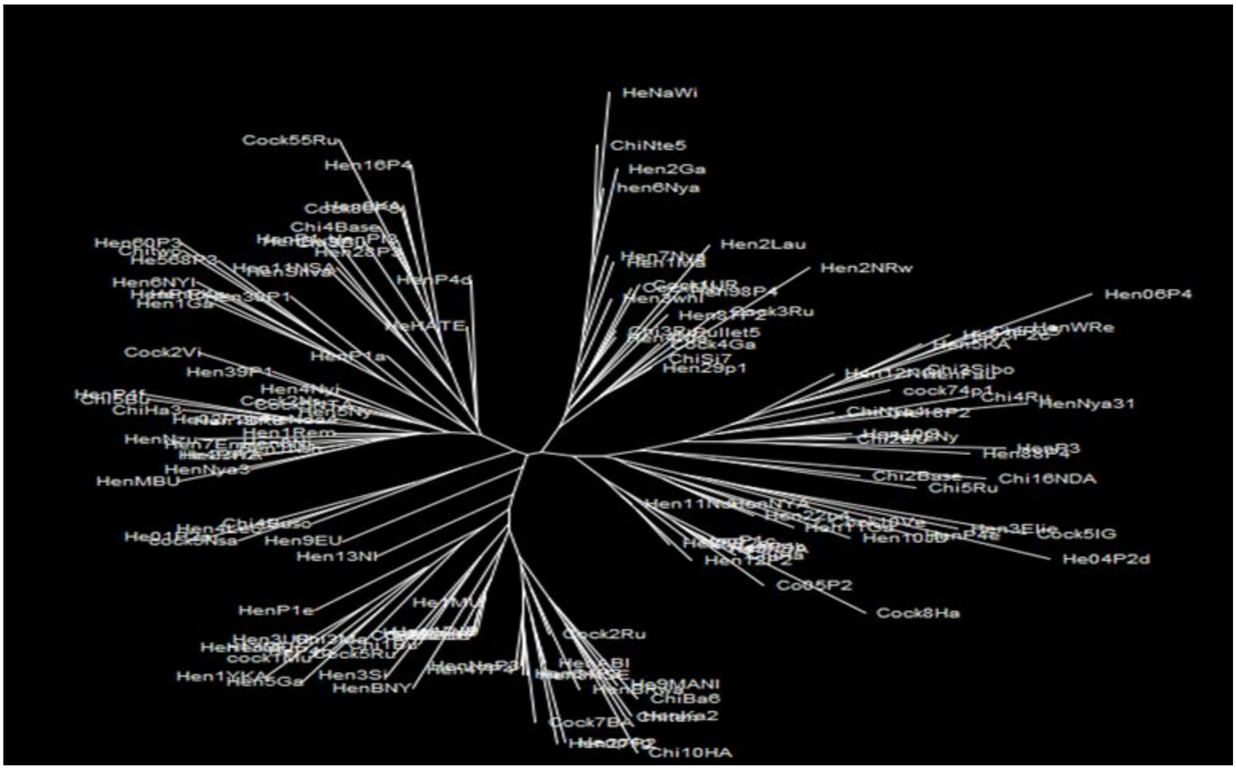

Fig 7: Neighbour-joining tree obtained from the distance matrix of tassel software among 142 indigenous chicken from the four agro-ecological zones; this tree was only for 20 variants extracted from chromosome 16.

Admixture analysis at chromosome 16

The Bayesian clustering analysis of ADMIXTURE resulted to K values from K1 to K4 and K1 was found to be with the lowest cross-validation error (Figure 8).this shows that at chromosome 16 level the indigenous chicken were grouped as one population.

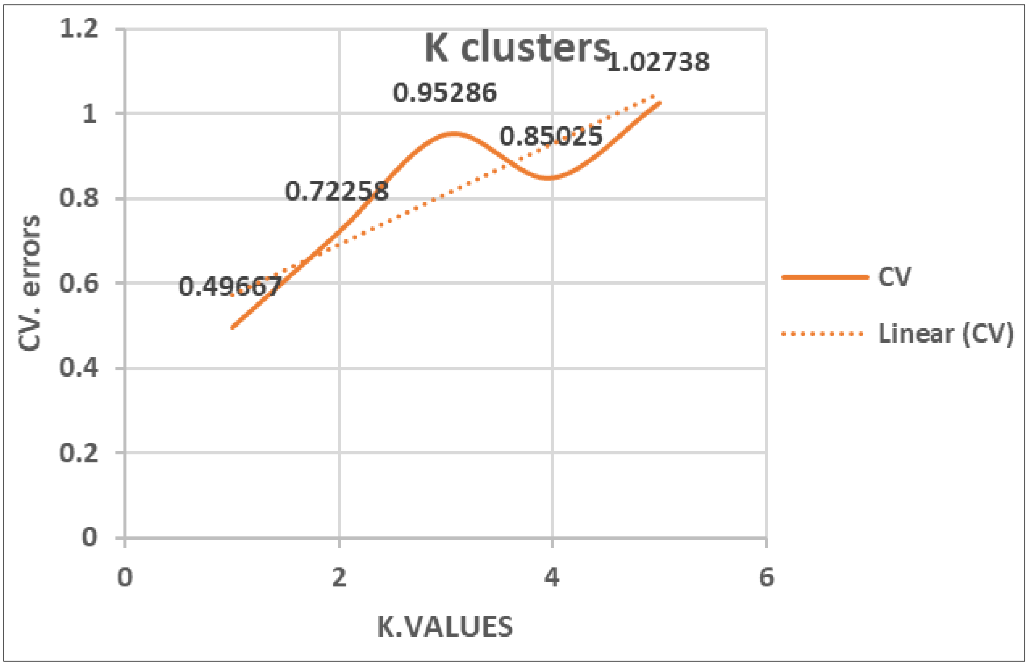

Fig 8: showing the cross-validation errors of the k- clusters

Basic Genetic Parameters of a Population

Observed heterozygosity (Ho) values averaged 17.55±0.12 across all the IC groups from the four agro- ecological zone. The expected heterozygosity values averaged 15.77 ± 0.02 across the four populations. The heterozygosity rate ranged from 0-0.3 across the populations, with 30 individuals having more than 0.2 heterozygosity rate as in Table 2. The average inbreeding coefficient per population was 0.43±0.03 across the four populations. The Inbreeding coefficients per agro-ecological zones were as follows central had the highest with 0.5 followed by eastern with 0.4 then northern with 0.4 and lastly southern with 0.3.

Table 2: Heterozygosity rates across the four agro ecological zones

Heterozygosity rate	0	0.10	0.05	0.15	0.20	0.21	0.25	0.26	0.30
No of Indv	18	51	4	40	18	2	6	1	3

SNP Marker Characteristics

Minor allele frequency (MAF) had an average of 0.16176 and SNP regions 100163035|F|0-13:G>A to 100021966|F|0-8:G>A-8:G>A had a MAF value that was below 0.05. An analysis of how the minor allele frequency were distributed across the 20 variants revealed that over 50% of the markers were within the 0–10% minor allele frequency threshold.

Table 3: Minor allele frequency of 20 variants extracted from chromosome 16

CHR	SNP	A1	A2	MAF	NCHROBS
16	100134902|F|0-45:G>A-45:G>A	G	A	0.4615	286
16	100149083|F|0-10:G>A-10:G>A	G	A	0.3169	284
16	100129173|F|0-17:C>A-17:C>A	A	C	0.003497	286
16	100034475|F|0-22:C>T-22:C>T	T	C	0.006993	286
16	100103111|F|0-39:T>C-39:T>C	C	T	0.003497	286
16	100094740|F|0-25:T>G-25:T>G	G	T	0.01748	286
16	100085316|F|0-26:T>A-26:T>A	T	A	0.465	286
16	100088251|F|0-14:A>G-14:A>G	A	G	0.01748	286
16	100163669|F|0-42:G>T-42:G>T	G	T	0.0979	286
16	100163035|F|0-13:G>A-13:G>A	A	G	0.0461	282
16	100125696|F|0-68:C>G-68:C>G	C	G	0.08741	286
16	100096683|F|0-36:G>T-36:G>T	T	G	0.1119	286
16	100021966|F|0-8:G>A-8:G>A	A	G	0.003497	286
16	100060608|F|0-32:A>G-32:A>G	G	A	0.09091	286
16	100077597|F|0-8:A>G-8:A>G	G	A	0.3741	286
16	100130892|F|0-21:T>C-21:T>C	C	T	0.3636	286
16	100147497|F|0-22:G>A-22:G>A	A	G	0.01049	286
16	100118266|F|0-26:G>C-26:G>C	G	C	0.5	266
16	100125885|F|0-14:G>A-14:G>A	A	G	0.01399	286
16	100033338|F|0-31:C>A-31:C>A	A	C	0.243	284

Discussion

This study focused on phenotypic and genomic characterization (at both whole genome and at chromosome level) of IC in Rwanda. The findings revealed both phenotypic and genotypic characterization at whole genome grouped the IC into two groups providing a deeper understanding of the structure of IC population to supplement the use of phenotypes for IC selection (Chiwanga *et al*., 2020). At chromosome level (chromosome 16) and the whole genome, the genetic diversity analysis revealed one and two genetic groups, respectively. These results indicate that at chromosome level, that there may be significantly less genetic variation than the variation within the whole genome of a population.

Phenotypic clustering

Regardless of extensive information of the genomic foundations of phenotypes the G. gallus, few and non-exhaustive studies have measured genotype and phenotype variations in populations of this species. In this study, measurements of antibody titers and Body weight were evaluated and compared among the IC obtained from the four agro-ecological zones. Phenotypic clustering grouped the IC into two clusters. The first cluster had a high mean body weight and low antibody titers as compared to cluster two which had a low mean body weight and high mean for antibody titers. Selecting for growth and production traits has been linked with reduced immunity. Selection for increased BW has been shown to be genetically associated with a reduction in disease resistance in chicken. (Bayyari *et al*., 1997; Wondmeneh *et al*., 2015) [4, 46]. This study's findings revealed that IC with high body weight have reduced antibody titers and vice versa when they have high antibody titers their body weight is reduced.

Genetic diversity at whole genome

The study reports the diversity of the genome of Rwanda IC through identifying and characterizing of 65,945 SNPs from 142 IC obtained from four agro-ecological zones that are northern, eastern, southern and central using whole-genome.
The analyses of the principal component and admixture revealed two ancestral gene pools across the Rwandan IC populations. A similar study done in Tanzania to investigate the population structure of Indigenous chickens using admixture grouped the selected IC into two gene pools (Mushi *et al*., 2020) [27]. In agreement with the Principal Component Analysis results, admixture analysis grouped all the indigenous chicken from the four agro-ecological zones into into two unlike a study done by Habimina *et al.* (2020) which revealed four gene pools using microsatellite. When K=2 the Rwandan indigenous chicken were grouped into central and Eastern, with more proportion of the central ancestry cluster probably because of interbreeding among the IC (; Mushi *et al.,* 2020) [27]. When K = 3, all samples were grouped into three clusters, each for pure IC from central and admixed. At K= 3, the IC from northen and central presented a homogenous cluster (Black and light blue respectively) which was not displayed by ICs from eastern and southern agro-ecological zones. In overall, the IC population were admixed with most birds being from the central agro ecological zone. Our results revealed that the Fst values for the whole population ranged from 0.071 to 0.218 thus indicating isolation between the IC populations, and this mightily likely mean that the IC populations are presently breeding with one another. Based on this study's findings, government should implement strategies that would conserve Rwanda indigenous chicken genetic diversity and its characteristics

Genetic diversity at chromosome 16(MHC region)

At chromosome 16, the investigation of the population structure by Principal Component, admixture analysis and neighbour joining approaches grouped the Rwanda indigenous chicken as one population. The means that there is horizontal gene flow probably due to small geographical size of the country. A study done in Kenya using microsatellite grouped IC based on MHC-linked markers with grouped Indigenous Chickens into three groups, composed of birds from dissimilar ecotypes while clustering based on non-MHC markers placed indigenous chicken into two gene pools (Ngeno *et al.*, 2015). The absence of geographical boundries, the purchasing of the seeder flocks from one geographical location to another and the free-range system of chicken management in the tropics might be the reason of increase in the interbreeding among the IC resulting in one Rwanda IC population. Common cultural practices like intermarriages among the tribes might have attributed to recurrent flow of gene in the locations. Equally, interactions of humans among countries like Kenya through trade lead to mixing up of IC (Mwacharo *et al.*, 2013b; Mushi *et al.*, 2020) [29, 27]. Chromosome 16 was analyzed because is houses MHC. Studies have reported that MHC is related with disease resistance and immune traits (Fulton *et al.*, 2016, 2017) [14]. Heterozygosity can also be used in the analysis og diversity of a population genetics. The mean of heterozygosity of a population indicates the level of its constancy. When the heterozygosity of a population is low, this notifies there is a high genetic constancy (Cheng, 2010) [8]. This study revealed that observed heterozygosity of the Indigenous Chicken population from the four agro ecological zones was ranging from 0 to 0.44 with an average of 0.11, while expected heterozygosity ranged from 0.007 to 0.5 with mean of 0.21. This study also revealed that the value of observed heterozygosity was lower than expected heterozygosity thus this could be attributed to forces like inbreeding and the F estimates (0.43). In this study classification according to immunological traits based on Newcastle disease antibody titers revealed selection pressure of indigenous chicken in indigenous chicken in Rwanda.

Conclusions

Phenotypic and genotypic clustering found 2 gene pools of IC in Rwanda. This shows that phenotype can also be used in the identification of different chicken ecotypes. Based on population structure analysis using SNPs the Rwanda Indigenous chicken belong to two genetically different groups at the whole genome. Population structure analysis at chromosome 16, placed the IC population as one clutter. The observed genetic diversity of the indigenous chicken for disease resistance should be well-thought-out when scheming a selection programme to ensure that the ICs population is sustainable, flexible and simultaneous improvement of this trait. Based on this study's findings government should implement strategies that conserve and maintain the genomic diversity of Rwanda indigenous chicken.

Ethics approval

This study used indigenous chicken to collect the data and the study was approved by the ethical committee of Egerton University, Nakuru, Kenya.

Author ORCIDs
E. Mbakaya https://orcid.org/0000-0003-1336-6426
K. Ngeno https://orcid.org/0000-0002-9271-7839
T. Muasya https://orcid.org/my-orcid?orcid=0000-0002-8758-7961

Author contributions
Conceptualization: E. Mbakaya, K. Ngeno, T. Muasya; methodology: E. Mbakaya, K. Ngeno, T. Muasya; software: E. Mbakaya, K. Ngeno, T. Muasya; Formal analysis: E. Mbakaya, K. Ngeno, T. Muasya; Visualization: E. Mbakaya; Writing- original draft: E. Mbakaya; Writing – review and editing: K. Ngeno, T. Muasya. All authors have read and agreed to the published version of the manuscript.

Declaration of interest: The authors declare no conflict of interest.

Acknowledgements
The authors gratefully acknowledge the colleagues in the Egerton University for their helpful comments on the manuscript. We would love to acknowledge Centre of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM) program for funding this project.

References
1. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. Genome research. 2009;19(9):1655-1664.
2. Alexander DH, Lange K. Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC Bioinformatics. 2011;12(1):1-6.
3. Amornbunchornvej C, Wangkumhang P, Tongsima S. ipADMIXTURE: R package for inferring sub-population clusters based on genetic admixture, Bio Rxiv, 2020. https://doi.org/10.1101/2020.03.21.001206.
4. Bayyari GR, Huff WE, Rath NC, Balog JM, Newberry LA, Villines JD, *et al.* Effect of the genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. Poultry Science. 1997;76(2):289-296.
5. Biscarini F, Nicolazzi EL, Stella A, Boettcher PJ, Gandini G. Challenges and opportunities in genetic improvement of local livestock breeds. Frontiers in Genetics. 2015;6:33.
6. Bishop SC, Woolliams JA. Genomics and disease resistance studies in livestock. Livestock Science. 2014;166:190-198.
7. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics. 2007;23(19):2633-2635.
8. Cheng HW. Breeding of tomorrow's chickens to improve well-being. Poultry Science. 2010;89(4):805-813.
9. Dar MA, Mumtaz PT, Bhat SA, Nabi M, Taban Q, Shah RA, *et al.* Genetics of disease resistance in chicken. Application of Genetics and Genomics in Poultry Science, 2018, 162-174.
10. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, *et al.* 1000 Genomes Project Analysis Group. The variant call format and vcftools. Bioinformatics. 2011;27(15):2156-2158.
11. Delany ME. Genetic variants for chick biology research: from breeds to mutants. Mechanisms of Development. 2004;121(9):1169-1177.
12. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software Structure: A simulation study. Molecular Ecology. 2005;14(8):2611-2620.
13. Eggen A. The development and application of genomic selection as a new breeding paradigm. Animal Frontiers. 2012;2(1):10-15.
14. Fulton JE, McCarron AM, Lund AR, Pinegar KN, Wolc A, Chazara O, *et al.* A high-density SNP panel reveals extensive diversity, frequent recombination and multiple recombination hotspots within the chicken major histocompatibility complex B region between BG2 and CD1A1. Genetics Selection Evolution. 2016;48(1):1-15.
15. Getabalew M, Alemneh T, Akeberegn D, Getahun D, Zewdie D. epidemiology, Diagnosis & Prevention of Newcastle disease in poultry. American Journal of Biomedical Science & Research. 2019;16(1):50-59.
16. Godinho R, Crespo EG, Ferrand N. The limits of mtDNA phylogeography: complex patterns of population history in a highly structured Iberian lizard are only revealed by the use of nuclear markers. Molecular Ecology. 2008;17(10):4670-4683.
17. Groenen MA, Wahlberg P, Foglio M, Cheng HH, Megens HJ, Crooijmans RP, *et al.* A high-density SNP-based linkage map of the chicken genome reveals sequence features correlated with recombination rate. Genome Research. 2009;19(3):510-519.
18. Habimana R, Okeno TO, Ngeno K, Mboumba S, Assami P, Gbotto AA, *et al.* Genetic diversity and population structure of indigenous chicken in Rwanda using microsatellite markers. PloS One. 2020;15(4):1-15.
19. Jie H, Liu YP. Breeding for disease resistance in poultry: opportunities with challenges. World's Poultry Science Journal. 2011;67(4):687-696.
20. Kapczynski DR, Afonso CL, Miller PJ. Immune responses of poultry to Newcastle disease virus. Developmental and Comparative Immunology. 2013;41(3):447-453.
21. Li YC, Ledoux DR, Bermudez AJ, Fritsche KL, Rottinghaus GE. The individual and combined effects of fumonisin B1 and moniliformin on performance and selected immune parameters in turkey poults. Poultry Science. 2000;79(6):871-878.
22. Luo C, Qu H, Ma J, Wang J, Li C, Yang C, *et al.* Genome-wide association study of antibody response to Newcastle disease virus in chicken. BMC Genetics. 2013;14(1):1-9.
23. Lu H, Giordano F, Ning Z. Oxford Nanopore MinION sequencing and genome assembly. Genomics, Proteomics & Bioinformatics. 2016;14(5):265-279.
24. Jain M, Olsen HE, Paten B, Akeson M. The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. Genome Biology. 2016;17(1):1-11.
25. Magothe TM, Okeno TO, Muhuyi WB, Kahi AK. Indigenous chicken production in Kenya: I. Current status. World's Poultry Science Journal. 2012;68(1):119-132.
26. Mujyambere V, Adomako K, Olympio SO, Ntawubizi M, Nyinawamwiza L, Mahoro J, *et al.* Local chickens in East African region: their production and potential. Poultry Science. 2022;101(1):101547.
27. Mushi JR, Chiwanga GH, Amuzu-Aweh EN, Walugembe M, Max RA, Lamont SJ, *et al.* Phenotypic variability and population structure analysis of Tanzanian free-range

local chickens. BMC Veterinary Research. 2020;16(1):1-10.
28. Mwacharo JM, Bjørnstad G, Mobegi V, Nomura K, Hanada H, Amano T, *et al.* Mitochondrial DNA reveals multiple introductions of domestic chicken in East Africa. Molecular Phylogenetics and Evolution. 2011;58(2):374-382.
29. Mwacharo JM, Nomura K, Hanada H, Han JL, Amano T, Hanotte O. Reconstructing the origin and dispersal patterns of village chickens across E ast A frica: insights from autosomal markers. Molecular Ecology. 2013;22(10):2683-2697.
30. Nxumalo N, Ceccobelli S, Cardinali I, Lancioni H, Lasagna E, Kunene NW. Genetic diversity, population structure and ancestral origin of KwaZulu-Natal native chicken ecotypes using microsatellite and mitochondrial DNA markers. Italian Journal of Animal Science. 2020;19(1):1277-1290.
31. Mwacharo JM, Jianlin H, Amano T. Native African chicken: valuable genetic resources for future breeding improvement. The Journal of Animal Genetics. 2006;34(2):63-69.
32. Mwacharo JM, Nomura K, Hanada H, Jianlin H, Hanotte O, Amano T, *et al.* Genetic relationships among Kenyan and other East African indigenous chickens. Animal Genetics. 2007;38:485-490.
33. Okoth Noah Okumu, Ngeranwa JJN, Binepal YS, Kahi AK, Bramwel WW, Ateya LO, *et al.* Genetic diversity of indigenous chickens from selected areas in Kenya using microsatellite markers. Journal of Genetic Engineering and Biotechnology. 2017;15(2):489-495.
34. Padhi MK. SWOT analysis of poultry meat chain in Romania. Romanian Journal of Economics. 2015;41(2(50):238-246.
35. Mwambene PL, Kyallo M, Machuka E, Githae D, Pelle R. Genetic diversity of 10 indigenous chicken ecotypes from Southern Highlands of Tanzania based on Major Histocompatibility Complex-linked microsatellite LEI0258 marker typing. Poultry science. 2019;98(7):2734-2746.
36. Saelao P, Wang Y, Chanthavixay G, Gallardo RA, Wolc A, Dekkers J, *et al.* Genetics and genomic regions affecting response to newcastle disease virus infection under heat stress in layer chickens. Genes. 2019;10(1):61-72.
37. Schmid M, Smith J, Burt DW, Aken BL, Antin PB, Archibald AL, *et al.* Third report on chicken genes and chromosomes. Cytogenetic and Genome Research. 2015;145(2):78-179.
38. Sodeland M, Kent MP, Olsen HG, Opsal MA, Svendsen M, Sehested E, *et al.* Quantitative trait loci for clinical mastitis on chromosomes 2, 6, 14 and 20 in Norwegian Red cattle. Animal Genetics. 2011;42(5):457-465.
39. Sweeney T, Hanrahan JP, Ryan MT, Good B. Immunogenomics of gastrointestinal nematode infection in ruminants–breeding for resistance to produce food sustainably and safely. Parasite Immunology. 2016;38(9):569-586.
40. Slifer SH. PLINK: key functions for data analysis. Current Protocols in Human Genetics. 2018;97(1):50-59.
41. Tian F, Sun D, Zhang Y. Establishment of paternity testing system using microsatellite markers in Chinese Holstein. Journal of Genetics and Genomics. 2008;35(5):279-284.
42. Van Der Waaij EH, Megens HJ, Kahi AK, Van Arendonk JAM, Crooijmans RPMA. Genetic diversity of different indigenous chicken ecotypes using highly polymorphic MHC-linked and non-MHC microsatellite markers. Animal Genetic Resources/Resources génétiques animales/Recursos genéticos animals. 2015;56(1):1-7
43. Walugembe M, Mushi JR, Amuzu-Aweh EN, Chiwanga GH, Msoffe PL, Wang Y, *et al.* Genetic analyses of Tanzanian local chicken ecotypes challenged with Newcastle disease virus. Genes. 2019;10(7):546-557.
44. Weir BS, Cockerham CC. Estimating F-statistics for the analysis of population structure. Evolution, 1984, 1358-1370.
45. Wollstein A, Lao O, Becker C, Brauer S, Trent RJ, Nürnberg P, *et al.* Demographic history of Oceania inferred from genome-wide data. Current Biology. 2010;20(22):1983-1992.
46. Wondmeneh E, Van Arendonk JAM, Van der Waaij EH, Ducro BJ, Parmentier HK. High natural antibody titers of indigenous chickens are related with increased hazard in confinement. Poultry Science. 2015;94(7):1493-1498.
47. Zhao S, Zhu M, Chen H. Immunogenomics for identification of disease resistance genes in pigs: a review focusing on Gram-negative bacilli. Journal of Animal Science and Biotechnology. 2012;3(1):1-13.