ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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International Journal of Poultry Science 13 (5): 279-291, 2014
ISSN 1682-8356

Genetic Diversity of the Cameroon Indigenous Chicken Ecotypes

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Abstract: Cameroon has a wide range of agro-ecological zones, having indigenous chicken populations which are thought to be adapted and diversified. Genetic diversity of the Cameroon chicken populations from agro-ecological zones I, II, III and IV was assessed using 25 microsatellite markers. A total of 314 chickens were genotyped, revealing 226 distinct alleles and 24 private alleles (10.62%). The mean polymorphic information content was 0.57. The average observed, expected and unbiased frequencies of heterozygote were 0.60, 0.62 and 0.65 respectively, with the mean Shannon index of 1.21. The global inbreeding coefficient among population was 0.13. Inbreeding coefficient varied significantly with 4.27% variation observed among ecotypes. Within ecotypes the highest diversity was observed in the Bafang-Bakou population having 7.92±4.22 alleles per locus, 168.80±4.73 gene copies, 9 private alleles and 0.68±0.02 expected heterozigosity. However the same region displayed the highest inbreeding coefficient (0.13). In all the populations, 67% of the loci did not deviate significantly from the Hardy-Weinberg. The neighbor-joining tree, UPGMA cladogram as well as the Evanno's population structure parameters revealed existence of 3 clusters in Cameroon chicken populations. The current study confirmed usefulness of microsatellites for studying genetic variation of the Cameroonian indigenous chicken. They demonstrate information on genetic variability of Cameroon local chicken populations, offer steps towards rational decision making prior to genetic improvement and conservation programs, without compromising the existence of each unique genotype.

Key words: Diversity, *Gallus gallus*, indigenous chicken, cameroon

INTRODUCTION

Cameroon has a wide range of agro-ecological zones, extending from the dense humid forest in the south to the semi-arid Sahel area in the northern part of the country. The variations of physico-geographic environmental parameters are suspected to affect animal species diversity and distribution and only the fittest will survive and perpetuate. Subpopulations of local chickens are found in all ranges of agro-ecological zones of Cameroon where they seem to be well adapted. Geographical isolation of the populations could lead to sub-structuring through drift, mutation and different natural selection forces (Muchadeyi *et al*., 2007). However, it is not known whether these ecotypes of chicken in Cameroon represent genetically distinct populations.

Indigenous chickens in Cameroon, as in other developing countries, play an important role to the livelihoods of smallholder families, as the main source of income, meat, egg, social and ritual values. Reports on the diversity of local chicken in Cameroon is restricted to phenotypic data, including adult body phaneroptic and measurements, weight, egg characteristics and production performances (Keambou *et al*., 2007; Fotsa *et al*., 2007; Keambou *et al*., 2009; Keambou and Manjeli 2009; Hako *et al*., 2009a, b; Keambou *et al*., 2010). These chicken populations have been kept over generations, but increasing adoption of commercial hybrids within rural backyard farming is eroding the genetic uniqueness of native breeds (Hosny, 2006), which need to be preserved and improved. Identification of unique and valuable genetic resources for breed improvement, evaluation of their genetic potential and contribution to the future strategies for sustainable management require a prior knowledge of the prevailing genetic diversity (Bordas *et al*., 2004).

The current study was carried out to assess the degree of diversity within and phylogenetic relationship between

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ecotypes and genotypes of Cameroon local chicken using 25 microsatellite markers.

MATERIALS AND METHODS

Sample collection and DNA extraction: Cameroon extends from 2° to 13° N which gives it almost all the characteristics of inter-tropical climates which generally include hot, humid and dry conditions. The south has an equatorial climate up to latitude 6° N; while between latitudes 6° and 13° N, has a tropical climate. Relief and oceanic effects modify local climates (Pamo, 2008). Samples were collected in four (Sudano Sahelian, Sudano Guinean, Western Highlands and humid forest with monomodal rainfalls) of the five different agro-ecological zones of Cameroon.

A total of 314 unrelated chickens were sampled, comprising five phenotypes of local chickens, two broilers and two layers commercial lines and a crossbred, a one generation selected local chicken. The description of the local chicken was reported earlier (Keambou *et al*., 2007). Populations were inferred based on Agro-ecological zone and phenotype. A drop of blood was sampled from the cubital vein of each bird onto Whatman FTA™ filter cards (Whatman International Ltd), allowed to dry under shade for about one hour and kept in separate envelop and room temperature until processing. Genomic DNA was isolated using a boiling method as described by Smith and Burgoyne (2004).

PCR amplification and DNA polymorphism: Twenty five fluorescently-labeled polymorphic microsatellite markers were selected, based on the degree of polymorphism and genome coverage (FAO, 2011). PCR reactions were carried out in a volume of 10 µL, containing 20 ng target DNA, 1X DreamTaq buffer, 0.2 µM dNTP's, 0.08 u/µL DreamTaq DNApolymerase and 0.2 µM of each forward and reverse primer. Thermal cycling was carried out in a GeneAmp® PCR System 9700 thermal cycler (Applied biosystems) with the following program: 1 min at 94°C followed by 35 cycles consisting of 30 sec at 94°C, 30 sec at 60°C, 30 sec at 72°C and a final extension step of 10 min at 72°C. Samples were analyzed on an ABI PRISM 377 DNA Sequencer. GeneScan™-500 LIZ® (Applied Biosystems) was used as internal size standard. The GeneMapper version 4.1 (Applied Biosystems) was used to determine the fragment sizes in base pairs.

Data analysis: Total number of alleles, allele frequencies and average number of alleles per locus, observed heterozygosity, expected heterozygosity, Polymorphism Information Content (PIC) and inbreeding coefficients (F_{IT}, F_{ST} and F_{IS}) and ANOVA were determined using the Arlequin version 1.1. and GenAlEx software version 6.3 (Peakall and Smouse, 2006). Pairwise FST (proportion of genetic variability due to population sub-structuring) values were computed for all pairs of ecotypes and populations using the Arlequin software package. Nei's standard genetic distances (Nei, 1972) were estimated among pairs of populations using the GenAlEx software version 6.3. The Evanno method (Evanno *et al*., 2005) of estimation of the more likely number of cluster was implemented according to Dent Earl and Bridgett (2011). The algorithm implemented in STRUCTURE was used to cluster individuals based on multilocus genotypes (Pritchard *et al*., 2000). The analysis involved an admixture model with correlated allele frequencies. The model was tested using 20,000 iterations (burn-in phase) and then 50,000 iterations for 2 = K = 8 with 100 runs for each K value, where K was the number of assumed clusters to be examined. A pairwise comparison of the 100,000 solutions was carried out. Solutions with over 95% similarity were considered identical. An unrooted Neighbor-Joining cladogram was obtained based on pair-wise kinship distance matrix between populations using the Neighbor-Joining program implemented in PHYLIP (Felsenstein, 1995). A consensus tree, evaluated by 1,000 bootstraps across the set of loci, was constructed.

RESULTS

Marker polymorphism and genetic composition of chicken populations: Marker polymorphism and genetic composition of the Cameroon chicken populations are presented in Table 1. The total number of alleles was 226 across populations, with an average of 9.04 alleles per microsatellite marker. The mean effective number of alleles was 3.13. The locus with the lowest number of alleles was MCW0103 with two) while LEI0192 gave 22 alleles. The least number of allele (81) was found in the Kaélé chicken population, while the highest (198) was found in Bafang-Bakou population. The mean polymorphic information content (PIC) was 0.57. There were 24 distinct private alleles (10.62%) which were mainly observed in the Bafang-bakou local chicken population.

The overall mean of observed and unbiased heterozigosity where respectively 0.60, 0.62 and 0.65 with the Shannon index of 1.21. The global inbreeding coefficients over all populations, among population and within population (F_{IT}, F_{ST} and F_{IS}) were 0.13; 0.08 and 0.03 respectively, leading to a fixation index of 0.03. The mean number of migrant per generation in the overall population and across all the loci was found to be 2.91. The intra-ecotype diversity (Table 2) of Cameroonian chicken inferred from mean ecotype expected heterozigosity varied from 0.51 to 0.68, while the observed heterozigosity varied from 0.50 to 0.68. The ecotypes could be classified as low-diversity class (ecotypes I, II and IV) and moderate diversity class (ecotypes III and selected local).

Table 1: Marker polymorphism across the studied Cameroonian chicken population

Primer name	Chr	N	SR (bp)	NA	Ne	PIC	Ho	UHe	I	F_{IT}	F_{ST}	F_{IS}	F	Nm
ADL0112	10	311	122-130	5	3.02	0.59	0.61	0.67	1.18	0.13	0.08	0.05	0.057	2.74
ADL0268	1	307	102-114	6	3.01	0.59	0.66	0.68	1.18	0.05	0.06	-0.01	-0.01	3.78
ADL0278	8	312	112-122	5	2.60	0.53	0.58	0.63	1.03	0.13	0.08	0.05	0.05	2.76
LEI0094	4	314	249-285	15	3.87	0.68	0.64	0.75	1.56	0.11	0.06	0.06	0.06	4.15
LEI0166	3	314	347-361	5	1.88	0.38	0.47	0.45	0.76	0.06	0.07	-0.01	-0.02	3.32
LEI0192	6	304	256-372	22	4.50	0.72	0.67	0.78	1.66	0.19	0.08	0.11	0.10	2.68
LEI0234	2	314	215-367	21	6.41	0.80	0.69	0.85	1.98	0.18	0.07	0.12	0.11	3.13
MCW0014	6	314	164-186	8	2.12	0.44	0.52	0.54	0.87	0.13	0.09	0.04	0.02	2.48
MCW0016	3	304	138-174	8	3.58	0.67	0.74	0.74	1.41	0.02	0.06	-0.04	-0.03	4.03
MCW0020	1	310	176-186	5	2.99	0.60	0.69	0.68	1.20	0.03	0.06	-0.03	-0.04	4.03
MCW0034	2	314	174-248	16	4.89	0.75	0.74	0.81	1.73	0.11	0.09	0.02	0.02	2.49
MCW0037	3	314	152-156	3	2.50	0.51	0.49	0.61	0.96	0.25	0.09	0.17	0.18	2.40
MCW0067	10	312	139-183	8	2.52	0.51	0.61	0.59	1.01	0.11	0.14	-0.03	-0.06	1.58
MCW0069	26	311	152-174	10	2.91	0.58	0.63	0.66	1.22	0.03	0.05	-0.02	-0.02	4.92
MCW0081	5	312	111-133	8	2.11	0.42	0.45	0.50	0.85	0.15	0.10	0.05	0.09	2.22
MCW0103	3	314	266-270	2	1.71	0.32	0.47	0.42	0.59	-0.01	0.08	-0.10	-0.09	2.94
MCW0104	13	298	186-226	17	4.41	0.72	0.69	0.78	1.71	0.15	0.09	0.06	0.05	2.41
MCW0123	14	314	76-94	9	3.45	0.65	0.65	0.72	1.40	0.08	0.05	0.04	0.03	5.13
MCW0165	23	310	101-131	9	3.10	0.60	0.47	0.67	1.29	0.37	0.10	0.30	0.32	2.20
MCW0183	7	307	296-322	10	3.41	0.64	0.79	0.71	1.36	-0.03	0.10	-0.143	-0.13	2.22
MCW0206	2	309	221-245	8	2.89	0.58	0.67	0.66	1.18	0.048	0.10	-0.06	-0.06	2.29
MCW0222	3	314	216-222	4	2.42	0.49	0.46	0.58	0.98	0.20	0.11	0.10	0.08	2.08
MCW0248	1	314	212-222	4	1.62	0.29	0.42	0.36	0.57	-0.01	0.11	-0.14	-0.09	2.02
MCW0295	4	309	85-103	9	3.22	0.63	0.69	0.70	1.34	0.06	0.09	-0.03	-0.03	2.54
MCW0330	17	307	248-288	9	3.08	0.61	0.53	0.69	1.28	0.20	0.10	0.11	0.13	2.21
Mean				9.04	3.13	0.57	0.60	0.65	1.21	0.13	0.08	0.03	0.03	2.91

N = number of genotyped individuals
Chr = chromosome
SR = observed allele size range
NA = observed number of alleles
Ne = effective number of alleles
PIC = polymorphic information content
HO = observed heterozygosity
HE = expected heterozygosity
I = Shannon index, FIT, FST
F = fixation index
FIS = inbreeding coefficient over all populations, among populations and within populations
Nm = number of migrants

Table 2: Standard Genetic diversity indices among Cameroon chicken ecotypes

	Number of							Gene diversity		p-value
Ecotypes	Loci	UL	PL	Alleles per locus	Gene copies	Ho	He	over loci	F_{IS}	(Rand F_{IS} = obs F_{IS})
I	25	24	24	3.24±1.16 (2-6)	7.92±0.40	0.60±0.29	0.64±0.18	0.63±0.36	0.07	0.304
II	25	25	24	3.72±1.77 (1-9)	24.00±0.00	0.50±0.26	0.51±0.23	0.51±0.27	0.07	0.104
III	25	24	24	8.80±4.96 (2-21)	452.76±7.17	0.60±0.13	0.68±0.13	0.66±0.33	0.11	0.000
IV	25	25	25	4.48±2.12 (2-9)	20.00±0.00	0.58±0.19	0.65±0.16	0.65±0.34	0.12	0.025
Local selected	25	22	22	4.72±1.84 (2-10)	25.68±0.94	0.65±0.14	0.68±0.11	0.67±0.34	0.03	0.277
Commercial	25	25	25	5.84±2.97 (2-13)	117.52±0.87	0.62±0.15	0.67±0.13	0.66±0.33	0.06	0.005

UL = usable loci
Ho = observed heterozigosity
PL = polymorphic loci, (3) range on number of alleles per locus
He = expected heterozigosity

All the loci were polymorphic in commercial strains and in chicken from the 4th agro-ecological zone of Cameroon. Conversely, the least number of PIC was found in local chicken (88%) which has undergone one generation selection, whereas their counterpart from zones I, II and III all displayed 96% of polymorphic loci. The minimal being observed in zone I (3.24), while the highest was from zone III which also showed the locus with the maximum number of alleles and genes copies. The chicken ecotypes from agro-ecological zones III and IV exhibited the highest degree of inbreeding (F_{IS}), hence 0.11 and 0.12, respectively; significant at 99.9% and 95% confidence interval respectively. Inbreeding observed in commercial strains (0.06) which is significant at 99% confidence interval.

The standard genetic diversity indices varied among Cameroon chicken ecotypes. The higher number of mean alleles per locus and genes copies in the 3rd agro-ecological zone as well as the lower values obtained in the 1st zone may have been influenced by the number of individuals sampled in each zone.

Table 3 present the analysis of molecular variance of all loci for ecotypes.

The analysis of molecular variance reveals that there is only 4.27% of variation among ecotypes of Cameroon local chicken and 10.36% variation among individuals within ecotypes. The greatest variability (85.36%) is within individuals.

A similar pattern of inbreeding, as displayed but F_{IS} and F_{ST} was observed using AMOVA analysis which indicated that 95.72% of the genetic variation was found among individuals within populations and the difference among ecotypes represented less than 5% of the total variability.

Despite the low variability among ecotypes, the proportion of genetic variability due to population sub-structuring (pairwise FST) among Cameroon ecotype population, showed almost a 95% confidence interval of significant distance.

The standard genetic diversity indices among Cameroon chicken population showed that there still a variation within ecotypes, same as for the Hardy-Weinberg equilibrium over loci. This diversity was confirmed by the heterozigosity and F-statistics in different chicken populations. In this study, the expected heterozigosity was higher than the observed in all populations, excepted for broiler, layer and their crossbreds. All populations were in Hardy-Weinberg equilibrium.

Matrix of pairwise genetic distances between ecotypes showed a no significant genetic distant between ecotype I and IV. The same was true for ecotype I and selected local chicken population On the other hand the genetic distance between all the other groups were significant ($p = 0.05$) (Table 4).

Table 5 shows the genetic diversity indices among Cameroon chicken populations.

The percentage of polymorphic loci within populations varied from 84% (Bafang-Bakou) to 100% (Dschang, Foumban, Buea-Nkongsamba, Broiler and Layer). The mean number of alleles per locus varied from 3.60 (crossbreds population) to 7.92 in the Bafang-Bakou population where the maximum number of gene copies (168.80) was found. However, the less diversity over loci was that of the Ngaoundéré population (0.51) whereas a highest diversity of 0.67 was commonly found in Balengou-Bangangté, Dschang, selected local and crossbred populations. The highest number of private alleles was found in Bafang-Bakou (9) followed by Balengou-Bangangté (4) and Babadjou group (3) population.

The Hardy-Weinberg equilibrium was tested against all loci and populations and is shown in Table 6.

Sixty seven percent (67%) of the overall loci do not deviate from the Hardy-Weinberg law. Only one monomorphic loci was found in Ngaoundéré population. The most equilibrated populations where the crossbreds, Buea-Nkongsamba and Kaélé, with only 1 (4%), 2 (8%) and 5 (20%) loci respectively deviating from the HWE, assuming that they still keep some characteristics of primary populations. Conversely, the most genetically unstable population was that of Bafang-Bakou, even though it had the highest diversity.

The observed number of alleles varied from 3.60 to 7.92. The highest number of alleles has been noticed in Bafang-Bakou, Babadjou block, Balengou-Bangangté, Foumban and Dschang, all populations of the 3rd agro-ecological zone of Cameroon. The effective number of alleles varied from 2.38-3.62. The Shannon index (I) expressing the population diversity in specific habitat is highest in Bafang-Bakou (1.42) and lowest in Kaélé (0.98) and Ngaoundéré (0.92). Further, the lowest observed heterozigosity is that of Ngaoundéré (0.48) while the greatest was found in selected local chicken population (0.65).According to populations, observed heterozigosity varied from 0.48 (Ngaoundéré) to 69 (crossbred). The P value of HWE displayed in Table 7 denoted that the observed and expected heterozigosity do not differentiate significantly, hence, considering all the loci, none of the local chicken population is diverging from the HW law. This is also confirm by the fixation index (F) and the inbreeding coefficient (F_{IS}) indices which are low. The negative values of these parameters expressed an excessive heterozigosity in broiler, layers, crossbreds, selected local and Kaélé chicken populations. However, none of these inbreeding coefficients is statistically different from the random F_{IS} value (Table 7).

The analysis of molecular variance presented in Table 8 demonstrated that 4.26% of the total variation was due to differences among populations, 8.99% among individuals within population and (86.74%) accounted for differences within individuals.

Table 3: Analysis of molecular variance over all loci for ecotypes

Source of variation	Sum of square	Variance components	Percentage variation
Among ecotypes	160.810	0.3621	4.27214
Among individuals within ecotypes	2840.539	0.91252	10.36227
Within individuals	2331.500	7.51741	85.36559
Total	5332.849	8.80614	

Table 4: Ecotypes pairwise Genetic distances (10100 permutations)

Ecotypes	I	II	III	IV	Local selected	Commercial
I	0.000					
II	0.09972*	0.000				
III	0.02852*	0.06665*	0.000			
IV	0.04102ns	0.10574*	0.01944*	0.000		
Local selected	0.01758ns	0.09655*	0.01701*	0.03988*	0.000	
Commercial	0.06923*	0.13781*	0.04034*	0.05787*	0.05818*	0.000

*p = 0.05, ns = non-significant

Genetic distance among populations: The pairwise population matrix of Nei genetic distance and identity is shown in Table 9, from which it comes that the genetic distances are very low within the first six populations, all from the 3rd agro-ecological zone of Cameroon. These distances increase when a comparison is made with populations of other agro-ecological zones and highest with exotic breeds. The highest genetic distance was found between layers and Kaélé chicken population, followed by that between broilers and Ngaoundéré, while the least genetic distance was found between Bafang-Bakou and Balengou-Bangangté population.

On the contrary of genetic distances, Nei genetic identities coefficient are highest among populations from the third agro-ecological zones.

Identity coefficients reduce progressively from the highest (0.964) found between the Babadjou group and Bafang-Bakou, to the lowest between layers and Ngaoundéré population (0.648).

The coancestry coefficients expressed as Reynolds distances and displayed in Table 10, reveal that the highest coefficient was obtained between crossbred and broiler (0.232), while the lowest (0.005) is found between Babadjou group and Bafang-Bakou, both populations of the 3rd agro-ecological zones of Cameroon.

The Wright coefficient, showing the standardized variance between populations is presented in Table 11. It is noticed that only coefficients between Buea-Nkongsamba against all other populations are statistically significant (p = 0.05). The highest significant Wright coefficient (0.163) is found between this pervious population and the crossbred chicken, followed by coefficients between broiler and Bue-Nkongsamba (0.133) and Dschang (0.128), respectively.

The pairwise FST values among population are lower than those obtained by Fotsa *et al.* (2011), but similar to those of Eltanany (2010). Most of these values are however significant reveal a certain level of differentiation. That differentiation is displayed by the neighbour joining tree and UPGMA cladogram, which when coupled to the Evanno population parameters reveals the existence of sub-structuring in the Cameroon local chicken population.

The best solution for structure (Pritchard *et al.*, 2000) from 2 = k = 8 was achieved. The Evanno table output describing population's structure parameters is presented in Table 12 and Fig. 1 and 2 illustrate the evolution of the mean estimate of ln probability of data and delta K estimating of the more likely number of cluster in Cameroonian local chicken population.

As demonstrated by Evanno *et al.* (2005), the L (K) did not show a clear mode for the true number of clusters, but the salient brake in slope of its evolution is noticed at the true K. however and ad hoc quantity based on the second rate of change of the likelihood function with respect to K did show the real peak at the true value of K. then, it is most probable for us to have 3 main distinct cluster in Cameroon local chicken.

The results obtained showed that the more likely number of cluster is 3. This is in agreement with the clades displayed by the neighbor-Net network and UPGMA methods (Fig. 3).

In agreement with Nei distances and Wright coefficients, the phylogenetic relationship by neighbour-Joining tree (a) and UPGMA cladogram (b) of Fig. 3 show that local chicken populations from the western highlands of Cameroon (agro-ecological zone III) tend to cluster together and with those from zone IV. Further, indigenous chicken from zones I and II are forming different distinct clades. These two groups of local chicken population are separated by the commercial chicken represented by Layers, broilers and their crossbreeds.

Most likely, the 3 groups of chicken's population in Cameroon are made of two locals (Northern (agro-zone I and II) and southern (agro zone II and IV) and a commercial group of broilers and layer). However, divergences within clusters were observed in the Neighbour-joining tree.

Table 5: Genetic diversity indices among Cameroon chicken populations

	Number of								Gene diversity
Population	Loci	UL	PL	Alleles	Alleles per locus	Gene copies	PA	Theta (H)	over loci
Bbj_Btchm_Mbda_Glm	25	22	22	160	6.40± 3.12	66.88±1.64	3	2.20	0.66±0.33
Bafang_Bakou	25	21	21	198	7.92±4.22	168.80±4.73	9	2.15	0.65±0.32
Blng_Bgnte	25	23	23	158	6.32±3.00	71.04±1.84	4	2.21	0.67±0.34
Buea_Nkong	25	25	25	112	4.48±2.12	20.00±0.00	0	1.90	0.65±0.34
Dschang	25	25	25	153	6.12/3.23	61.68±0.748	1	2.09	0.67±0.33
Foumban	25	25	25	164	6.56±3.241	57.52±0.87	2	1.98	0.65±0.33
Kaélé	25	24	24	81	3.24±1.16	7.92±0.40	0	1.78	0.63±0.36
Ngaoundéré	25	25	24	93	3.72±1.77	24.00±0.00	0	1.05	0.51±0.27
Local selected	25	22	22	118	4.720±1.84	25.68±0.94	1	2.13	0.67±0.34
Broiler	25	25	25	121	4.84±1.89	57.84±0.55	2	1.70	0.63±0.31
Layer	25	25	25	112	4.48±1.78	49.76±0.66	2	1.51	0.60±0.30
Crossbred	25	24	24	90	3.60±1.12	9.92±0.40	0	2.08	0.67±0.37

UL: Usable loci, PA = private alleles, PL: Polymorphic loci

Table 6: Tests for Hardy-Weinberg equilibrium probability of loci in cameroon chicken populations

Locus	Bbdj Btchm Mbda Glm	Bfng Bkou	Blng Bgnte	Buea Nkng	Dschang	Foumban	Kaélé	Ndere	Lcl sel	Broiler	Layer	Crossebred
ADL0112	0.000***	0.000***	0.599^{ns}	0.761^{ns}	0.000***	0.794^{ns}	0.046*	0.001***	0.037*	0.086^{ns}	0.000***	0.528^{ns}
ADL0268	0.401^{ns}	0.000***	0.109^{ns}	0.818^{ns}	0.921^{ns}	0.985^{ns}	0.261^{ns}	0.237^{ns}	0.157^{ns}	0.840^{ns}	0.144^{ns}	0.710^{ns}
ADL0278	0.511^{ns}	0.195^{ns}	0.282^{ns}	0.245^{ns}	0.417^{ns}	0.001***	0.046*	0.277^{ns}	0.768^{ns}	0.000***	0.125^{ns}	0.801^{ns}
LEI0094	0.031*	0.000***	0.000***	0.640^{ns}	0.006**	0.558^{ns}	0.046*	1.000^{ns}	0.728^{ns}	0.039*	0.000***	0.544^{ns}
LEI0166	0.378^{ns}	0.000***	0.710^{ns}	0.880^{ns}	0.297^{ns}	0.081^{ns}	0.775^{ns}	0.880^{ns}	0.914^{ns}	0.046*	0.593^{ns}	0.708^{ns}
LEI0192	0.991^{ns}	0.000***	0.911^{ns}	0.003**	0.780^{ns}	0.030*	0.695^{ns}	0.049*	0.271^{ns}	0.242^{ns}	0.022*	0.451^{ns}
LEI0234	0.019*	0.000***	0.030*	0.425^{ns}	0.959^{ns}	0.000***	0.382^{ns}	0.001***	0.003**	0.616^{ns}	0.000***	0.353^{ns}
MCW0014	0.000***	0.000***	0.000***	0.190^{ns}	0.000***	0.000***	0.505^{ns}	0.842^{ns}	0.046*	0.576^{ns}	0.000***	0.223^{ns}
MCW0016	0.503^{ns}	0.521^{ns}	0.040*	0.675^{ns}	0.947^{ns}	0.300^{ns}	0.586^{ns}	0.138^{ns}	0.523^{ns}	0.102^{ns}	0.512^{ns}	0.756^{ns}
MCW0020	0.396^{ns}	0.000***	0.106^{ns}	0.425^{ns}	0.096^{ns}	0.001***	0.230^{ns}	0.006**	0.565^{ns}	0.597^{ns}	0.008**	0.396^{ns}
MCW0034	0.003**	0.091^{ns}	0.773^{ns}	0.949^{ns}	0.023*	0.161^{ns}	0.586^{ns}	0.723^{ns}	0.066^{ns}	0.933^{ns}	0.010*	0.125^{ns}
MCW0037	0.728^{ns}	0.093^{ns}	0.217^{ns}	0.741^{ns}	0.839^{ns}	0.063^{ns}	0.172^{ns}	0.704^{ns}	0.864^{ns}	0.134^{ns}	0.139^{ns}	0.180^{ns}
MCW0067	0.000***	0.000***	0.190^{ns}	0.329^{ns}	0.964^{ns}	0.605^{ns}	0.046*	0.195^{ns}	0.037*	0.957^{ns}	0.416^{ns}	0.576^{ns}
MCW0069	0.122^{ns}	0.690^{ns}	0.952^{ns}	0.287^{ns}	0.057^{ns}	0.830^{ns}	0.506^{ns}	0.929^{ns}	0.934^{ns}	0.516^{ns}	0.944^{ns}	0.442^{ns}
MCW0081	0.940^{ns}	0.000***	0.014*	0.245^{ns}	0.646^{ns}	0.000***	0.339^{ns}	0.001***	0.005**	0.799^{ns}	0.979^{ns}	0.576^{ns}
MCW0103	0.093^{ns}	0.772^{ns}	0.960^{ns}	0.527^{ns}	0.337^{ns}	0.356^{ns}	0.775^{ns}	0.488^{ns}	0.166^{ns}	0.184^{ns}	0.158^{ns}	0.576^{ns}
MCW0104	0.001**	0.000***	0.000***	0.246^{ns}	0.001**	0.511^{ns}	0.387^{ns}	0.484^{ns}	0.045*	0.520^{ns}	0.000***	0.890^{ns}
MCW0123	0.002**	0.000***	0.000***	0.084^{ns}	0.315^{ns}	0.000***	0.696^{ns}	0.780^{ns}	0.673^{ns}	0.029*	0.008**	0.847^{ns}
MCW0165	0.000***	0.000***	0.000***	0.007**	0.000***	0.000***	0.349^{ns}	0.032*	0.024*	0.000***	0.000***	0.103^{ns}
MCW0183	0.836^{ns}	0.148^{ns}	0.637^{ns}	0.250^{ns}	0.003**	0.974^{ns}	0.285^{ns}	0.062^{ns}	0.888^{ns}	0.145^{ns}	0.003**	0.427^{ns}
MCW0206	0.599^{ns}	0.000***	0.855^{ns}	0.223^{ns}	0.035*	0.723^{ns}	0.931^{ns}	0.017*	0.862^{ns}	0.272^{ns}	0.702^{ns}	0.019*
MCW0222	0.045*	0.048*	0.007**	0.910^{ns}	0.013*	0.880^{ns}	1.000^{ns}	0.977^{ns}	0.781^{ns}	0.863^{ns}	0.395^{ns}	0.475^{ns}
MCW0248	0.658^{ns}	0.000***	0.015*	0.958^{ns}	0.439^{ns}	0.848^{ns}	0.046*	Mono.	0.279^{ns}	0.578^{ns}	0.470^{ns}	0.708^{ns}
MCW0295	0.042*	0.000***	0.002**	0.141^{ns}	0.212^{ns}	0.693^{ns}	0.227^{ns}	0.182^{ns}	0.632^{ns}	0.758^{ns}	0.170^{ns}	0.411^{ns}
MCW0330	0.000***	0.213^{ns}	0.004**	0.081^{ns}	0.789^{ns}	0.628^{ns}	0.245^{ns}	0.236^{ns}	0.868^{ns}	0.160^{ns}	0.000***	0.116^{ns}

ns = not significant *p<0.05 **p<0.01 ***p<0.001 mono = monomorphic

Table 7: Populations' genetic parameters: heterozygosity, f-statistics and polymorphism by population

Populations	Na	Ne	I	Ho	He	HWE P-value	F	F_{IS}	P-value (Rand FIS = obs FIS)
Bbdj_Btchm_Mbda_Glm	6.40±0.62	3.56±0.27	1.38±0.09	0.62±0.03	0.68±0.03	0.292^{ns}	0.08±0.03	0.082	0.292
Bfng_Bkou	7.92±0.84	3.62±0.33	1.42±0.09	0.58±0.02	0.68±0.02	0.111^{ns}	0.14±0.02	0.137	0.111
Blng_Bgnte	6.32±0.60	3.62±0.31	1.37±0.09	0.60±0.03	0.68±0.02	0.296^{ns}	0.11±0.03	0.110	0.300
Buea_Nkng	4.48±0.42	3.16±0.30	1.19±0.09	0.58±0.19	0.65±0.34	0.442^{ns}	0.07±0.04	0.070	0.442
Dschang	6.12±0.65	3.48±0.31	1.34±0.09	0.62±0.03	0.67±0.03	0.364^{ns}	0.06±0.02	0.063	0.364
Foumban	6.56±0.65	3.44±0.35	1.34±0.09	0.61±0.03	0.65±0.03	0.401^{ns}	0.07±0.03	0.069	0.401
Kaélé	3.24±0.23	2.58±0.20	0.98±0.07	0.60±0.29	0.64±0.18	0.406^{ns}	-0.09±0.08	-0.090	0.406
Ngaoundéré	3.72±0.35	2.38±0.22	0.92±0.09	0.50±0.26	0.51±0.23	0.370^{ns}	0.06±0.06	0.057	0.370
Lcl_selected	4.72±0.37	3.19±0.22	1.25±0.07	0.65±0.14	0.68±0.11	0.445^{ns}	-0.01±0.04	-0.003	0.445
Broiler	4.84±0.38	2.90±0.17	1.18±0.07	0.63±0.04	0.62±0.03	0.399^{ns}	-0.02±0.04	-0.018	0.399
Layer	4.48±0.36	2.83±0.22	1.11±0.08	0.59±0.04	0.59±0.03	0.232^{ns}	-0.01±0.05	-0.008	0.232
Crossebred	3.60±0.22	2.81±0.17	1.09±0.06	0.69±0.05	0.61±0.03	0.478^{ns}	-0.12±0.06	-0.124	0.478

NA = observed number of alleles
Ne = effective number of alleles
Ho = observed heterozygosity
He = expected heterozygosity
I = Shannon index
F = fixation index
ns = not significant deviation from HWE
F_{IT}, F_{ST} and F_{IS} = inbreeding coefficient over all populations, among populations and within populations

Table 8: Analysis of molecular variance for all loci

Source of variation	Sum of square	Variance components	Percentage variation	F-Statistics over all loci
Among population	297.075	0.36915	4.26080	F_{IS} = 0.094
Among individuals within population	2705.394	0.77901	8.99160	F_{ST} = 0.043
Within individuals	2331.500	7.51563	86.74759	F_{IT} = 0.132
Total	5333.969	8.66380		

Table 9: Matrix of Nei Genetic Distance (below diagonal) and identity (above diagonal)

Ecotypes	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
Bbdj_Btchm_Mbda_Glm (1)	-	0.964	0.958	0.901	0.951	0.950	0.833	0.848	0.900	0.826	0.801	0.855
Bfng_Bkou (2)	0.037	-	0.967	0.906	0.963	0.964	0.852	0.886	0.911	0.817	0.789	0.866
Blng_Bgnte (3)	0.043	0.034	-	0.911	0.954	0.953	0.838	0.860	0.903	0.875	0.819	0.884
Buea_Nkng (4)	0.105	0.099	0.094	-	0.897	0.894	0.797	0.801	0.843	0.807	0.721	0.794
Dschang (5)	0.050	0.037	0.048	0.108	-	0.953	0.805	0.841	0.906	0.828	0.785	0.840
Foumban (6)	0.051	0.037	0.048	0.112	0.048	-	0.835	0.867	0.930	0.798	0.773	0.836
Kaélé (7)	0.183	0.160	0.177	0.227	0.216	0.181	-	0.788	0.842	0.723	0.673	0.759
Ngaoundéré (8)	0.165	0.121	0.151	0.222	0.173	0.142	0.239	-	0.818	0.691	0.648	0.758
Lcl_selected (9)	0.105	0.094	0.102	0.170	0.098	0.072	0.172	0.201	-	0.758	0.753	0.801
Broiler (10)	0.191	0.202	0.133	0.214	0.189	0.226	0.324	0.369	0.277	-	0.724	0.794
Layer (11)	0.222	0.237	0.199	0.327	0.242	0.258	0.396	0.435	0.284	0.323	-	0.827
Crossebred (12)	0.156	0.144	0.123	0.231	0.175	0.179	0.276	0.276	0.222	0.231	0.190	-

DISCUSSION

The average number of alleles per marker obtained in this study 9.04 is higher than that reported by Fotsa *et al.* (2011) 7.09 in the 5th agro-ecological zone of Cameroon, by Berthouly *et al.* (2008) for the local European and Asian breeds and that mentioned in Ghana 7.8, Iran 5.4, China 3.8, Egypt 7.3 and Vietnam 5 (Liu *et al*., 2008; Osei-Amponsah *et al.*, 2010; Mohammadabadi *et al.*, 2010; Cuc *et al.*, 2010; Eltanany *et al.*, 2011). However, the values obtained in the present study are in the same range as those from the four varieties of Pakistani Aseel chicken (Babar *et al.*, 2012), Brazilian and Ethiopian chicken ecotypes (Clementino *et al.*, 2010; Nigussie *et al.*, 2011). The mean number of effective alleles (3.13) is 50% less than that obtained by Babar *et al.* (2012) but in conformity with Pandey *et al.* (2003).

Heterozigosity is also known as gene diversity. The level of mean population heterozigosity reflects the degree of population consistency (Chen *et al.*, 2004). The lower the population heterozigosity, the higher population genetic consistency and vice versa. The present work showed that the mean observed heterozigosity of the different chicken population in 25 microsatellites loci ranged from 0.42 to 0.79, while the expected and unbiased heterozigosity ranged from 0.34-0.82 and 0.36-0.81, respectively. This showed that the genetic diversity of chicken in Cameroon is very high. These observations are consistent to that of Fotsa *et al.* (2011) in the 5th agro-ecological zone of Cameroon. Based on these observations it can be stated that the diversity of chicken population in Cameroon is higher than that obtained for local European and Asian chicken breeds (Berthouly *et al.*, 2007), in Chinese native and Pakistani Aseel chicken populations (Chen *et al.*, 2004; Babar *et al.*, 2012). Further, Cameroonian indigenous chicken populations have a comparable level of diversity as their Ethiopian and Egyptian counterparts (Nigussie, 2011; Eltanany *et al.*, 2010), but have a lower diversity as compared to observations made in the southern china (Yu Ya-Bao *et al.*, 2006).

It is considered that loci are highly informative when PIC>0.5, 0.25<PIC<0.5 indicates reasonably informative locus and PIC<0.25 indicates a slightly informative locus (Bostein *et al.*, 1980; Vanhala *et al.*, 1998). As such, 80% of loci studied where highly informative. The highest value of PIC of 0.8 was that of LEI0234 and the mean polymorphism information content of 0.57 indicates that generally the microsatellites loci chosen in this study are of reasonable high quality information on the diversity of Cameroonian chicken populations. The PIC values obtained are higher than those (0.31-0.49) of 11 Chinese local chickens reported by Wu *et al.* (2004), comparable to those of Fu-Xiang *et al.* (2010) but lower as compared to those obtained by Babar *et al.* (2012) for the Pakistani Aseel chicken and for the 12 Chinese

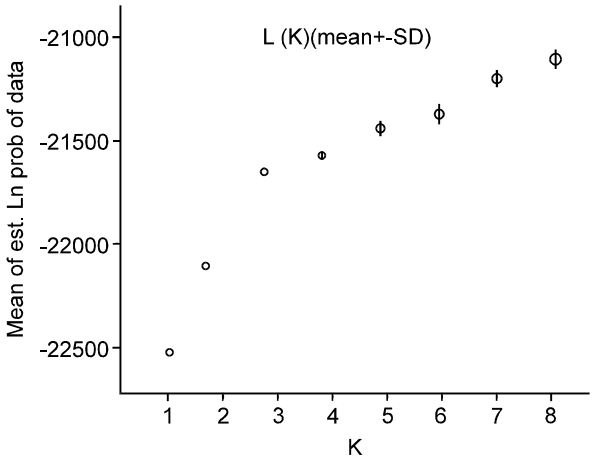

Fig. 1: Evolution of the mean estimate of ln probability of data with the number of clusters (K) in Cameroonian local chicken population

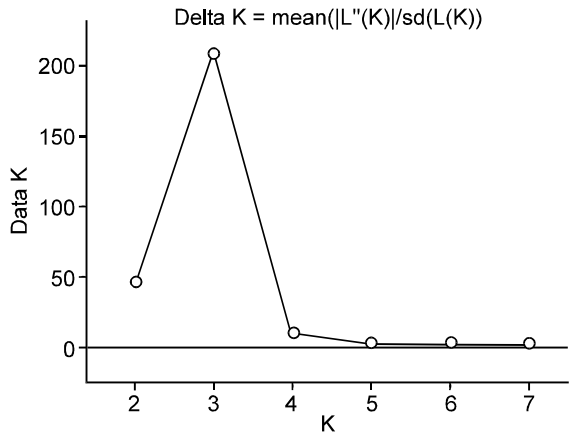

Fig. 2: Evolution of delta K estimating of the more likely number of cluster in Cameroonian local chicken population

indigenous black-bone chicken breeds (0.67) and Bian chicken (0.67) (Tang *et al.*, 2005; Bai *et al.*, 2004).

The F_{ST} value revealing the diversity between Cameroon chicken populations is the double of the 0.048 obtained by Nigussie (2011) for Ethiopian local chicken ecotypes and Mwacharo *et al.* (2007) for Kenyan local chicken (0.003-0.040).

The overall Wright's F-statistics parameters, denoting the inbreeding coefficient obtained in the present study (0.03) is much more lower than those shown in the Aseel chicken (Babar *et al.*, 2012), but similar to values obtained in many varieties and populations of local chickens (Berthouly *et al.*, 2008; Eltanany *et al.*, 2010; Yu Ya-Bo *et al.*, 2006). The F_{ST} allows estimation of the number of migrant individuals according to loci in a population per generation (Nm). In the Cameroonian

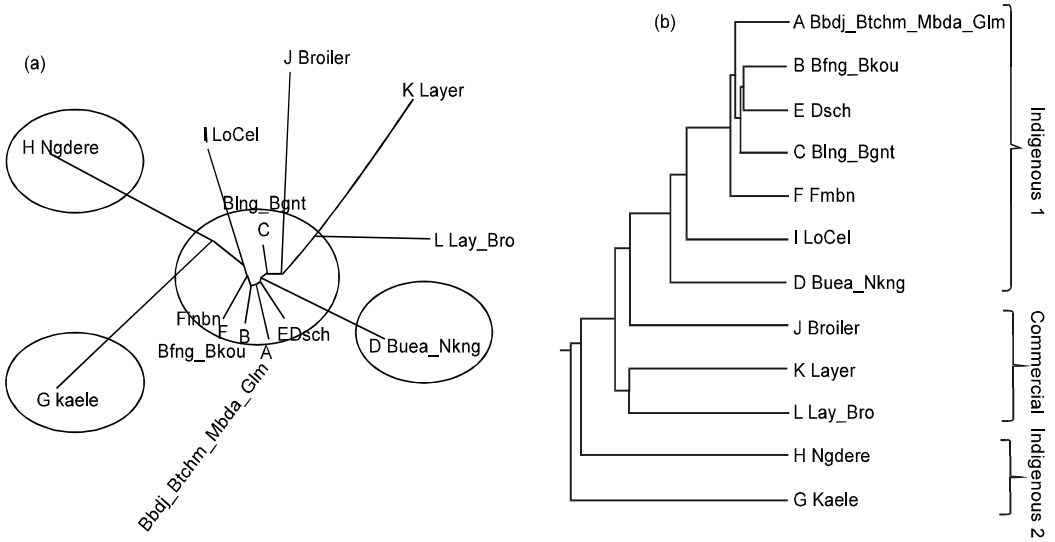

Fig. 3: Neighbour-Joining tree constructed using DA genetic distance matrix (a) and UPGMA cladogram (b) showing relationship among Cameroon local chicken population

chicken population, this number varies from 1.58-4.92, with an average of 2.91. This value is higher as compared to that mentioned by Babar *et al*. (2012) in all varieties of Aseel chicken.

The variation of observed and expected heterozigosity may be adduced to differences in location, sample size, population structure and sources of microsatellite markers (Kaya *et al*., 2008). The gene diversity over loci observed in Cameroon chicken ecotypes are all higher than the 0.47 reported by Hillel *et al*. (2003) within 52 populations across 22 loci. This difference may be due to the uniqueness of genetic composition of the ecotypes. However, further investigations must be carried out particularly in the 1st and 2nd agro-ecological zone of Cameroon. The low genetic diversity in zone II may be attributed to the production system and importance accorded in the area to other domestic animal species like cattle and small ruminant to the detriment of local chicken kept as small close flocks by women. Among Cameroon local chicken, ecotypes III and IV showed significant high degree of inbreeding, respectively 0.11 and 0.12 this may have an impact on traits fixation in the populations. This degree of inbreeding are highly are higher to those reported by Tadano *et al*. (2007) for 12 commercial chicken lines based on 40 microsatellite loci, while lower than reported by Kaya *et al*. (2008) for Turkish native chicken (0.301) with 10 SSR loci.

The AMOVA finding is similar to that of Nigussie (2011) for Ethiopian local chicken ecotypes and slightly higher to the 92% reported by Shabatzi *et al*. (2007) for native Iranian chicken populations. According to Tixier-Boichard *et al*. (2009), the variety of motivations of village farmers for keeping chickens, including product quality, adaptation to environment and cultural uses shows that within population diversity is a major objective of keeping village chickens. This statement may also be true in Cameroon where it has been observed that improved local chicken with uniform light plumage pattern tend to be rejected by local small farmers (Keambou, unpublished).

The FST values are similar to observations of Nigussie (2011) and Mwacharo *et al*. (2007). Even though small variation among ecotypes, the variability among individuals within ecotypes is above 10%. This brought the necessity to evaluate the diversity within ecotypes, particularly the populations of the 3rd agro-ecological zone which showed both the highest diversity and inbreeding coefficient.

The standard diversity indices result is similar to that of Yu Ya-Bo *et al*. (2006). As displayed by the AMOVA, the Cameroonian chicken studied exhibited higher intra-population genetic diversity than European fancy and purebred commercial lines (Eltanany *et al*., 2010).

Low genetic distances indicate a close genetic relationship whereas large genetic distances indicate a more distant genetic relationship. Within a population genetic distance can be used to measure the divergence between different sub-populations. Nei's standard genetic distance measure assumes that genetic differences arise due to mutations and genetic drift whereas Reynolds distance assumes that genetic differences arise due to genetic drift only. The Nei genetic distances obtained in this study varied from 0.037-0.435, while the genetic identity varied from 0.673-0.967. These values are similar to that of Yu Ya-Bo *et al*.

Table 10: Pairwise Reynold's genetic Matrix distances (coancestry coefficients) as t/M=-ln(1-F_{ST})

Ecotypes	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
Bbdj_Btchm_Mbda_Glm(1)	-	-	-	-	-	-	-	-	-	-	-	-
Bfng_Bkou(2)	0.006	-	-	-	-	-	-	-	-	-	-	-
Blng_Bgnte(3)	0.005	0.004	-	-	-	-	-	-	-	-	-	-
Buea_Nkng(4)	0.066	0.072	0.044	-	-	-	-	-	-	-	-	-
Dschang(5)	0.022	0.023	0.017	0.071	-	-	-	-	-	-	-	-
Foumban(6)	0.023	0.023	0.008	0.055	0.047	-	-	-	-	-	-	-
Kaélé (7)	0.008	0.005	0.005	0.062	0.021	0.031	-	-	-	-	-	-
Ngaoundéré (8)	0.008	0.007	0.006	0.080	0.025	0.035	0.006	-	-	-	-	-
Lcl_selected(9)	0.032	0.026	0.029	0.096	0.042	0.044	0.048	0.033	-	-	-	-
Broiler(10)	0.094	0.103	0.089	0.142	0.137	0.051	0.101	0.112	0.147	-	-	-
Layer(11)	0.022	0.022	0.019	0.090	0.041	0.040	0.019	0.008	0.018	0.113	-	-
Crossebred (12)	0.085	0.066	0.077	0.177	0.112	0.127	0.089	0.075	0.105	0.232	0.101	-

Table 11: Pairwise among-population differentiation (F_{ST} values)

Ecotypes	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
Bbdj_Btchm_Mbda_Glm(1)	-	-	-	-	-	-	-	-	-	-	-	-
Bfng_Bkou(2)	0.006*	-	-	-	-	-	-	-	-	-	-	-
Blng_Bgnte(3)	0.005^{ns}	0.004^{ns}	-	-	-	-	-	-	-	-	-	-
Buea_Nkng(4)	0.064*	0.069*	0.043*	-	-	-	-	-	-	-	-	-
Dschang(5)	0.022*	0.023*	0.017*	0.068*	-	-	-	-	-	-	-	-
Foumban(6)	0.023^{ns}	0.022^{ns}	0.008^{ns}	0.053*	0.046^{ns}	-	-	-	-	-	-	-
Kaélé (7)	0.008*	0.005*	0.005^{ns}	0.060*	0.021*	0.031*	-	-	-	-	-	-
Ngaoundéré (8)	0.008*	0.007*	0.006^{ns}	0.076*	0.025*	0.034*	0.006^{ns}	-	-	-	-	-
Lcl_selected(9)	0.031^{ns}	0.025^{ns}	0.028^{ns}	0.092*	0.041^{ns}	0.043^{ns}	0.047*	0.033*	-	-	-	-
Broiler(10)	0.090*	0.098*	0.083*	0.133*	0.128*	0.050*	0.096*	0.106*	0.136*	-	-	-
Layer(11)	0.022*	0.022*	0.019*	0.086*	0.040*	0.040*	0.019*	0.008^{ns}	0.017^{ns}	0.107*	-	-
Crossebred (12)	0.082*	0.063*	0.074*	0.163*	0.106*	0.119*	0.085*	0.072*	0.100*	0.207*	0.096*	-

*P = 0.05, ns = non-significant

Table 12: Evanno population's structure parameters

K	Reps	LnP(K)	LnP(K) SD	Ln'(K)	|Ln"(K)|	Delta K
1	3	-22517.8	0.0577	NA	NA	NA
2	3	-21995.1	1.6166	522.7667	73.1	45.2189
3	3	-21545.4	1.7321	449.6667	366.7667	211.7528
4	3	-21462.5	5.1962	82.9	55	10.58476
5	3	-21324.6	22.8007	137.9	75.13333	3.295227
6	3	-21261.8	44.8413	62.76667	111.0667	2.476883
7	3	-21088	35.1606	173.8333	84.76667	2.41084
8	3	-20998.9	39.9526	89.06667	NA	NA

(2006) and Eltanany *et al.* (2010), respectively in 12 Chinese and Egyptian indigenous chickens. They are lower than those obtained in four varieties of Pakistani Aseel chicken by Babar *et al.* (2012), but higher than that of Fu-Xiang *et al.* (2010) on Chinese Bian chicken. On the other hand, the Reynolds genetic distances between local chicken populations, meaning distances only related to drift, are very low and similar to observations of Eltanany *et al.* (2010). This information is important to devise effective breeding strategies for genetic improvement of Cameroonian local chicken populations depending upon the nature of market demand for higher growth rate, free range poultry meat and eggs as stated by Babar *et al.* (2012).

When using allele frequency-based estimates of genetic differentiation under such conditions, four forces can account for genetic divergence between populations: mutation, genetic drift, migration and selection (Graur and Li, 2000). While mutation is important in the long term, genetic drift plays a significant role during short-term evolution in situations where populations are reproductively isolated (Laval *et al.*, 2002). The indigenous chicken populations exhibited isolation by distance and seemed to be at equilibrium under dispersal and genetic drift. It is probable that these chickens did not arrive in their current locations recently, because there would not have been sufficient time for isolation by distance to take effect and, that long distance gene dispersal is not sufficiently common to prevent genetic divergence.

Conclusion: The current study has established genetic uniqueness within the Cameroonian local chicken ecotypes. The phenotypic variability of Cameroon chicken populations is still visible at the molecular level, even if a clear sub-structuring is not observed. This diversity is observed from the allele's number and types, through Standard genetic parameters, to UPGMA and Neighbour trees and Structure parameters. These results bring in available objective information on genetic variability of Cameroon local chicken populations and offer the basic step towards rational decision making prior to the genetic improvement and conservation programs, without compromising the existence of each unique genotype. The variability shown by the genetic diversity parameters, distances and trees and confirm by the peak of delta K at 3 allow us to propose that there should be established at least two centers of conservation of local chicken populations in Cameroon, one in the northern and the other in the western highlands of Cameroon. The results of this study also confirmed the usefulness of microsatellites for the study of genetic variation and divergence of the Cameroonian indigenous chicken.

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support provided to the Biosciences eastern and central Africa Hub-the International Livestock Research Institute (BecA-ILRI Hub) by the Australian Agency for International Development (AusAID) through a partnership between Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the BecA-ILRI Hub and by the Syngenta Foundation for Sustainable Agriculture (SFSA); the Bill and Melinda Gates Foundation (BMGF) and the Swedish Ministry of Foreign Affairs through the Swedish International Development Agency (Sida), which made this work possible. We sincerely thank the farmers who participated in the project, Tsafack Antoine and Denis from the livestock extension services who actively contribute in sample collection in zones 3 and 4. A special acknowledgement is addressed to Moses Njahira and Martina Kyalo for the guidance during the laboratory work.

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