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Assessing the Morphological Diversity of Ethiopian Indigenous Chickens Using Multivariate Discriminant Analysis of Morphometric Traits for Sustainable Utilization and Conservation

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Abstract

This study aimed to differentiate indigenous chicken populations of four administrative zones including Kaffa, Sheka, Metekel, and Bale based on morphometric measurements using multivariate analysis. Data on quantitative traits were collected from 3069 adult indigenous chickens of both sexes. Live weight (LW), body length (BL), breast circumference (BC), wingspan (WS), shank length (SL), shank circumference (SC), keel length (KL), back length (BkL), and neck length (NL) were recorded. A cluster and discriminant analysis was applied to identify the combination of variables that best differentiate among chicken populations. Results indicated that Metekel chickens were characterized by higher LW, BL, KL, and BkL and differed from other groups ($P < 0.05$). Sheka chickens demonstrated the highest BC, WS, SL, SC, and NL being different from others ($P < 0.05$). Cluster analysis generated two distinct groups in which chickens of Bale and Sheka were clustered in one group while those of Metekel and Kaffa in another group each separated with sub-clusters. All Mahalanobis distances among the four chicken populations were significant being the shortest between Sheka and Bale chickens and the longest between those of Metekel and Bale ($P < 0.0001$). Three statistically significant ($P < 0.001$) canonical variables (CAN) were extracted of which CAN1 and CAN2 accounted for 73.2 and 14.6% of the total variations, respectively. The scatter plot generated by canonical discriminant analysis showed that CAN1 effectively discriminated between chickens of Metekel and Kaffa while the CAN2 best discriminated against those of Bale and Sheka. The discriminant analysis correctly classified 95.3, 94.9, 92.3, and 82.2% of Metekel, Bale, Kaffa, and Sheka chickens into their origin population, respectively. The current study revealed that multivariate analysis of morphometric traits provided a practical basis for differentiating the indigenous chicken populations into different groups. However, the authors recommend genetic characterization studies to validate the detected morphometric-based differentiation in chicken populations.

Introduction

Domestic chickens are the most widely distributed genetic resources in many rural and peri-urban regions of Africa and Asia. Due to their short generation interval, reproductive efficiency, and potential to adapt in a wide range of agro-ecological zones, chickens are considered the most suitable livestock species for many smallholder farmers (Moula *et al.*, 2011; Melesse, 2014). Most rural communities of Ethiopia keep few to a large number of chickens. Moreover, recent socio-economic studies have indicated that chicken rearing has been reported

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to enhance food security through an increased supply of animal source products such as meat and eggs of high quality (Wodajo *et al.*, 2020).

According to CSA (2018/19), there are about 59.42 million chickens in Ethiopia, of which 90.8%, 4.39%, and 4.76 % are indigenous, exotic, and crossbred, respectively. The dominance and widespread distribution of indigenous chickens in contrasting production systems are characterized by their adaptive potentials to various types of environmental and diseases related stressors (Hassen *et al.*, 2007; Egahi *et al.*, 2010; Al-Qamashoui *et al.*, 2014; Habimana *et al.*, 2020). Thus, they broadly signify the possession of genetic materials that would enable them to survive, reproduce and produce in a wide range of production environments (Dana *et al.*, 2010; Melesse and Negesse, 2011; Melesse, 2014; Getachew *et al.*, 2016). However, the uncontrolled distribution of exotic chicken breeds to the local community without a systematic genetic improvement strategy has resulted in a dramatic loss of genetic diversity in the indigenous chickens by diluting their genetic makeup (Wölders *et al.*, 2006; Melesse and Negesse, 2011).

Various researchers have reported that assessment of the genetic characteristics of populations is a prerequisite for successful planning of genetic improvement programs (Al-Qamashoui *et al.*, 2014; Azimu *et al.*, 2018; Habimana *et al.*, 2020). However, in regions where molecular-based genetic characterization is not affordable, morphological measurements have been used to explore the characteristics of local livestock populations. Results of such studies provide useful information in the classification of indigenous animal genetic resources in their source of origin based on differences in their morphological traits, which is an essential step for developing sustainable genetic improvement and conservation programs (Dahloum *et al.*, 2016; Getachew *et al.*, 2016; Arandas *et al.*, 2017).

Many statistical tools are available for assessing the morphological profiles of indigenous chicken populations. In this regard, multivariate analysis of morphological traits has been successfully used to estimate the existence of genetic variations within and between indigenous chicken populations (Ajayi *et al.*, 2012; Egena *et al.*, 2014; Daikwo *et al.*, 2015; Ikpeme *et al.*, 2016; Yakubu and Ari, 2018; Neto *et al.*, 2019). Among others, canonical discriminant analysis has been reported to be the most suitable statistical tool to differentiate indigenous animal populations using morphological traits (Rosario *et al.*, 2008; Al-Atiyat, 2009; Gwaza *et al,* 2013; Ogah, 2013; Daikwo *et al.*, 2015; Dahloum *et al.*, 2016; Al-Atiyat *et al.*, 2017). It can be applied to discriminate various livestock types when all measured morphological variables are considered simultaneously and thus helpful in exploring the genetic diversity study of local animal genetic resources. Moreover, the classification of indigenous livestock populations based on morphometric traits supports the clustering of these animals to the same group by using molecular tools (Hassen *et al.*, 2016).

In Ethiopia, there were many morphological characterization studies conducted to describe the existence of phenotypic variations among the indigenous chicken populations (Dana *et al.*, 2010; Melesse and Negesse, 2011; Getu *et al.*, 2014; Negassa *et al.* 2014; Getachew et al, 2016). These studies have produced valuable information to the local research community involved in the field as well as to the policymakers in the livestock sector for designing and implementing proper interventions. However, most of these studies were focused on specific districts with inadequate sample populations delivering limited information that could be further utilized in developing sustainable genetic improvement and conservation programs at a larger scale. In addition, to the authors' knowledge, only a few authors (Getu *et al.*, 2014; Getachew *et al.*, 2016) have applied multivariate analysis to differentiate the Ethiopian indigenous chicken populations based on their morphological traits. Therefore, the present study aimed to assess the existence of phenotypic diversity among indigenous chicken populations of four administrative zones consisting of ten districts based on their morphometric traits by applying canonical discriminant analysis in combination with cluster and discriminant analysis approaches.

Materials and Methods

Site selection and sampling techniques

This study was conducted in Sheka, Kaffa, Bale, and Metekel administrative zones representing contrasting agro-ecological zones of Ethiopia. Sheka and Kaffa zones are located in the wet humid zone of western Ethiopia. Bale zone is located in the east-central highland of Ethiopia while Metekel falls in the western part of Ethiopia.

Multi-stages purposive with proportional sampling techniques were applied to select the representative districts and kebeles (the smallest administrative units within a district) within each zone. In the first stage, ten districts were selected purposively based on their potential for chicken production. Accordingly, three districts each from Kaffa and Sheka zones and 2 districts each from Bale and Metekel zones were selected. In the second stage of sampling, 44 kebeles were purposively selected from all zones based on the distribution of the chicken population. Accordingly, 15 kebeles from each of Kaffa and Sheka zones, 8 kebeles from Metekel, and another 6 from Bale zones were proportionally selected (Table 1). In the third stage, from the total list of households who possess at least

three adult chickens of both sexes and have long enough experiences in chicken rearing, 1064 households were randomly selected based on proportional to the population size to selected kebeles. Collectively, 3069 chickens (959 males and 2110 females) were sampled from all zones (Table 1).

Table 1. The sample size of districts, kebeles, households, and chickens

Zones	GPS coordinates	Sampled districts	Sampled kebeles	Sampled households	Number of sampled chickens		
					Male	Female	Overall
Kaffa	7.3361° N & 35.7407° E	3	15	300	300	600	900
Sheka	7.5618° N & 35.6533° E	3	15	280	282	564	846
Metekel	10.7803°N & 35.5658° E	2	8	304	201	402	603
Bale	6.7606° N & 40.3089° E	2	6	180	176	544	720
	Total	10	44	1064	959	2110	3069

Data collection procedures

Data on nine morphometric traits were scored following the descriptor list of FAO (2012) for phenotypic characterizations of chickens. Accordingly, the following traits were measured: live weight (LW), body length (BL), breast circumference (BC), back length (BkL), keel length (KL), wingspan (WS), length of the shank (SL), the circumference of the shank (SC), and length of the neck (NL). The LW of individual chickens was measured using a portable digital balance fitted with a holder made of a carton designed to hold the birds calmly while taking the weight. All other linear measurements were taken in a centimeter unit using measuring tapes made of textile material. Live weight and linear measurements were taken from adult chickens as recommended by FAO (2012).

Data analysis

After double-checking for any types of errors or outliers, data were subjected to GLM procedures of Statistical Analysis System (SAS 2012, ver. 9.4) by fitting zone and sex as independent variables. When F-test was declared significant at 0.05 levels, least-square means for more than two levels of fixed effects were then separated using the adjusted Tukey-Kramer test to account for missing data of specific traits.

The degree of morphological similarity or divergence among the indigenous poultry populations was determined using multivariate analysis. The procedure of the Cluster Analysis was performed and a dendrogram was constructed using the single method based on the minimum distances between the chicken populations of the four zones to group them into their morphological similarity. Moreover, the stepwise discriminant analysis procedure (STEPDISC) was conducted to rank the morphometric traits by their discriminating power. Selected traits were then subjected to canonical discriminant analysis using the CANDISC to determine the existence of population-level phenotypic differences between the studied populations of the four zones. The TEMPLATE and SGRENDER procedures were also applied to create a plot of the first two canonical variables in a scatter graph for visual interpretation. The discriminant analysis of the DISCRIM procedure was also conducted to determine the percentage classification of chickens into their source populations using the quadratic discriminant function for unequal covariance matrices within classes after conducting Bartlett's homogeneity test. The cross-validation option was finally applied to evaluate the accuracy of the classification with a minimum bias. All multivariate analyses were performed using the Statistical Software of SAS (2012, ver. 9.4). Since data were not available for specific traits of some zones, only seven variables (LW, BL, BC, WS, SL, SC, and KL) were considered in the multivariate analysis. Moreover, four independent researchers representing each zone have collected the data by applying the analogous data collection procedure.

Results

The least-square means for the morphometric traits, the significance of the zone, and sex effects are presented in Table 2. The effects of zone and sex were highly significant for all traits studied. Accordingly, chickens of Metekel had higher LW, BL, KL, and BkL than those of other chicken populations ($P < 0.001$). The BC, WS, SL, SC, and NL values for Sheka chickens were highest as compared with the other populations. However, the Kaffa chickens had the lowest LW and WS values. Similarly, the Bale chickens had the lowest BL, KL, and BkL values compared with the other populations. The effect of sex was significant for all traits being higher in males than in female chickens ($P < 0.001$).

In male chickens, all investigated morphometric traits positively correlated with LW ($P < 0.01$; Table 3). Moreover, except BL which negatively correlated with NL, all other traits positively correlated with each other ($P < 0.01$)**.** In female chicken populations, there was a strong positive correlation of LW with other morphometric traits. Similarly, a positively strong association was observed among all traits except BL, SL, SC, and BkL (Table 3). On the other hand, SL and SC demonstrated a significant negative correlation with BL and BkL in female chicken populations ($P < 0.01$).

Table 2. Least square means of live weight (kg) and linear body measurements (cm) in indigenous poultry populations as affected by zone and sex (N = 3069)

Parameter	LW	BL	BC	WS	SL	SC	KL	BkL	NL
Zone	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Kaffa	1.35^{d}	39.1^{b}	26.4^{b}	39.5^{d}	7.74^{c}	3.69^{c}	10.5^{c}	18.9^{b}	NA
Sheka	1.53^{b}	38.2^{c}	27.9^{a}	46.4^{a}	8.75^{a}	4.23^{a}	11.7^{b}	NA	15.9^{a}
Bale	1.42^{c}	37.4^{d}	24.6^{c}	43.0^{b}	8.13^{b}	3.91^{b}	9.44^{d}	17.3^{c}	10.7^{c}
Metekel	1.64^{a}	43.5^{a}	26.5^{b}	42.1^{c}	7.84^{c}	3.71^{c}	12.2^{a}	22.1^{a}	12.2^{b}
Sex	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Male	1.61	41.4	27.8	45.2	8.87	4.20	11.5	20.4	13.7
Female	1.37	37.8	25.0	40.3	7.36	3.56	10.4	18.5	12.2

[a-d]Means with different superscript letters within the same column and class are statistically different at P < 0.05; NA = data not available; LW = live weight; BL = body length; BC = breast circumference; WS = wingspan; SL = shank length; SC = shank circumference; KL = keel length; BkL = back length; NL = neck length

Table 3. The correlation coefficients of morphometric traits for male (above diagonal, N = 959) and female chickens (below diagonal, N = 2110)

Traits	LW	BL	BC	WS	SL	SC	KL	BkL	NL
LW	-	0.39**	0.42**	0.35**	0.20**	0.40**	0.47**	0.55**	0.19**
BL	0.45**	-	0.26**	-0.03^{ns}	-0.01^{ns}	0.07*	0.47**	0.83**	-0.13**
BC	0.28**	0.17**	-	0.54**	0.24**	0.54**	0.63**	0.69**	0.62**
WS	0.42**	0.05*	0.12**	-	0.43**	0.63**	0.46**	0.27**	0.73**
SL	0.13**	-0.06*	0.19**	0.39**	-	0.38**	0.27**	0.29**	0.47**
SC	0.13**	-0.25**	0.23**	0.45**	0.43**	-	0.46**	0.35**	0.57**
KL	0.51**	0.54**	0.34**	0.25**	0.15**	0.01^{ns}	-	0.82**	0.55**
BkL	0.61**	0.82**	0.20**	0.15**	-0.02^{ns}	-0.35**	0.79**	-	0.69**
NL	0.14**	0.06*	0.45**	0.12**	0.23**	0.15**	0.45**	0.71**	-

*$P < 0.05$; **$P < 0.01$; ns = non-significant

LW = live weight; BL = body length; BC = breast circumference; WS = wing span; SL = shank length; SC = shank circumference; KL = keel length; BkL = back length; NL = neck length

The dendrogram (Figure 1) established using the cluster analysis showed two large distinct clusters: cluster one included chickens of Agarfa and Goba districts (representing Bale zone) as independent sub-group and those of Masha, Gecah, and Yeki districts (representing Sheka zone) as a separate sub-group. Cluster two included chickens of Bulen and Pawe districts (representing Metekel zone) as a separate sub-group and those of Decha, Gimbo, and Chena districts (representing Kaffa zone) in another sub-group.

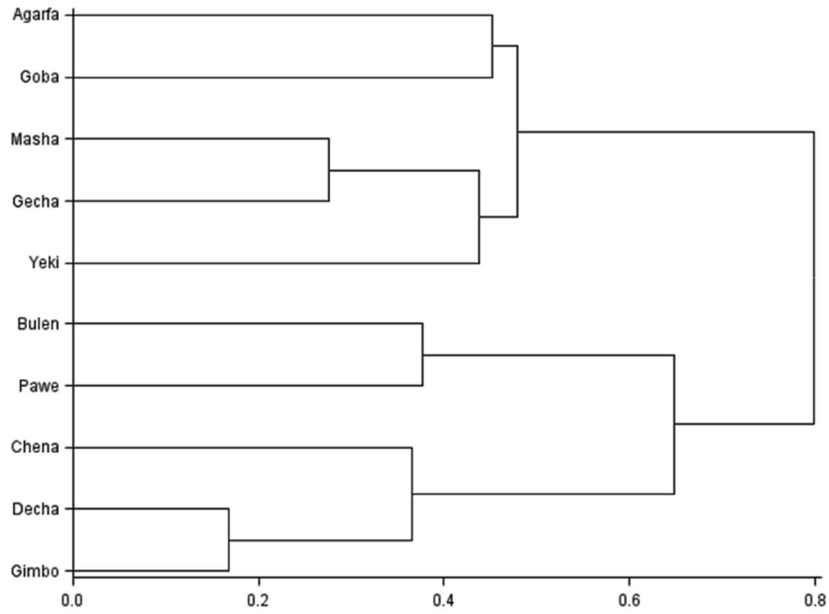

Figure 1. Dendrogram based on minimum distances between indigenous chicken populations of the districts using morphometric traits

Seven quantitative variables with complete data for both sexes were subjected to the STEPDISC procedure and all of them were identified as the best discriminating variables (Table 4). The contribution of the selected variables was tested by Wilk's lambda and validated their discriminating power to significantly differentiate the studied population into separate groups. All the seven variables were then subjected to canonical discriminant analysis, which performed the uni- and multivariate analysis, the Mahalanobis distances, and eigenvalues of extracted canonical variables. Results of the analysis indicated that the univariate statistics testing the hypothesis that class means are equal validated that each quantitative variable in the sampled populations significantly contributed to the total variation ($P < 0.0001$). Population differences between zones as tested by the multivariate analysis were also found to be significant ($P < 0.0001$). Wilk's Lambda further tested the hypothesis that assumes zones' means are equal across the chicken populations and found to be highly significant, which confirms that differences observed among populations of the four zones were statistically different from zero.

Table 4. Summary of stepwise discriminant analysis for selection of traits with the highest discriminating power among the chicken populations

Step	Variables entered	Partial R^2	F-value	Pr > F	Wilks' Lambda	Pr < Lambda	ASCC	Pr > ASCC
1	Body length	0.543	1220	<0.0001	0.557	<0.0001	0.148	<0.0001
2	Wingspan	0.445	811	<0.0001	0.253	<0.0001	0.292	<0.0001
3	Keel length	0.296	428	<0.0001	0.178	<0.0001	0.376	<0.0001
4	BC	0.219	285	<0.0001	0.139	<0.0001	0.442	<0.0001
5	SC	0.143	170	<0.0001	0.119	<0.0001	0.458	<0.0001
6	Live weight	0.119	137	<0.0001	0.105	<0.0001	0.486	<0.0001
7	Shank length	0.034	35.7	<0.0001	0.102	<0.0001	0.490	<0.0001

BC = breast circumference; SC = shank circumference; ASCC = average squared canonical correlation

Table 5 showed significant Mahalanobis distances between zones based on morphometric measurements sorted by mean distances ($P < 0.0001$). The shortest Mahalanobis distance was observed between Sheka and Bale chickens followed by that of Sheka and Kaffa. On the other hand, the longest distance was noted between Metekel and Bale followed by that of Metekel and Sheka. The distance among the other zones was intermediate ranging from 9.12 to 9.37.

Table 5. Mahalanobis distances between chicken populations of the four zones based on morphometric traits

Zones	Kaffa	Sheka	Bale	Metekel
Kaffa	0	7.08	9.12	9.37
Sheka		0	4.39	19.2
Bale			0	23.9
Metekel				0

All distances are significant at $P < 0.0001$

The variable which is defined by the linear combination is the first canonical variable (CAN1). In the current study, the CAN1 showed the highest possible multiple correlations with the groups which were relatively high (0.87; Table 6). The process of extracting the rest canonical variables that are needed for the separation purposes will be repeated until the number of variables equals the number of classes/groups minus one. In the present study, since there were four zones (Kaffa, Sheka, Bale, and Metekel), the maximum number of CANs to be extracted for separation purposes would be $4 - 1 = 3$. This is evident in Table 6 where CAN1, CAN2, and CAN3 explained 73.2%, 14.6%, and 12.2% of the total variation, respectively being highly significant ($P < 0.0001$). However, among the identified canonical variables, both CAN1 and CAN2 explained about 88.0% of the total variation, which was used to plot the individual birds over the scatter plane as displayed in Figure 2. The plot clearly showed that CAN1 discriminated between chickens of Metekel and Kaffa zones while the CAN2 best discriminated against among those of Bale and Sheka zone. The null hypothesis that assumes the current canonical correlations and all smaller ones are zero has been rejected based on the likelihood ratio test (Table 6).

As indicated in Table 7, the first canonical variable CAN1 loaded highly for BL and KL with the respective canonical discriminant function score of 1.51 and 0.61, while the CAN2 loaded for WS, KL, and LW with a canonical discriminant function score of 0.89, 0.74 and 0.68, respectively. Results of the canonical structure were also in line with that of total standardized canonical coefficients in which BL and SC dominated CAN1, while WS, LW, and KL showed the largest influence on CAN2. The CAN3 of

total canonical structure and coefficients was influenced by BC. Values of the standardized canonical coefficient signify the contribution of each variable to the discriminant function.

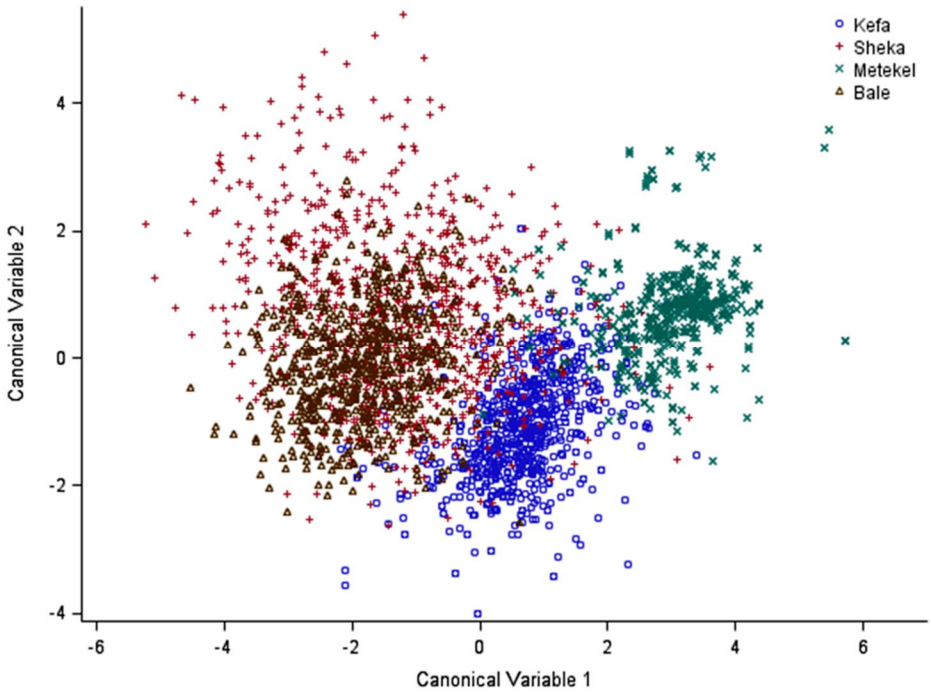

Figure 2. The canonical representation of indigenous chicken populations across the four zones

Table 6. Summary of canonical correlations and eigenvalues

Functions	Canonical correlations	Eigenvalues			Likelihood ratio	Approximate F-value	Pr > F
		Eigenvalue	Proportion	Cumulative			
CAN1	0.868	3.049	0.732	0.732	0.102	508	<0.0001
CAN2	0.615	0.601	0.146	0.878	0.412	284	<0.0001
CAN3	0.580	0.509	0.122	1.000	0.663	311	<0.0001

CAN1 = canonical variable 1; CAN2 = canonical variable 2; CAN3 = canonical variable 3

Table 7. Standardized canonical coefficients and canonical structures based on morphometric variables of indigenous chickens sampled from four zones

Variables	Standardized canonical coefficients			Canonical structures		
	CAN1	CAN2	CAN3	CAN1	CAN2	CAN3
Live weight	0.156	**0.681**	-0.534	0.251	**0.713**	-0.015
Body length	**1.510**	-0.389	-0.652	**0.738**	0.295	-0.032
Breast circumference	-0.010	-0.582	**1.128**	0.069	0.210	**0.692**
Wingspan	-0.953	**0.886**	-0.329	-0.331	**0.734**	0.106
Shank length	-0.250	-0.187	0.036	-0.240	0.288	0.180
Shank circumference	-0.592	-0.240	-0.139	0.382	0.330	0.189
Keel length	**0.611**	0.743	0.910	0.422	**0.675**	0.519

The most important variables within the CAN1, CAN 2 and CAN3 are highlighted with boldface

The discriminant analysis assumes that the individual group covariance matrices are equal (homogeneity within covariance matrices) and by default, it uses the linear discriminant function for classification. In the current discriminant analysis, equality of covariance matrices within groups was tested using Bartlett's test of homogeneity for all traits and was significant (χ^2 = 6120; P < 0.0001). Accordingly, the null hypothesis that assumes all four covariance matrices within the chicken populations are equal was rejected. Therefore, the within-group covariance matrices were used to derive the quadratic discriminant function criterion for the classification of the four chicken populations. The quadratic discriminant function correctly classified 95.3%, 94.9%, 92.3%, and 82.2% of individual chickens into their respective source population of Metekel, Bale, Kaffa, and Sheka zones with an overall average correct classification of 91.2% (Table 8). The accuracy of the classification was further cross-

validated in which 94.3, 94.0, 92.1, and 81.9 of Metekel, Bale, Sheka, and Kaffa chickens were correctly assigned to their origin populations with an overall average of 90.6% (Table 8).

Table 8. Percent of individual chickens classified into their respective zones and cross-validation of classification based on morphometric variables (values in brackets are number of chickens)

Zones	Kaffa	Sheka	Bale	Metekel	Total
Re-substitution					
Kaffa	92.3 (683)	4.57 (41)	0.67 (6)	2.45 (22)	100 (898)
Sheka	6.28 (53)	82.2 (694)	10.2 (86)	1.30 (11)	100 (844)
Bale	0.83 (6)	4.17 (30)	94.9 (683)	0.14 (1)	100 (720)
Metekel	1.50 (9)	3.0 (18)	0.17 (1)	95.3 (572)	100 (600)
Error count estimate	0.077	0.178	0.055	0.047	0.088
Cross-validation					
Kaffa	92.1 (827)	4.79 (43)	0.67 (6)	2.45 (22)	100 (898)
Sheka	6.52 (55)	81.9 (691)	10.3 (87)	1.30 (11)	100 (844)
Bale	0.97 (7)	4.86 (35)	94.0 (677)	0.14 (1)	100 (720)
Metekel	1.83 (11)	3.67 (22)	0.17 (1)	94.3 (566)	100 (600)
Error count estimate	0.0791	0.1813	0.0597	0.0567	0.0942
Priors	0.25	0.25	0.25	0.25	

As further showed in Table 8, the quadratic discriminant function calculated the misclassified observations via re-substitution and cross-validation options. Accordingly, the misclassification error level among the four chicken populations was negligible with an overall error count of 0.088 (8.80%) for all observations. The overall error count estimates for cross-validation analysis were 9.42%, which provided a nearly unbiased estimate but with a relatively large variance. As showed in Table 8, 10.2 and 6.28% of Sheka chickens were misclassified to Bale and Kaffa zones, respectively while about 4.6% of Kaffa chickens were misclassified to Sheka.

Discussion

Quantitative traits

Designing strategies for genetic improvement and the consequent sustainable conservation of indigenous animal genetic resources should be based on the assessment of the phenotypic characteristics of populations under consideration. Results of the analysis of the morphometric traits revealed clear variations in the studied morphometric traits among the four chicken populations. Chickens of Metekel were characterized by higher LW, BL, KL, and BkL than those of the other zones. Likewise, Sheka chickens demonstrated higher BC, WS, SL, SC, and NL values as compared with the other populations. This indicates that both Metekel and Sheka chickens were the most divergent ecotypes from the others. The findings further suggest that the genetic merits of chickens from Metekel and Sheka zones could be improved by taking into account these traits in a well-designed breeding program.

The effect of sex was significant ($P < 0.001$) for all traits being higher in males than in female chickens and is in good agreement with the reports of Guni *et al.* (2013) and Getachew *et al.* (2016) for indigenous chickens of Nigeria and Ethiopia, respectively. Osei-Amponsah *et al.* (2012) and Rotimi *et al.* (2016) also reported similar findings in which indigenous male chickens were superior in growth performance traits than females across all genotypes. The superiority of males over females could be attributed to sexual dimorphism due to differences in the level of male sex hormones, which is responsible for larger muscle development in males than in females (Melesse *et al.*, 2013; Rotimi *et al.*, 2016).

Getu *et al.* (2014) reported an overall mean of 1.46 kg LW and 3.78 cm SC, which is in close agreement with the current findings. However, the same authors reported lower overall mean values for BL, KL, and WS than observed in the present study. Studies reported by Getachew *et al.* (2016) for WS (top), BL, BC, and LW in females were consistent with the current observations of the same sex. Similarly, WS (top), BL, BC, and SC values in male chickens reported by the same authors are in line with the current findings. Nevertheless, SL and LW values in males and that of SL in female chickens observed in the current study were lower than reported by Getachew *et al.* (2016). Since these two traits are essential parameters to categorize chickens into layer or meat type, the incidence of such differences could be attributed to the genetic makeup of the chicken populations. Moreover, as most local communities do not practice systematic selection, indigenous chicken populations might have been subjected to high natural selection pressure resulting in considerable variations among indigenous livestock populations.

The live weight of Sheka chickens is comparable with that of Ogah (2013) for Normal feathered and naked neck Nigerian indigenous chicken genotypes. In another study, Daikwo *et al.* (2015) reported comparable values for SL and BC for normal feathered Nigerian chicken ecotypes. However, the same authors have reported a much lower BL value

for normal and frizzle feathered Nigerian chickens than obtained from the current study (27.5 vs. 38.7). Such differences could be arising due to the type of chicken breed and their management systems used by various individual communities. Phenotypic variations present in a population may also arise due to genotypic and environmental effects and their interactions, which are essential components in acquiring genetically induced long-term adaptation in a given production environment.

The results of correlation analysis are essential in determining the degree of relationship between the studied morphometric traits. Most of the quantitative traits are naturally correlated due to genetic (pleiotropy and linkage disequilibrium) and non-genetic environment-related effects (Rosario *et al.*, 2008). Thus, understanding the association between quantitative traits is of paramount importance in designing sustainable genetic improvement programs through selection within the local animal populations. In the current study, significant positive associations were observed in most of the morphometric traits studied, which implies that these traits are influenced by similar genes in the same direction making the selection process of correlated traits more effective in any genetic improvement programs (Ikpeme *et al.*, 2016). Moreover, the significant positive relationships observed between LW and other morphometric traits could be utilized as a practical selection criterion where information on heritability estimates of quantitative traits is unavailable due to a lack of pedigree and performance records. Besides, LW could be predicted from linear body measurements in rural areas where weighing scales are not available or unaffordable (Fayeye *et al.*, 2014; Yakubu and Ari, 2018).

Multivariate analysis

The dendrogram (Figure 1) clustered the studied chicken populations into two distinct clusters in which chickens of Bale and Sheka zones grouped in one cluster and those of Metekel and Kaffa in the second cluster. This observation suggests the existence of a strong morphological relationship among chicken populations of the districts within each zone. Wilk's lambda test also confirmed that all the selected variables in the stepwise discriminant analysis had a highly significant contribution to discriminate the total population into separate groups. However, based on the values of Wilk's lambda and the average squared canonical correlation, BL has shown the highest level of significant discriminating power while SL had the least in differentiating the chicken populations of the four zones.

Most of the discriminating variables (LW, BL, BC, SL, NL, KL) in the present study are similar to those reported by Daikwo *et al.* (2015) for Nigerian indigenous chickens and that of Ajayi *et al.* (2012) for Algerian chickens. Consistent with the current findings, Neto *et al.* (2019) reported that BL, WS, and LW showed the greatest variability to discriminate between the Brazilian fighting cocks and naturalized roosters. On the contrary, Getu *et al.* (2014) reported SL as the most important variable to discriminate among three chicken ecotypes reared in North Gondar of Ethiopia. Such variations among different studies may arise from the sample size and birds' age considered under individual study.

Determining the morphological distances will help in understanding the genetic diversity of the indigenous animal genetic resources to initiate programs aiming at conservation and sustainable utilization under their production environments. In this regard, the Mahalanobis distance is the most commonly used distance measure for quantitative traits of livestock breeds. In the current study, all the Mahalanobis distances were highly significant ($P < 0.001$), indicating the existence of large variations among the quantitative traits of the studied populations. These observations are in good agreement with those of Daikwo *et al.* (2015) who reported similar findings for North-Central Nigerian chicken breeds. The Wilks' lambda test for the sampled population in the current study was 0.1017 (10.2%) indicating the existence of phenotypic variations (about 90%) between the indigenous chicken populations rather than within populations. Moreover, the analysis results of the quadratic discriminant function provided complementary information in which about 91.0% of the individual chickens were correctly classified to their source population indicating genetic homogeneity within a population rather than between populations. Getachew *et al.* (2016) reported 83.5% of the variability in the discriminator variables, which is lower than observed in the current study. The genetic diversity available between animal breeds is a key element while setting conservation priorities among the indigenous animal genetic resources in any of the genetic improvement programs.

The longest Mahalabois distance was observed between Bale and Metekel chicken ecotypes implying the sampled chicken populations from both zones were much different in quantitative features considered in the current study. On the other hand, the distance between Sheka and Bale chicken populations is comparatively low which might be attributed to the sharing of similar genetic identities as a result of the non-selection, presence of inbreeding, and migration among these ecotypes over many generations (Daikwo *et al.*, 2015).

The Mahalabois distances reported by Ogah (2013) among the Nigerian normal feather, frizzle feather, and naked neck chickens ranged from 3.37 to 4.64, which is similar to that of Sheka and Bale chicken populations. Daikwo *et al.* (2015) reported a

Mahalabois distance of 11.3 between normal and frizzle feathered chicken breeds of Nigeria, which is comparable to those observed between Kaffa to Bale and Kaffa to Metekel. On the other hand, higher Mahalabois distances of 434, 430, and 38.3, respectively were reported for layers, broilers, and indigenous chicken populations of Jordan (Al-Atiyat, 2009). Such large variations might arise in the methods applied for computing the Mahalanobis distances. For example, pairwise distances computed using canonical discriminant analysis might be different from the one analyzed using the discriminate function that produces squared Mahalanobis distances. Moreover, the number of samples used in the discriminant analysis would influence the outcome of the Mahalanobis distances being higher in a smaller sample size than in a larger (Melesse *et al.*, unpublished data).

The canonical discriminant analysis extracted three canonical variables of which CAN1 and CAN2 accounted for 73.2 and 14.6% of the total variations, respectively, in which the former canonical function accounting for the largest amount of the between-population variability. This observation is somehow comparable with that of Getachew *et al.* (2016), who reported that CAN1 and CAN2 accounted for 81.8 and 16.5% of the total variation, respectively for indigenous chicken populations of Western Ethiopia. In a study undertaken on local chickens of northwestern Ethiopia, Getu *et al.* (2014) reported lower CAN1 and CAN2 values that accounted for 66.7 and 33.3% of the variation, respectively than observed in the current study. In another study conducted on Nigerian indigenous chicken populations, CAN1 and CAN2 accounted for 59.7 and 40.3% of the total variations, respectively (Ogah, 2013). Such differences might be attributed to the type of morphometric variables used in the canonical analysis (some of these authors used beak length, comb length and width, body width, wing length, and thigh length, which were not considered in the current study).

The first canonical variable CAN1 loaded highly for BL and KL while the CAN2 weighted for WS, KL, and LW. These traits that loaded high in the two CAN1 and CAN2 demonstrated their relevance in differentiating between the studied chicken populations. Canonical discriminant analysis has been further successfully proven in identifying variation of morphometric traits among the studied chicken populations. Getu *et al.* (2014) reported that KL and WS showed the highest loading on CAN2 which is in good agreement with the current findings. The same authors reported that SL and beak length showed the highest loading on CAN1 that differs from the present observation. According to the reports of Ogah (2013) on Nigerian indigenous chickens, body width and body weight dominated CAN1 with negative values while body length, thigh length, and keel length influenced CAN2. Age and genetic make of the birds might be responsible for differences reported in various literature.

The proportion of correctly classified individuals gives a measure of the morphological distinctness of the sampled populations. Overall, about 91.0% of the sampled chicken populations were correctly assigned to their source population for those morphometric variables included in the discriminant analysis. Accordingly, all the sampled chicken populations were distinct from each other indicating homogeneity within populations. The Bale and Metekel chicken populations were mostly isolated from each other and all other ecotypes. They were particularly the most diverged from the other sample populations, which might be associated with the greatest distances observed between chicken populations of Bale and Metekel zones that might have resulted in restricted intermingling and subsequent morphological differentiation. Also, the indigenous chickens of the Metekel zone might have been selected naturally for adaptation to warm semi-arid to sub-humid conditions, whereas those of the Bale zone could be more tolerant to cool sub-humid conditions of the tropical climates.

The misclassification among the four chicken populations was negligible with an overall error count of 0.09 for all observations. The highest count error estimates for the assignment were observed in Sheka chickens in which about 10% of them were misclassified to Bale while 4% of Bale to Sheka, which could be a possible explanation for the observed short Mahalanobis distance between both zones as discussed above. Such misclassifications to source populations could be also related to the possibility of recent individual migration due to exchange programs between chicken populations of the two zones. Moreover, previous works by Turan *et al.* (2005) and Al-Atiyat *et al.* (2017) have indicated the difficulty of obtaining a 100% correct assignment of animals into their original population of the same animal species based on phenotypic traits. The lowest misclassification errors observed in Blae and Metekel chicken populations could be an indication of more uniformity because of more genetic homogeneity of these populations than the Sheka chickens.

According to the reports of Yakubu and Ari (2018), 84.0, 82.0, and 100% of Sasso, Kuroiler, and Fulani chicken breeds were correctly classified into their source population with 86.7% accuracy, which is lower than observed in the current findings. Based on the findings of Daikwo *et al.* (2015), 100% of the Nigerian normal and frizzle feathered local chickens were correctly classified into their source populations, which contradicts the assumptions made by Turan *et al.* (2005) and Al-Atiyat *et al.* (2017).

Conclusion

The studied chicken populations demonstrated a distinct differentiation reflecting the existence of high genetic variability among them. The observed genetic distances between the studied indigenous chicken populations could be used to describe the existence of morphological differences and predict potential gains from them through the application of sustainable breeding and conservation programs. From the morphometric traits considered in the canonical discriminant analysis, body weight, body length, wingspan, breast circumference, and keel length were found to effectively differentiate the studied chicken populations. About 91% of chickens were correctly classified into their source population indicating genetic homogeneity within a population. Metekel chickens demonstrated a better body weight and length profile as measured by their live weight, body length, keel length, and back length. Sheka chickens were characterized by enhanced breast circumference, wingspan, shank length and circumference, and neck length. Thus, promoting the genetic potentials of these indigenous chickens using a well-designed breeding program would bring a swift genetic improvement for their future sustainable conservation and utilization. However, the authors recommend that these findings should be validated through molecular-based genetic characterization studies.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

The datasets analyzed during the current study are available from the corresponding author on request.

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