

## Cluster-Based Assessment of Growth Performance in Cameroon Local Chicken

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### Abstract

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This study integrates clustering and network analyses to identify performance-based groups and their connections to phenotypes, providing insights to optimize local chicken breeding programs in Cameroon. A total of 113 birds were grouped based on growth patterns between 16 and 22 weeks, assessed using total weight gain (TWG) and leg circumference gain (LCG). Repeated measures and Welch ANOVA were used to test performance differences, while Generalized Least Squares (GLS) ANCOVA identified growth predictors. Although phenotypic diversity was observed, TWG and LCG did not differ significantly across phenotypes ( $P > 0.05$ ), whereas final body weight (BW) and leg circumference (LC) at 22 weeks did ( $P < 0.01$ ). Cluster analysis identified four distinct performance cluster groups independent of phenotype, with significant divergence in performance. Birds in Clusters 3 and 4 consistently outperformed ( $P < 0.001$ ) their counterparts in the other Clusters 1 and 2, showing superior TWG and LCG while sexual dimorphism was in favour of males ( $P < 0.05$ ). Cluster 4 exhibited the highest final BW, whereas Cluster 3 had the greatest total weight gain, indicating a distinct tendency for early and late-stage growth, which could be strategically optimized for selective crossbreeding to combine their complementary traits. Network analysis indicates historical gene flow and possible heterozygosity within the population, with Normal and Feathered shank phenotypes potentially serving as genetic bridges for performance traits, while the distinct peripheral positioning of Feathered leg and Naked neck, linked only to Clusters 1 and 2, suggests genetic distinctiveness. GLS-ANCOVA confirmed Cluster 3 and 4, as the most significant predictors of TWG ( $P < 0.001$ ), alongside sex ( $P < 0.05$ ) and LCG ( $P < 0.05$ ). Integrating cluster and network analysis can enhance sustainable breeding strategies in low-input systems, balancing growth efficiency with genetic diversity. Breeders and policymakers are encouraged to adopt systematic performance recording practices and promote cross-cluster crossbreeding within local flocks.

### Introduction

With the global increase in food prices, food insecurity remains a significant challenge, particularly in developing countries with limited resources. Poultry farming in these regions plays a pivotal role in supporting rural livelihoods, enhancing food security, and alleviating poverty, especially

through the breeding of indigenous chicken stocks, which are a valuable resource for both meat and egg production (Besbes, 2009; Birhanu *et al.*, 2023). In Cameroon, the poultry industry has experienced significant growth over the years, with current annual meat and egg production standing at 123,000 tonnes and 88,000 tonnes, respectively (FAOSTAT, 2022).

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However, the industry's heavy reliance on imported stock poses a real threat to national sovereignty, especially during global pandemics characterized by trade and movement restrictions (GIZ, 2018; Rother *et al.*, 2022). Consequently, valorizing indigenous genetic resources is seen as a better and more sustainable alternative for a resilient livestock sector (Mapiye *et al.*, 2019; Hako and Yoniwo, 2023a).

Cameroon local chicken represents a valuable genetic resource, renowned for its adaptability to local environmental conditions and resilience to diseases and climate stress, particularly in low-input farming systems (Hako Touko *et al.*, 2021; Hako and Yoniwo, 2023b). This unique adaptability is underpinned by considerable genetic diversity, which constitutes an important reservoir for breeding and selection programs aimed at improving growth performance, egg production, and disease resistance (Keambou *et al.*, 2015). The richness of the gene pool of Cameroon local chickens has been underscored through genetic studies (Fotsa *et al.*, 2011; Hako Touko *et al.*, 2015). Analysis of 25 microsatellite markers revealed a diverse genetic structure, harbouring tropical genes that can be harnessed for genetic improvement programs (Keambou *et al.*, 2014). Despite their adaptability, the local chickens often display slow and inconsistent growth performance, which is inherently influenced by their genetic makeup and phenotypic traits in combination with environmental and management factors (Kpomasse *et al.*, 2023). Notably, there is a considerable level of genetic admixture and introgression within the population, which contributes to overlapping traits and variations in growth and productivity outcomes (González Ariza *et al.*, 2021; Leroy *et al.*, 2012). Phenotypic traits such as body weight, feed conversion ratio, average daily gain and other morphometric parameters, including shank length and circumference, have been reported to be greatly correlated with growth rate and productivity in chicken (Nosike *et al.*, 2021; Miyumo *et al.*, 2023).

The use of genetic selection tools such as QTLs has significantly enhanced production efficiency in broiler chickens. For example, selecting for low residual feed intake (RFI) has improved feed conversion ratios and reduced waste (Li *et al.*, 2020a; Zhang *et al.*, 2021). However, the implementation of QTL-based selection is limited in many low-income contexts due to significant technical and economic constraints. Moreover, intensive trait-specific selection can also lead to a loss in genetic diversity, potentially reducing the overall resilience and adaptability of the population (Malomane *et al.*, 2021).

In such settings with limited access to these advanced breeding tools, complementary phenomics-based selection strategies like clustering offer cheap alternatives for grouping and selecting animals based

on performance metrics such as growth outcomes. While previous studies have primarily used clustering to classify chicken breeds based on genetic markers (Rosenberg *et al.*, 2001; Vakhrameev *et al.*, 2023) or phenotypic characteristics (Kochish *et al.*, 2023), this study innovates by applying performance-based clustering to identify growth-optimized subgroups within a single indigenous population. Rosario *et al.* (2008) in their study demonstrated that clustering analysis on performance and morphometric traits is effective for assessing phenotypic variability and can help identify key traits for breeding programs. They further concluded that body weight was the most important morphometric trait for clustering analysis in indigenous chicken populations. However, few studies have applied clustering analysis to group and select birds based on growth performance data in Africa, and fewer still have linked these clusters to phenotypes. Our study builds on such work by classifying Cameroon local chickens using hierarchical and K-means clustering of growth traits and visualizing cluster-phenotype relationships using network plots. Furthermore, unlike previous approaches, we also complement this by identifying significant predictors of weight gain using Generalised Least Squares Analysis of Covariance (ANCOVA). We hypothesize that performance-based clustering can identify biologically meaningful subgroups not apparent through phenotype alone, and these subgroups (clusters) interpreted through network analysis can inform more effective and sustainable selection practices. By employing this hybrid approach, we aim to assess growth variation, examine cluster-phenotype interactions, and provide practical selection recommendations to optimize meat production while supporting the sustainability and resilience of local poultry farming in Cameroon.

## Materials and methods

The animal experiment was approved by the University of Dschang's Department of Animal Sciences Ethics Committee (Ethics No: DZOO/CE/01322). The study was conducted at the Teaching and Research Farm of the University of Dschang, which is located between latitudes 5° and 7° North and longitudes 8° and 20° East in the Cameroon Western Highlands. A total of 177 day-old chicks were obtained by incubating eggs randomly collected from farmer households in the Western region of Cameroon. These were reared together under uniform conditions until 16 weeks of age, as part of a broader research initiative focused on the performance evaluation and selective breeding of local chickens for growth and egg production traits. For the current analysis, a subset of 113 birds was selected based on health status and the need to represent all phenotypes present in the population.

At week 16, the birds were phenotyped, sexed, tagged and housed in separate pens with slatted floors based on phenotype. Phenotyping was done by visually classifying chickens into different morphological categories, while tagging was done using leg bands. The five phenotypes under study were the Naked neck (4), Crested (7), Feathered leg (4), Feathered shank (4) and Normal feathered chickens (86), representing 3.5%, 6.2%, 3.5%, 10.6% and 76.2% of the sample size. These proportions reflect the natural occurrence of these phenotypes among the local chicken population in Africa as observed by Fotsa *et al.* (2010), Bembidé *et al.* (2013) and Dahloum *et al.* (2016). All birds were served the same commercial feed (Table 1), provided once a day, ensuring that each bird received at least 80 g, which was deemed appropriate for local chickens aged 16 weeks and above (Yi *et al.*, 2018). Water was always made available in drinking troughs.

**Table 1:** Feed composition and calculated nutritional content

Ingredient	Unit	Quantity
Maize	%	53.25
Wheat bran	%	24.25
Soybean meal	%	17.50
Premix	%	5.00
<i>Calculated nutritional composition</i>		
Proteins	%	18.17
Energy	kcal/kg	2638
Fibers	%	5.32
Calcium	%	0.32
Phosphorus	%	0.60
Lysine	%	0.91
Methionine	%	0.36

#### Data Collection

Body weight (BW) and leg circumference (LC) were measured weekly from 16 to 22 weeks using a Camry digital kitchen scale (5 kg capacity, 0.1 g precision) and a calliper (0.1 mm precision), respectively. To quantify growth performance, both weekly and total gains were computed. Weekly body weight gain (WWG) (equation 1) and weekly leg circumference gain (WLC) (equation 2) were calculated by subtracting the values of the previous week from the current week's values, reflecting short-term increment in growth. Total weight gain (TWG) (equation 3) and total leg circumference (LCG) (equation 4) of the birds were equally computed to assess cumulative performance over the study period and served as the primary input for subsequent cluster and network analysis.

$$\text{WWG} = \text{BW}_{\text{Current week}} - \text{BW}_{\text{Previous week}} \quad (\text{Equation 1})$$

$$\text{WLC} = \text{LC}_{\text{Current week}} - \text{LC}_{\text{Previous week}} \quad (\text{Equation 2})$$

$$\text{TWG} = \text{BW}_{22 \text{ weeks}} - \text{BW}_{16 \text{ weeks}} \quad (\text{Equation 3})$$

$$\text{LCG} = \text{LC}_{22 \text{ weeks}} - \text{LC}_{16 \text{ weeks}} \quad (\text{Equation 4})$$

#### Statistical analysis

A hybrid clustering approach was employed to classify chickens based on total weight gain (TWG) and total leg circumference gain (LCG) from 16 to 22 weeks (Galdino and da Silva, 2024). First, hierarchical cluster analysis (HCA) using Ward's method was applied to explore the overall data structure and guide the choice of cluster number (Murtagh & Legendre, 2014). This was followed by K-means clustering, which finalized group assignments based on proximity to cluster centroids (Vakhrameev *et al.*, 2023). The combination of these two approaches, which are commonly used in exploratory biological studies, balances the visual interpretability of HCA while leveraging the partitioning efficiency of K-means (Galdino and da Silva, 2024). Although K-means alone is suitable for larger datasets, HCA was used here as an exploratory step to assess natural groupings and inform cluster selection. The optimal number of clusters was determined and confirmed using the Elbow Method and Silhouette Analysis (Zhao *et al.*, 2018; Humaira & Rasyidah, 2020). The Elbow Method examines the point at which the reduction in within-cluster sum of squares begins to level off, indicated by the "elbow" point (Syakur *et al.*, 2018), while the Silhouette score, which ranges from -1 to 1, assesses how well each point fits within its cluster (Ezugwu *et al.*, 2022). The following equation (5) represents the objective function for optimizing K-means clustering, by minimizing the total within-cluster variance (Ikotun *et al.*, 2023).

$$J_{ck} = \sum_{i=1}^K \sum_{x \in C_i} \|x - \mu_i\|^2 \quad (\text{Equation 5})$$

Where: K = The number of clusters;  $C_i$  = The set of points in the  $i^{th}$  cluster;  $x$  = A data point;  $\mu_i$  = The centroid of the  $i^{th}$  cluster (mean of all points in  $C_i$ );  $\|x - \mu_i\|^2$  = The squared Euclidean distance between the data point  $x$  and the cluster centroid  $\mu_i$ .

Additionally, network analysis was conducted to explore relationships between clusters and phenotypes. This helped to visualize shared performance traits and possible overlap among groups, offering insight into potential genetic connections or historical crossbreeding patterns. In this network bipartite graph, the nodes represent phenotypes and clusters, while edges indicate their connections (Zhang *et al.*, 2014). The network was analyzed using degree centrality, which quantifies how connected each phenotype is across multiple clusters, offering insights into performance distribution (Venturini *et al.*, 2021). The clarity of visual representation was enhanced by applying a spring layout algorithm to optimize node positioning, and highlight the interconnections (Bendahman & Lotfi, 2024).

To assess whether growth and leg circumference changed significantly over time, a repeated measures ANOVA was conducted. Prior to analysis, the Shapiro-Wilk normality test (Shapiro & Wilk, 1965) confirmed that the residuals followed a normal distribution ( $W = 0.9881$ ,  $p$ -value = 0.4292). However, given the unequal sample sizes across phenotypes, Welch's ANOVA was used to compare total weight gain (TWG) and total leg circumference gain (LCG) across phenotypes, clusters, and sex. Unlike traditional ANOVA, this method is robust to violations of variance homogeneity and adjusts degrees of freedom using the Welch-Satterthwaite equation (Delacre *et al.*, 2017; Celik, 2020). Post hoc pairwise comparisons were performed using Games-Howell tests, which account for unequal variances and sample sizes without assuming pooled variance (Games & Howell, 1976). The choice of Welch ANOVA and Games-Howell tests was based on the clear imbalance in group sizes and the inherent biological variability expected in field data from indigenous chicken populations, which may trigger variance heterogeneity in performance data. A generalized Welch ANOVA model is specified as follows (Equation 6).

$$Y_{ijk} = \mu + (A)_i^1 + B_j + (C)_k^2 + (AB)_{ij}^* + e_{ijk} \quad (\text{Equation 6})$$

Where:  $Y_{ijk}$  = TWG or LCG for the  $k^{\text{th}}$  individual in the  $i^{\text{th}}$  Cluster and  $j^{\text{th}}$  sex group;  $\mu$  = Overall mean;  $(A)_i^1$  = Fixed effect cluster ( $i$  varying from 1 to 4);  $B_j$  = Fixed effect of sex ( $j$  varying from 1 to 2);  $(C)_k^2$  = Fixed effect of phenotype ( $k$  varying from 1 to 5);  $(AB)_{ij}^*$  = Interaction effect between sex and cluster (*applies only in cluster-based comparisons*);  $e_{ijk}$  = Residual error term:  $e_{ijk} \sim N(0, \sigma_{ijk})$ , where  $\sigma_{ijk}$  represents within-group-specific variance that differs across levels of sex, cluster and or phenotype.

Superscripts <sup>1</sup> and <sup>2</sup> indicate that the respective terms are only included in models testing cluster-based and phenotype-based comparisons, respectively. Interaction involving phenotype and sex was not tested in phenotype-based models due to a considerable imbalance in sex distribution across phenotypes. For instance, the Naked neck group (4) had just one cock and 3 hens. This skewed distribution limited the ability to draw statistically meaningful conclusions from sex-phenotype interactions.

Generalized least squares (GLS) analysis of covariance (ANCOVA) was used to assess the determinants of growth in chicken with TWG as the response variable. The experimental design consisted of a one-factor fixed-effects model with cluster, sex, and phenotype treated as fixed factors, and leg

circumference gain (LCG) included as a covariate to adjust for variation in structural growth. The model accounted for heteroscedasticity across groups by applying variance weighting to estimate a separate variance component for each group, as indicated by Pinheiro & Bates (2000). Interaction terms between cluster and sex were also included to assess sex-specific performance patterns. GLS-ANCOVA was favoured over standard ANCOVA due to its flexibility in handling unequal group variances, and it yielded a slightly better fit ( $AIC = 1270.56$  vs. 1325.49). The AIC is a common metric for model comparison in biological studies, where a lower AIC value indicates a better fit to the data (Portet, 2020; Sutherland *et al.*, 2023). While the absolute AIC difference (~4%) is modest and not definitive on its own, the choice of GLS was also guided by the known variance heterogeneity in the dataset. We therefore interpret this improvement cautiously, acknowledging that it complements, but does not solely justify, the use of GLS in this context. The GLS ANCOVA model was specified as shown in equation (7).

$$BWG_{ijk} = \mu + C_i + S_j + P_k + (\beta \cdot LCG_{ijk}) + \sigma_k \cdot e_{ijk} \quad (\text{Equation 7})$$

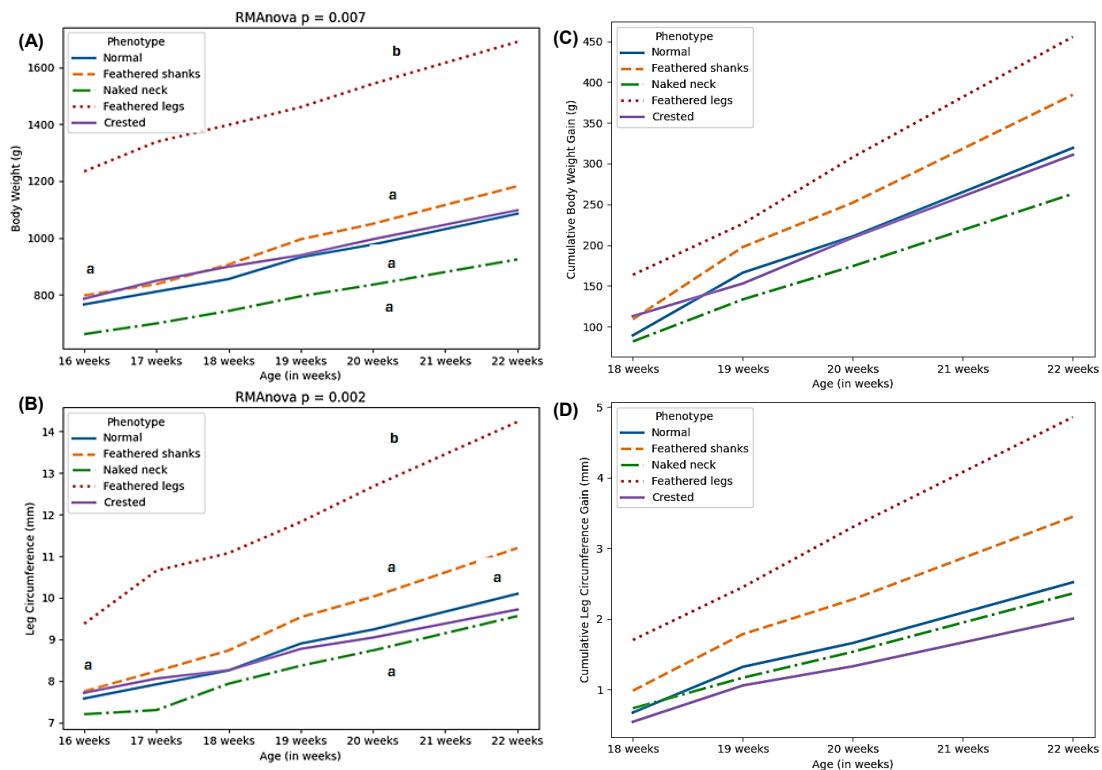
Where:  $BWG_{ijk}$  = Observed total weight gain for the  $k^{\text{th}}$  individual in the  $i^{\text{th}}$  Cluster and  $j^{\text{th}}$  Phenotype.;  $\mu$  = Overall mean;  $C_i$  = Fixed effect cluster ( $i$  varying from 1 to 4);  $S_j$  = Fixed effect of sex ( $j$  varying from 1 to 2);  $P_k$  = Fixed effect of phenotype ( $k$  varying from 1 to 5);  $\beta \cdot LCG_{ijk}$  = LCG (leg circumference gain) as covariate, where  $\beta$  is the regression coefficient;  $\sigma_k$  = Variance component specific to each phenotype group;  $e_{ijk}$  = Random error or residual effect for the  $k^{\text{th}}$  individual.

Finally, Pearson correlation was used to assess the relationship between the leg circumference and body weight of the studied chickens. Descriptive summary statistics and repeated measures ANOVA was performed with JASP 0.19.3 software (JASP Team, 2024), while Python (colab.research.google.com) was used to perform both clustering and network analysis, Pearson correlation and to generate graphical output of growth trends. Finally, R Studio 4.2.2 (R Core Team, 2022) was employed for Welch-ANOVA and GLS-ANCOVA modelling.

## Results

### Growth performance of local chicken phenotypes

Figure 1 illustrates the weekly evolution and cumulative gains in body weight and leg circumference for the studied Cameroon local chicken phenotypes, providing a detailed comparison of growth patterns.



**Figure 1.** Weekly evolution in body weight (A) and leg circumference (B), and body weight gain (C) and leg circumference gain (D) from 16 to 22 weeks in Cameroon local chickens. Line plots show weekly means.

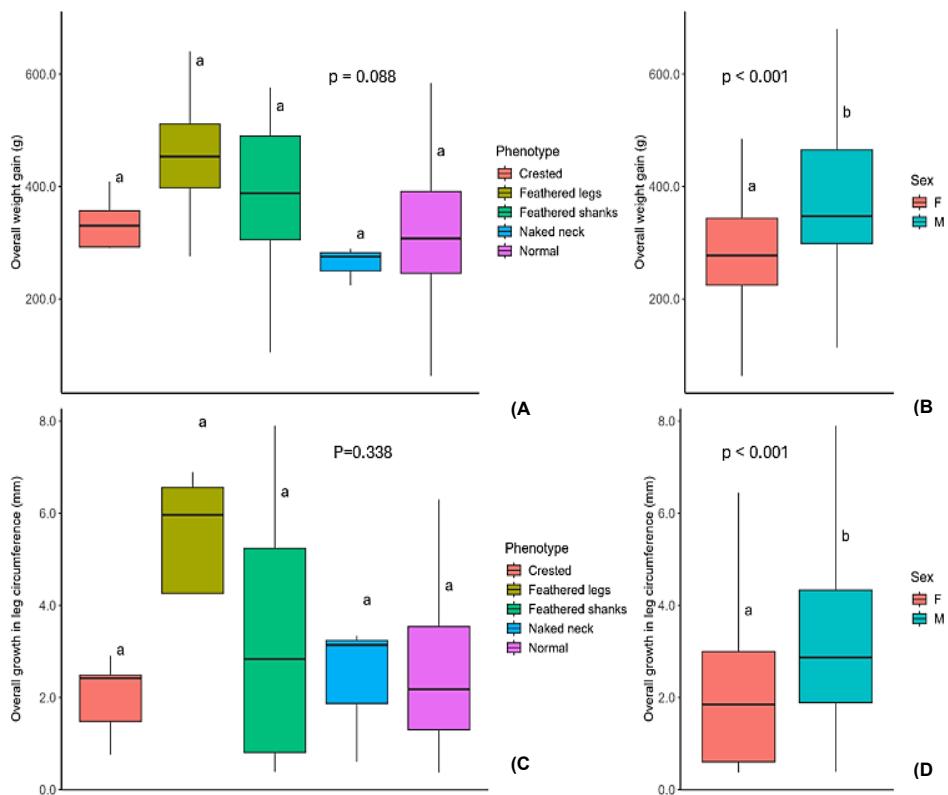
Repeated measures ANOVA p-values are reported; different letters indicate significant pairwise differences based on Games-Howell tests.

The results of repeated-means ANOVA revealed significant differences in the phenotype's growth rate ( $P < 0.01$ ) and leg circumference ( $P < 0.01$ ). All phenotypes displayed a steady increase in body weight, but with distinct growth rates (Figure 1A). The feathered leg chickens, for instance, exhibited the highest growth, reaching 1689.9 g by 22 weeks, followed by the feathered shanks (1182.2 g), crested (1097.3 g) and normal (1085.6 g) chickens. The naked neck chicken had the lowest final weight (924.6 g), but was not statistically different from the crested, normal and feathered shank chickens. Similarly to the body weight, the feathered leg chickens exhibited the largest leg circumference (14.2 mm) by 22 weeks, followed by the feathered shanks (11.2 mm), normal (10.1 mm) and crested (9.7 mm) chickens (Figure 1B). The naked neck chicken equally had the smallest final leg circumference (9.6 mm). However, this was not statistically different from the feathered shanks, naked neck, crested and normal chickens. The cumulative gains in live weight (Figure 1C) as well as the cumulative gains in leg

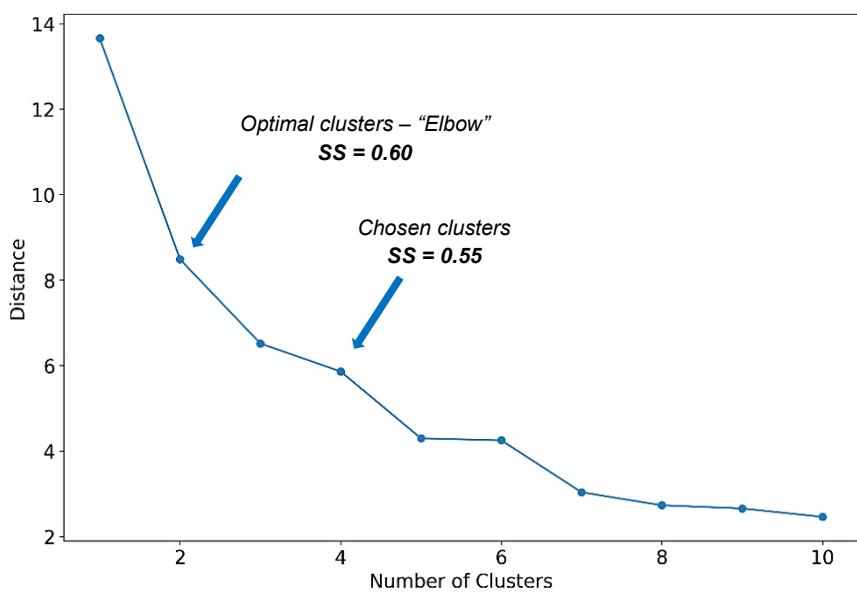
circumference (Figure 1D) mirror the evolution trends as the feathered leg and feathered shank chickens consistently outperform the other phenotypes between 16 and 22 weeks.

The total weight gain (TWG) and overall leg growth (LCG) across phenotypes as well as sexes at the end of the 22 weeks are presented in Figure 2. Welch's ANOVA showed a non-significant difference in the TWG ( $P > 0.05$ ) and LCG ( $P > 0.05$ ). However, the feathered shank chickens showed a superior mean TWG (455.7 g) followed by the feathered shank chickens (384.6 g). Conversely, the naked neck chickens recorded the least average total weight gain (263.0 g) (Figure 2A). Likewise, the feathered leg (4.9 mm) and feathered shank (3.5 mm) chickens equally had slightly higher overall leg growth (LCG) compared to the other phenotypes (Figure 2C). Similarly, the naked neck (2.4 mm) and crested chickens (2.0 mm) also had the least TWG.

In contrast to phenotypes, differences in TWG and LCG by sex were statistically significant ( $P < 0.001$ ), with cocks ( $\delta$ ) achieving a higher overall weight gain of 378.9 g (Figure 2B) and leg circumference growth of 3.3 mm compared to 282.6 g and 2.1 mm respectively in the hens ( $\varphi$ ) (Figure 2D).



**Figure 2.** Total weight gain (TWG) by phenotype (A) and sex (B), and leg circumference gain (LCG) by phenotype (C) and sex (D). Boxplots show medians and interquartile ranges. Welch's ANOVA p-values are reported; different letters denote significant pairwise differences based on Games-Howell post hoc tests.



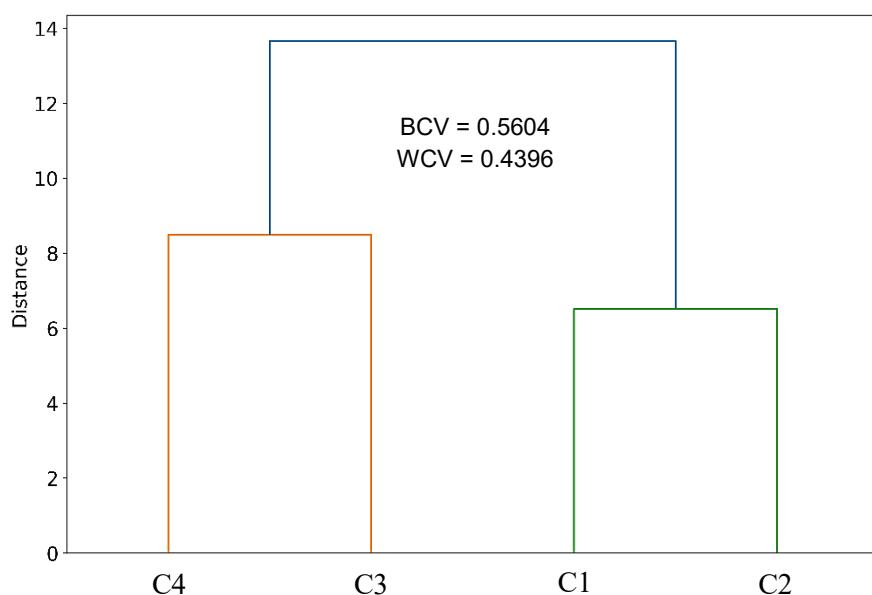
**Figure 3.** Determination of the optimal number of clusters using the Elbow method and Silhouette score (SS). The plot shows the reduction in within-cluster distance as the number of clusters increases from 1 to 10. The inflection points (elbow) suggest the optimal cluster number. Four clusters were selected based on a balance between interpretability and performance, despite the highest SS being observed at two clusters.

### Cluster and network analysis

Although the two-cluster solution had the highest average silhouette score (0.60), we opted for a four-cluster configuration (score = 0.55) to better capture subtle but meaningful biological variations in growth performance. The two-cluster grouping was statistically compact but too coarse, and obscured important growth distinctions. Contrastingly, the four-cluster solution allowed us to detect meaningful differences in performance, particularly between Clusters 3 and 4 and Clusters 1 and 2, thus offering a more practical basis for selection. This decision balanced statistical fit with biological relevance and

aligned with exploratory clustering practices in livestock studies (Palarea - Albaladejo & McKendrick, 2020). Moreover, the very slight difference of 0.05 in the Silhouette score highlights the fact that the four-cluster solution remains reasonably well separated and meaningful for subsequent analysis.

The dendrogram of performance clusters obtained from agglomerative hierarchical clustering of the total weight gain and leg circumference gain data points using Ward's linkage method is illustrated in Figure 4.



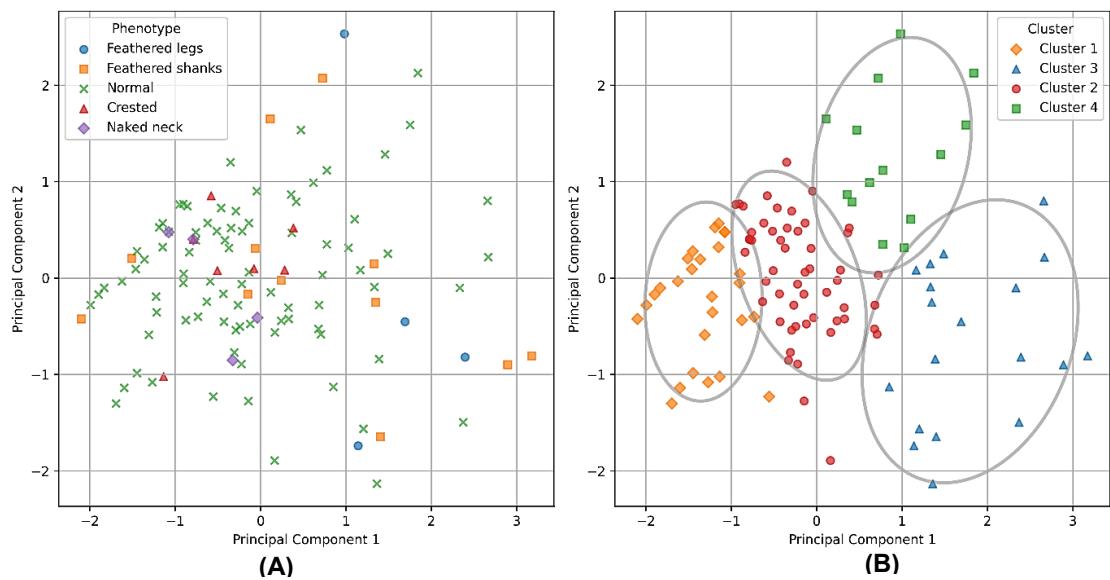
**Figure 4.** Hierarchical clustering dendrogram showing four performance clusters (C1–C4) derived from total weight gain (TWG) and leg circumference gain (LCG). Ward's method with Euclidean distance was applied. Vertical distances reflect dissimilarity between clusters. BCV and WCV represent the calculated between-cluster variance and within-cluster variance.

It is observed that Cluster 3 (C3) and Cluster 4 (C4) merge at approximately 8, indicating a moderate level of similarity between these two clusters. On the other hand, Clusters 1 (C1) and Cluster 2 (C2) merge at a shorter distance of around 6, suggesting a higher degree of similarity between them compared to C3 and C4. Ultimately, all four clusters (C1, C2, C3, and C4) merge at a greater distance of approximately 14, signifying the point at which all data points are grouped into a single cluster. The analysis reveals that the between-cluster variance (BCV) accounts for 56.04% of the total variance, highlighting the distinct differences between the clusters. Meanwhile, the within-cluster variance (WCV) is 43.96%, reflecting the variability within each cluster.

The PCA plot (Figure 4A) illustrates the distribution of the different phenotypes based on the first two principal components, which capture most of

the variance in the data. The normal, crested, and naked neck chickens appear to cluster more centrally, although some normal feathered chickens are equally scattered widely across the plot. This indicates a diverse range of phenotypic expressions within the normal category. However, the feathered shank and feathered leg chickens are more peripheral, suggesting they could be more distinct. The K-means plot (Figure 4B) shows the aggregation of the four performance clusters on the PCA space mapped by cluster markers. Overall, clusters appear to be comprised of different phenotypes. For instance, the normal feathered chickens appear to be found in all clusters, whereas feathered leg chickens are distinctly located in either Cluster 3 or Cluster 4. Also, the Crested and naked neck chickens seem to be more concentrated in cluster 1 and cluster 2.



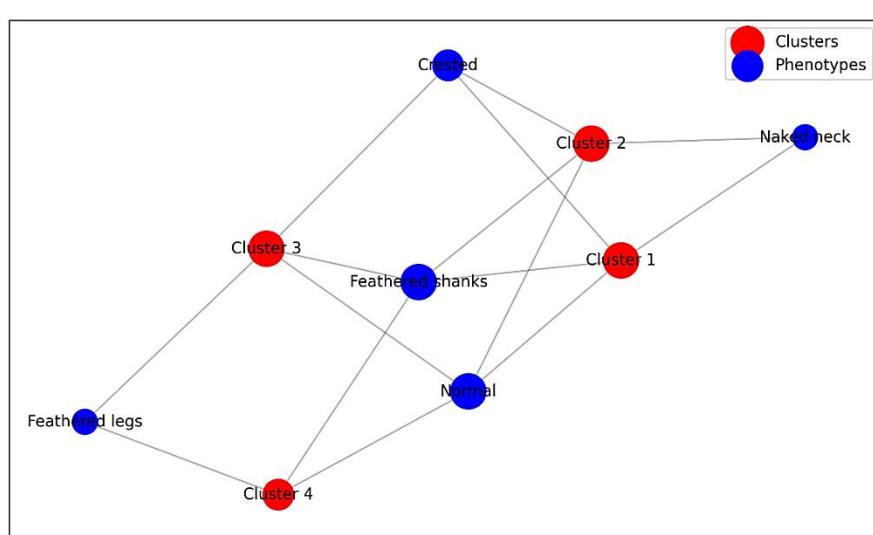


**Figure 5.** Principal component analysis (PCA) biplots. (A) Distribution of birds by phenotype across the first two principal components. (B) K-means cluster assignments overlaid on PCA-reduced space, with ellipses indicating cluster dispersion. The plot illustrates how performance-based clusters partially overlap with phenotypic classifications, reflecting both convergence and divergence in growth traits

Additionally, Cluster 3 and Cluster 4 consist of more dispersed chickens along the horizontal axis (PC1), suggesting greater variability in the principal component 1 dimension. In contrast, Cluster 1 and Cluster 2 seem to be closer to the origin of the plot and are more distinct from each other, indicating less variability in PC1 but potentially more in PC2.

A simplified visualization of the association between performance clusters and local chickens phenotypes is presented on the bipartite network plot (Figure 6). Results confirm that the feathered legs

phenotype is peripherally located while the normal feathered chickens is centrally located, as previously identified by the K-means cluster results (Figure 4). Additionally, Clusters 3 and 4 are more associated with distinct phenotypes, including feathered legs and feathered shanks, while crested, normal and naked necks are more represented across Clusters 1 and 2. However, the normal chickens and feathered chickens are also connected to all four clusters, equally confirming the K-means clustering results.

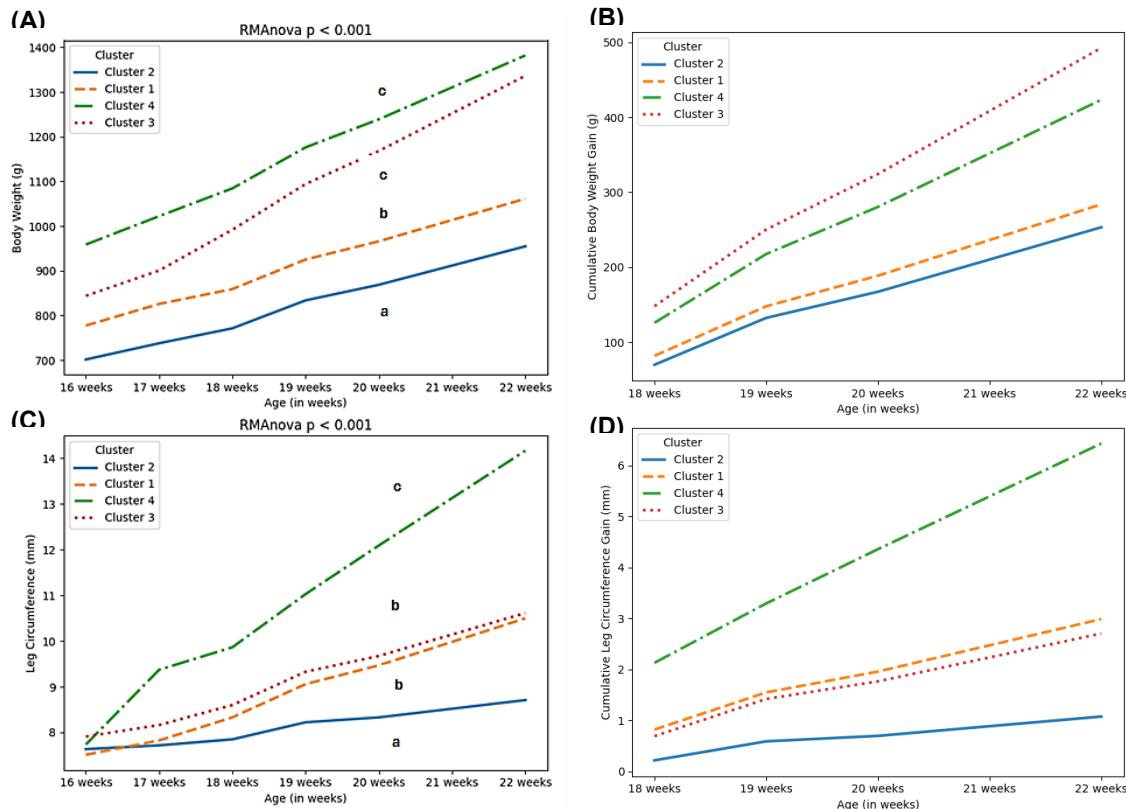


**Figure 6.** Bipartite network plot showing associations between phenotypes and performance-based clusters. Node colour indicates category (red = phenotype, blue = cluster), and edge thickness reflects the frequency of phenotype occurrence within each cluster. The central position of Normal and Feathered Shank phenotypes suggests a broad distribution across clusters.

#### Growth performance of local chickens clusters

Results on the weekly trend and the cumulative gains in body weight (BW) and leg circumference (LC)

between weeks 16 and 22 for the performance clusters are presented in Figure 7.



**Figure 7.** Weekly evolution in body weight (A) and leg circumference (B), and body weight gain (C) and leg circumference gain (D) from 16 to 22 weeks in local chickens clusters. Line plots show weekly means. Repeated measures ANOVA p-values are reported; different letters indicate significant pairwise differences based on Games-Howell tests.

There was a very significant difference in the growth profile ( $P<0.001$ ) and leg circumference ( $P<0.001$ ) of the different groups. A general trend can be observed across clusters showing a steady increase in body weight (Figure 7A) and leg circumference (Figure 7C), but at varying rates. Cluster 3 and Cluster 4 recorded the highest live weights (1381.9 g and 1336.4 g, respectively) by 22 weeks. Conversely, Cluster 1 and Cluster 2 chickens showed the lowest but distinct evolution in body weight, with a final weight of 1061.3 g and 954.7 g, respectively, by 22 weeks. Similarly, the largest leg circumference by 22 weeks was observed for Clusters 4 (14.2 mm) and 3 (10.6 mm) while Clusters 1 and 2 had the smallest leg circumferences (10.6 mm and 8.7 mm). The cumulative weekly gains in body weight (Figure 7B) and leg circumference (Figure 7D) equally mirror the evolution trends as local chickens in Clusters 3

followed by Cluster 4 consistently gained more weight than their counterparts in Clusters 2 and 1.

Welch ANOVA revealed significant differences in TWG ( $P<0.001$ ) and LCG ( $P<0.001$ ) for performance clusters with chickens Cluster 3 and Cluster 4, respectively, gaining an average of 478.9 g and 413.2 g in live weight between 16 and 22 weeks (Table 2). The least cumulative gains were recorded for Clusters 1 (284.5 g) and lastly Cluster 2 (258.8 g). However, subjects in Cluster 4 (6.4 mm) had superior overall leg growth, followed by those in Cluster 1 (3.0 mm) and Cluster 3 (2.7 mm). Chickens in Cluster 2 had the lowest ( $P<0.001$ ) mean LCG (1.07 mm) compared to the subjects in other clusters.

Although there was no significant difference ( $p > 0.05$ ) in the TWG and LCG across sexes within each cluster group, results show that overall, cocks ( $\delta$ ) outperform the hens ( $\varphi$ ) both in terms of TWG and

LCG. However, ANOVA revealed a significant interaction effect ( $P<0.001$ ) between sex and clusters (S\*C), indicating that the impact of sex on performance is not independent of the cluster grouping.

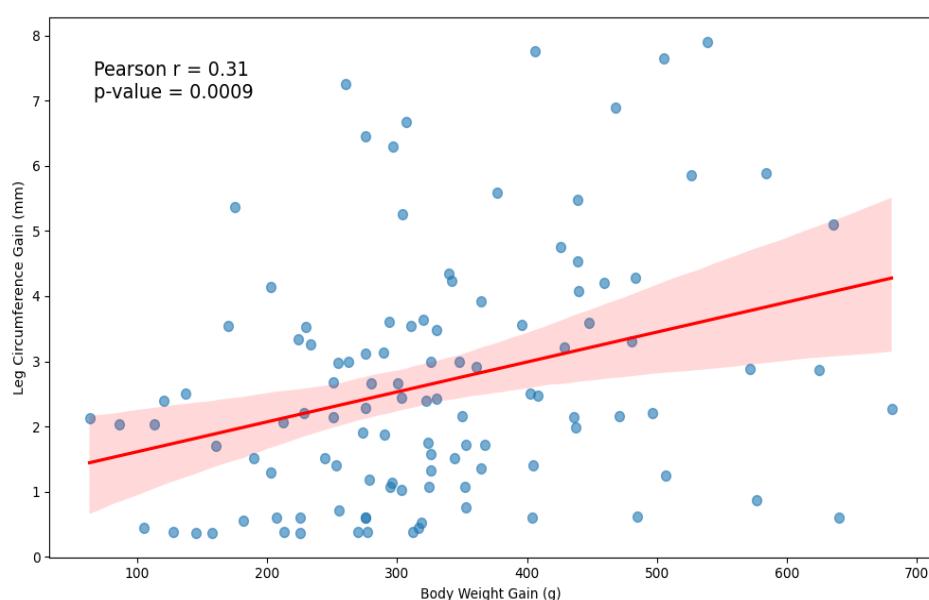
To assess the relationship between the increase in leg circumference and the gain in body weight, a

correlation analysis was performed by plotting TWG against LCG, as shown in Figure 8. The results revealed a statistically significant ( $P<0.001$ ) positive correlation between TWG and LCG. However, the relatively weak correlation coefficient ( $r = 0.31$ ) suggests that the association is not very strong.

**Table 2:** Summary statistics of total weight (TWG) and leg circumference (LCG) gain of local chickens by cluster and sex from 16 to 22 weeks

Variable	Cluster	Sex	N	Mean	SD	CV	p - C	p - S*C
TWG	Cluster 2 <sup>a</sup>	Hen (♀) <sup>a</sup>	30	246.6 <sup>a</sup>	93.3	0.38		
		Cock (♂) <sup>a</sup>	12	271.1 <sup>ab</sup>	95.8	0.35		
		<i>Subtotal C2</i>	42	258.8	94.5	0.38		
	Cluster 1 <sup>a</sup>	Hen (♀) <sup>a</sup>	19	272.5 <sup>ab</sup>	51.3	0.19		
		Cock (♂) <sup>a</sup>	17	296.4 <sup>ab</sup>	57.4	0.19		
		<i>Subtotal C1</i>	36	284.5	54.3	0.19	<0.001	<0.001
	Cluster 4 <sup>b</sup>	Hen (♀) <sup>a</sup>	4	389.9 <sup>abc</sup>	85.0	0.22		
		Cock (♂) <sup>a</sup>	10	436.5 <sup>bc</sup>	137.7	0.32		
		<i>Subtotal C4</i>	14	413.2	111.3	0.27		
LCG	Cluster 3 <sup>b</sup>	Hen (♀) <sup>a</sup>	5	453.0 <sup>c</sup>	33.9	0.08		
		Cock (♂) <sup>a</sup>	16	504.8 <sup>c</sup>	86.7	0.17		
		<i>Subtotal C3</i>	21	478.9	60.3	0.13		
	Cluster 2 <sup>a</sup>	Hen (♀) <sup>a</sup>	30	1.0 <sup>b</sup>	0.6	0.62		
		Cock (♂) <sup>a</sup>	12	1.4 <sup>b</sup>	0.7	0.48		
		<i>Subtotal C2</i>	42	1.2	0.6	0.55		
	Cluster 1 <sup>b</sup>	Hen (♀) <sup>a</sup>	19	2.9 <sup>a</sup>	0.6	0.21		
		Cock (♂) <sup>a</sup>	17	3.1 <sup>a</sup>	1.1	0.35		
		<i>Subtotal C1</i>	36	3.0	0.8	0.19	<0.001	<0.001
	Cluster 4 <sup>c</sup>	Hen (♀) <sup>a</sup>	4	6.1 <sup>c</sup>	0.7	0.11		
		Cock (♂) <sup>a</sup>	10	6.6 <sup>c</sup>	1.0	0.16		
		<i>Subtotal C4</i>	14	6.4	0.9	0.14		
	Cluster 3 <sup>d</sup>	Hen (♀) <sup>a</sup>	5	2.5 <sup>ab</sup>	1.4	0.54		
		Cock (♂) <sup>a</sup>	16	2.8 <sup>a</sup>	1.3	0.46		
		<i>Subtotal C3</i>	21	2.7	1.3	0.50		

Note. N (frequency), SD (standard deviation), CV (coefficient of variation), p (p-value for Welch ANOVA, not assuming equal variance), C (Cluster), S\*C (Interaction).



**Figure 8.** Scatter plot showing the relationship between body weight gain (TWG, g) on the x-axis and leg circumference gain (LCG, mm) on the y-axis in Cameroon local chickens. The red line represents the line of best fit with a 95% confidence interval shaded in pink. Pearson correlation coefficient (r) and p-value are displayed in the top-left corner.

### Factors influencing growth

A general least squares analysis of covariance was performed to understand the factors influencing growth performance in local chickens. Total weight

gain (TWG) was used as the dependent variable, leg circumference gain (LCG) as the covariate, and phenotype, cluster and sex were included as factors (Table 3).

**Table 3:** GLS-ANCOVA results for factors influencing overall weight gain

Variable	Value	SE	t-value	p-value
<b>Cluster</b>				
Cluster 2	-52.92	26.03	-2.03	0.045*
Cluster 3	192.37	24.33	7.91	< 0.001***
Cluster 4	192.50	43.48	4.43	< 0.001***
<b>Sex</b>				
Cock	42.03	18.23	2.31	0.023*
<b>Phenotype</b>				
Feathered legs	19.29	65.64	0.29	0.769
Feathered shanks	-0.31	44.58	-0.01	0.994
Naked neck	-0.59	41.59	-0.01	0.989
Normal	-10.31	36.66	-0.28	0.779
<b>Leg circumference gain</b>				
	-19.14	9.37	-2.04	0.044*
<b>Model parameters</b>				
Intercept	326.36	45.87	7.12	< 0.001***
Model Nagelkerke R <sup>2</sup>	0.11			
Model AIC	1270.56			
ANCOVA AIC	1325.49			

SE, standard error; R<sup>2</sup>, coefficient of determination. \*\* and \* significant at  $P<0.001$ ,  $P<0.01$  and  $P<0.001$ , respectively.<sup>1</sup>

Cluster groups significantly affected weight gain, with Cluster 3 ( $P<0.001$ ) and Cluster 4 ( $P<0.001$ ) each gaining approximately 192 g more than the Cluster 1 chickens between weeks 16 and 22. Contrastingly, Cluster 2 chickens significantly ( $P<0.05$ ) gained 52.9 g less than Cluster 1 chickens during the same period. Sex is also a significant ( $P<0.05$ ) predictor of growth performance in adult local chickens with cocks gaining 42 g more than hens. Further, gain in leg circumference (LCG) has a small but significant negative effect ( $P<0.05^*$ , -19.2) on weight gain, indicating there is 19.2 g less in body weight per mm gain in leg circumference. Conversely, phenotype is not an important ( $P> 0.05$ ) indicator of growth performance in adult local chickens. Nonetheless, Feathered legs chickens gained slightly more (approximately 19.3 g and 29.7 g) body weight compared to the crested and normal feathered counterparts when corrected for growth in leg circumference (LCG).

### Discussion

Cameroon local chickens display substantial genetic diversity as evidenced by the variety of phenotypes observed post-incubation. The underrepresentation of Naked Neck, Crested, and Feathered Legs/Shanks

phenotypes in our sample aligns with known patterns of low frequency for Na/, Pt/, and Cr/\_ adaptive genes across African indigenous chickens (Nigussie *et al.*, 2010; Dahloum *et al.*, 2016; Kindie & Tamiru, 2021). However, we observe a slightly higher occurrence of chickens with leg feathering (14.1%) compared to earlier studies by Fotsa *et al.* (2010). This change could be motivated by the perception that feathered leg chickens grow faster than local chickens but retain the latter's desirable organoleptic qualities, which are highly valued by consumers (Castellini *et al.*, 2008).

Observed differences in body weight and leg circumference across phenotypes may reflect complex interactions between genetic makeup, sex, and other subtle micro-environmental factors such as in-pen competition for feed. The consistently higher growth performance in males supports well-documented patterns of sexual dimorphism in poultry (Bembidé *et al.*, 2013; Halima *et al.*, 2007), which is often attributed to hormonal and physiological factors, such as higher circulating levels of testosterone in males, which promote muscle growth and protein synthesis (Osei-Amponsah *et al.*, 2011). Furthermore, males generally exhibit more efficient feed conversion and greater appetite compared to

females, contributing to faster weight gain (Fotsa *et al.*, 2011; Mustofa *et al.*, 2021). The presence of sex-linked growth genes and differential energy allocation strategies may also play a role in shaping these observed differences. The leg circumference values observed suggest a modest skeletal development relative to heavier indigenous breeds. When contextualized against similar studies from Central Africa (Bembidé *et al.*, 2013), Algeria (Dahloum *et al.*, 2016), and Nigeria (Adeleke *et al.*, 2010), the performance of Cameroon local chickens appears intermediate, which underscores the need for targeted selection if productivity is to be improved.

The superior growth profile of feathered leg/shank chickens was demonstrated between 16 and 22 weeks, corroborating the findings of Keambou *et al.* (2013) and Bembidé *et al.* (2013). This may point to a potential genetic predisposition for superior growth in this phenotype. Yang *et al.* (2019) highlight the association between phenotypic traits like leg feathering and important economic traits like growth rate. Candidate genes like the forelimb (PITX1) and hindlimb (TBX5), specific transcription factors have been identified to influence skeletal development and growth performance in domestic chickens; however, the mechanism remains poorly understood (Li *et al.*, 2020b). It could be that the feathered leg phenotype may instead present a marker of polygenic traits associated with muscle development and hormonal regulation rather than a single candidate gene. Meanwhile, the least evolution for the Naked Neck chickens supports previous findings that associate their thermoregulatory efficiency with adaptation to harsh environments rather than growth performance (Hako & Yoniwo, 2023a, 2023b). Furthermore, the lack of significant differences ( $P>0.05$ ) in TWG and LCG across phenotypes may be attributed to several factors. First, this could be due to underlying genetic admixture and shared ancestry among local chickens populations, where visible traits do not necessarily reflect differences in growth-related genes (Ren *et al.*, 2023). Secondly, morphological features like crests or feather patterns are often controlled by major genes (*Na*, *Pti*, *Cr*, *Fr*) which are not directly related to the polygenic traits governing growth in chickens (Tixier-Boichard, 2002). Additionally, the small sample sizes for rare phenotypes (Feathered leg, Crested and Naked neck), uniform experimental conditions that limit genotype-by-environment interactions, and the potential compensatory growth effect during the later stages may have minimized observable performance differences. Moreover, the uneven sex distribution within each phenotype, despite sex being a well-known growth determinant (Nguyen Van *et al.*, 2020), may have further confounded these comparisons. These findings highlight the fact that phenotype alone may not be a very reliable predictor of growth performance in

chickens, and alternative performance-based selection methods can be more effective.

### Cluster and Network Analysis

Our results confirm that clustering based on performance traits can identify biologically relevant subgroups that phenotype-based classification may overlook. However, as Vakhrameev *et al.* (2023) demonstrated in their work on divergently selected global chickens breeds, clustering outcomes are highly sensitive to methodological choices, including clustering algorithms, trait combinations, and levels of genetic admixture. Their proposed EY/W indicator and trait-based sorting by inflection points emphasize the importance of carefully selecting input variables and understanding trait interactions. While our study focused on growth-related traits (TWG and LCG), the implications of their work suggest that integrating broader phenotypic or genomic indicators could further refine performance-based classification in local chickens populations. Rosario *et al.* (2008) identified three initial clusters based on body weight, while we revealed two clusters using Silhouette analysis before refining to four for greater biological interpretability. The difference in clustering outcomes could be attributed to factors such as the age of birds analyzed and the choice of linkage methods. Rosario *et al.* employed Mahalanobis distance, while our study used Ward's method with Euclidean distance. These methodological differences underscore how the structure of clustering results may vary based on design choices, dataset properties, and the stage of growth under investigation (Palarea - Albaladejo & McKendrick, 2020).

With the modified four-cluster solution, Clusters 3 and 4 consistently demonstrate superior outcomes. The similarity observed between these two clusters and their clear distinction from Clusters 1 and 2 suggests a potential concentration of favourable growth traits within certain groups, possibly reflecting underlying genetic patterns. This divergence is particularly meaningful because it emerged independently of phenotypic classification, reinforcing the value of data-driven clustering. The consistently better performance in Clusters 3 and 4 may reflect a more efficient resource utilization, better adaptation to the semi-intensive and intensive system, or an inherited advantage linked to unidentified genetic factors. Sexual dimorphism was also in favour of cocks under performance-based groupings who demonstrated superior TWG and LCG irrespective of phenotypes. These results further support the practical application of performance-based grouping for improving productivity in local poultry breeding programs, especially where molecular tools are unavailable (Rosario *et al.* 2008).

Furthermore, these findings unlock valuable information for local poultry breeding programs in

Cameroon, with potential complementary effects on growth. For instance, while Cluster 4 expressed the highest live weight by 22 weeks, Cluster 3 demonstrated the highest TWG between 16 and 22 weeks. This may be due to the better early growth for birds in Cluster 4 and the best late growth for subjects in Cluster 3. This distinct performance dynamics observed where one excels in early growth and the other in sustained weight gain could be optimized by selective crossbreeding to combine these complementary traits. Such an approach could produce offspring (F1) that achieve faster initial growth while maintaining high cumulative gains, which is desirable for meat production. Meanwhile, the clear divergence between Clusters 1 and 2 opens the possibility of divergent selection pathways, where one group may be optimized for growth and the other for traits like egg production or resilience in low-input systems. The significant difference in leg circumference among sexes and clusters reinforces the ranking of cluster performance, and their alignment with body weight trends supports their use as a secondary selection trait in this study. The above patterns demonstrate that cluster-based selection can reveal biologically meaningful subgroups, enabling targeted selection without relying on phenotypic appearance alone. Importantly, this strategy offers the advantage of enhancing the likelihood of preserving within-population genetic diversity by avoiding uniform selection pressure on single traits or phenotypes.

Network analysis provides additional insights into the relationship between phenotypes and performance-based clusters. Rather than clustering by strict physical appearance, phenotypes were distributed across multiple clusters, pointing to possible genetic exchange between groups from historical crossbreeding. Genetic admixture is very common among indigenous chicken populations reared under extensive poultry systems, as indicated by Keambou *et al.* (2014). Studies by Leroy *et al.* (2012) and Bembidé *et al.* (2024) have reported high genetic diversity and heterozygosity levels exceeding 45% in scavenging chicken populations across Cameroon and the Central African Republic, as determined using microsatellite markers. The Normal and Feathered shank chicken were centrally distributed across all four clusters. This centrality indicates these phenotypes may serve as genetic bridges across cluster groups, facilitating the spread of advantageous traits through natural and planned crossbreeding. Furthermore, their consistent presence in Clusters 3 and 4 highlights their potential usefulness in developing breeding lines focused on growth optimization. From a breeding strategy perspective, this means that selection decisions informed by network analysis can help maintain broad genetic diversity while strategically combining

high-growth potential from different phenotype groups. On the other hand, peripheral phenotypes like Naked Neck and Feathered Legs, which appeared in fewer clusters, particularly Clusters 1 and 2, may possess niche traits such as fast growth rate, disease resistance or adaptation to specific ecological zones that are also valuable depending on breeding goals. This peripheral placement may be due to their small sample sizes in the current study, but could also point to limited gene flow or historical underutilization by breeders, which makes them good reservoirs for potentially unique alleles for resilience and adaptability. Other studies have confirmed high intra- and inter-breed gene flow, contributing to the adaptability and heterozygosity observed in scavenging populations (Ren *et al.*, 2023). By integrating cluster-based analysis and network structure, breeding programs can prioritize fast-growing birds while also preserving genetic diversity. This framework supports sustainable breeding by promoting both productivity and conservation of genetic resources, especially in contexts where formal pedigree or genomic data may be lacking. In practical terms, this dual approach empowers breeders to design crossing schemes that balance growth efficiency with genetic stability, thereby ensuring that selection does not lead to narrow genetic pools or unintended loss of traits essential for survival in low-input systems.

### Factors Influencing Growth

GLS ANCOVA assessed the influence of cluster grouping, sex, phenotype and leg circumference gain (LCG) on total weight gain (TWG). The model reveals a low  $R^2$  value (0.112) yet captures statistically significant effects, which means that although it captures meaningful patterns, a large portion of variability remains unexplained, possibly due to unmeasured biological or environmental factors. Notably, phenotype did not significantly influence TWG, suggesting that external morphology alone is not a reliable proxy for growth performance in adult local chickens. Feathered leg birds showed a marginal advantage, gaining approximately 19.3 g more than Crested and Normal birds, though this difference was not statistically conclusive and warrants further investigation. Clusters 3 and 4 were the strongest predictors of higher TWG, with birds in these birds in clusters gaining over 190 g more than Cluster 1 ( $P<0.001$ ). In contrast, Cluster 2 birds gained about 52.9 g less than those in Cluster 1, reinforcing divergent growth potential across performance-based groups. Sex was also a significant factor, with cocks gaining an average of 42 g more than hens, consistent with established sexual dimorphism in poultry growth (Keambou *et al.*, 2013; Nguyen Van *et al.*, 2020).

Although Pearson correlation revealed a weak but significant positive relationship between LCG and TWG ( $r = 0.31, P < 0.001$ ), the GLS model showed a small but significant negative effect of LCG on TWG. This suggests a possible trade-off where birds allocating more resources to skeletal growth and development may do so at the expense of body mass accumulation. These findings mirror those of Liswaniso *et al.* (2024), who reported weak positive correlations between shank circumference and body weight and a negative, non-significant relationship with growth rate in Chinese Sasso chickens. Such trade-offs emphasize that structural traits like leg circumference should be considered as supplementary, not primary, indicators in breeding programs.

### Conclusion

This study confirms the utility of cluster-based selection for optimizing growth performance traits in Cameroon local chickens, offering a potentially more effective alternative to traditional phenotype-based selection. Clusters 3 and 4 represent promising candidates for targeted breeding efforts due to their superior body weight and weight gain profile. In parallel, network analysis revealed overlapping relationships between clusters and phenotypes, suggesting opportunities for strategic cross-cluster mating to enhance productivity while maintaining genetic diversity. To capitalize on these findings, we recommend that local poultry breeders and policymakers adopt systematic performance

recording practices and promote crossbreeding strategies within local flocks. Notable limitations include the study's moderate sample size, absence of genomic data, and a model that explained only 11.2% of total variation in TWG, indicating the need to account for additional biological and environmental factors. Future research should integrate molecular markers and a broader range of performance traits to improve classification accuracy and predictive power. Overall, the combination of clustering and network analysis presents a scalable, evidence-based framework for improving growth efficiency and genetic resilience in local poultry production systems.

### CRedit authorship contribution statement

**Yoniwo SN:** Conceptualisation, Methodology, On-farm experimentation and data collection, Formal analyses, Writing the original draft and Editing; **Yato Katte NK:** On-farm experimentation and data collection, Writing the original draft and Editing; **Hako Touko BA:** Co-supervision, Conceptualisation, Methodology, Reviewing and Validation; **Muluh DW:** On-farm experimentation and data collection. **Awah Ndukum J:** Supervision, Reviewing and Validation.

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