REVISTA DELOS DESARROLLO LOCAL SOSTENIBLE

Breed characterization of native chickens based on their head shape and size

Caracterização racial de galinhas autóctones com base na forma e tamanho da cabeça

DOI: 10.55905/rdelosv16.n45-026

Recebimento dos originais: 27/07/2023
Aceitação para publicação: 25/08/2023

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ABSTRACT
Native chickens have unique characteristics related to adaptation to the most diverse breeding systems. The phenotypic characterization of different chicken ecotypes is necessary in view of the current situation of loss of local genetic resources. Development of methodologies capable of supporting morphometric studies is very useful in native domestic breeds conservation. Thus, geometric morphometrics is a recently proposed approach for the quantification of shape and size variation separately, which has shown advantages over the traditional method. The objective of this study was to characterize female chickens of the Peloco, Barbuda, and Caneluda breeds based on their head shape and size by using geometric morphometric. Images of the head of 85 chickens were captured, and 13 landmark and semi-landmark were set in the beak, nostril and eye regions. The three chicken breeds evaluated showed significant differences ($p < 0.01$) regarding the head shape. Most variations occurred in the eyes and beak; the Peloco breed presented wide and curved beak, and round eyes. According to the grouping analysis, the Peloco and Caneluda breeds had similar head shape and were allocated to the same group; and the Barbuda breed was allocated separated from the others by differing from them. The breeds presented significant differences ($p < 0.01$) in size, and the Caneluda breed had the largest head size. The geometric morphometrics allowed the distinction of the breeds by identifying shape variations, especially in the beak and eye regions. Thus, it is promising for studies on phenotypic diversity and breed characterization of these birds.

Keywords: geometric morphometrics, phenotypic diversity, genetic resources.

RESUMO
As galinhas nativas têm características únicas relacionadas com a adaptação aos mais diversos sistemas de reprodução. A caracterização fenotípica de diferentes ecótipos de galinhas é necessária, tendo em conta a atual situação de perda de recursos genéticos locais. O desenvolvimento de metodologias capazes de apoiar estudos morfométricos é muito útil na conservação de raças domésticas nativas. Assim, a morfometria geométrica é uma abordagem recentemente proposta para a quantificação da forma e variação de tamanho separadamente, que tem mostrado vantagens sobre o método tradicional. O objetivo deste estudo foi caracterizar frangos fêmeas das raças Peloco, Barbuda e Caneluda com base na forma da cabeça e tamanho, utilizando morfometria geométrica. Imagens da cabeça de 85 galinhas foram capturadas, e 13 marcos e semimarcos anatômicos foram definidos nas regiões do bico, narina e olho. As três raças avaliadas apresentaram diferenças significativas ($p < 0,01$) quanto à forma da cabeça. A maioria das variações ocorreu nos olhos e bico; a raça Peloco apresentou bico largo e curvo, e

olhos redondos. De acordo com a análise de agrupamento, as raças Peloco e Caneluda tinham forma de cabeça semelhante e foram alocadas para o mesmo grupo; e a raça Barbuda foi alocada separada das outras por diferir delas. As raças apresentaram diferenças significativas (p < 0,01) de tamanho, e a raça Caneluda teve o maior tamanho de cabeça. A morfometria geométrica permitiu a distinção das raças identificando variações de forma, especialmente nas regiões do bico e do olho. Assim, é promissor para estudos sobre diversidade fenotípica e caracterização da raças dessas aves.

Palavras-chave: morfometria geométrica, diversidade fenotípica, recursos genéticos.

1 INTRODUCTION

Native chickens are widely used in family poultry. These chickens are good foragers, present maternal ability—important in the natural incubation process—and have high survival rate, with minimum handling requirement. In addition, they can survive with few resources and adjust well to fluctuations in food availability (Salako & Ige, 2006; Mengesha & Tsega, 2011). Therefore, they are especially useful for small breeders, and sometimes their only source of income, by producing good quality animal protein for both consumption and commercialization (Kaya & Yildiz, 2008; Alders & Pym, 2010).

The great phenotypic diversity of these birds is extremely important for food safety; they are considered as a reservoir of genes regarding adaptability and resistance to diseases with potential for future use (Boettcher et al., 2010). Although these chickens are less productive than commercial breeds and lines, they present unique characteristics of economic and cultural value to the locations where they have developed. These characteristics allowed the sustainability of family poultry, especially in developing countries.

The chicken breeds found in Brazil originated from crosses between European and Asian breeds introduced during the colonial period—14th to 19th centuries. However, many of these ecotypes are in an imminent state of extinction due to the intense use of genetically improved lineages, the introduction of exotic breeds, and indiscriminate crosses. According to Iqbal et al. (2015), the fact that some native breeds can be extinct before we even know about their characteristics and potentialities denotes the urgent need to characterize these resources for their use and conservation.

In general, the first step in breed characterization is the identification of populations based on morphological descriptors (Ajayi et al., 2012). Thus, linear morphometry has been used for phenotypic characterization and also for selection of superior animals, using linear distances between body structures. The measures usually used to describe the shape and size of the birds are: body length, width, circumference, and weight (Cabarles Jr. et al., 2012; Akililu et al., 2013; Salces et al., 2015). However, this type of description is not accurate, since it does not evaluate the shape as a whole, making this assessment difficult through linear measurements.

Geometric morphometrics comprises a set of techniques capable of analyzing the shape of the structures by obtaining landmarks, which are geometrically homologous points between the structures (Tofilsky, 2008), making it possible to evaluate the effects of shape and size separately (Monteiro & Reis, 1999; Mitteroecker & Gunz, 2009).

In geometric morphometrics, measurements are made from images, thus, it is possible to photograph the individuals, archive the images, and measure them later. This method is advantageous compared to linear measures, which can be extremely laborious and depending on the structure used (Perés-Casanova & Martinez, 2013). Studies on animal production using geometric morphometrics are scarce in the literature, thus, the objective of this work was to assess the variations in head shape and size among three native chicken breeds, aiming their breed characterization, and conservation of these genetic resources.

2 MATERIALS AND METHODS

2.1 Local and experimental animals

A total of 85 females chickens—12 Barbuda, 22 Caneluda, and 51 Peloco—were evaluated. These chickens were from the Native Poultry Breeding Center of the Poultry Sector of the State University of Southwest of Bahia, located in Itapetinga in the state of Bahia, Brazil.

2.2 IMAGE COLLECTION AND DATA COLLECTION

Images of the left profile of the birds' head were captured using a digital camera (Sony Cyber-Shot 16.1 MP) coupled to a tripod, positioned at the beak height of each bird to take their photograph. A graph paper was fixed as background to asses the scale and later size analysis. The images were converted from the original format (JPEG) to the TPS format in the tpsUtil program (Rohlf, 2015). Five anatomical type-I landmark and seven semi-landmark were set over the beak, nostril and eye regions, using the tpsDig2 program (Rohlf, 2015) (Figure 1).

Subsequently, the semi-landmarks were align, transforming them into precise landmarks using the tpsRelw program (Rohlf, 2015).

2.3 STATISTICAL ANALYSIS

After the measurements, the Cartesian coordinates were analyzed in the MorphoJ-2.0 software (Klingenberg, 2011). A Procrustes analysis was performed to obtain the fit of the mean or a consensus configuration. A canonical variables analysis was performed with the purpose of reducing the sample space to few variables capable of explaining the original variation. Then, a discriminant analysis was used to verify the distinction of the breeds. Subsequently, a cross-validation was performed to verify the accuracy of the data and how many individuals were classified correctly in their respective breeds.

After obtaining the shape variables, a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) grouping analysis was performed using the PAST-2.03 software (Hamer et al., 2001).

The size analysis was performed from the centroid size, which is a general measure of size defined as the square root of the sum of the squares of the distances between each reference point and the center of mass (Bookstein, 1991).

Analysis of variance (ANOVA) was performed for the size from the centroid size, and the Tukey's test was used to compare the means. The variation of the shape as a function of size, i.e., the shape changes with size, was evaluate through regression analyses.

3 RESULTS

The Peloco, Barbuda and Caneluda breeds showed significant differences ($p < 0.001$). The first two canonical variables explained 94.33% of the shape variation among the birds, and the first and second canonical variate axis (CVA1 and CVA2) explained 62.15% and 32.18% of the variation between breeds, respectively. This shows a greater distinction of the Peloco breed in the CVA1, and the distinction between Caneluda and Barbuda in the CVA2 (Figure 2A).

The analysis of the deformation grid in relation to the consensus configuration, which represents the average shape among all the birds of the three breeds, allowed to identify the shape variations in beak, nostril and eye of the chickens. The deformation grids associated to the CVA1 (Figure 2B and 2C) showed that the main variations were in the initial portion of the beak and around the eyes. The Barbuda and Caneluda chickens were distributed at the negative end of the CVA1 (Figure 2A), i.e., they had negative scores in the CVA1. On the other hand, deformation grids associated with the CVA2 (Figure 2D and 2E) showed that the variation in shape comprises changes in the eye and beak regions, i.e., they had positive scores in the CVA2.

According to the dissimilarity between the breeds, based on the distances of Procrustes and Mahalanobis, the distances of shape were significant ($p < 0.01$) (Table 1), with a greater morphological proximity between the Peloco and Caneluda chickens.

According to the cross-validation test, 78.13% of the chickens were correctly classified in their respective breeds, especially the Peloco breed, which had the highest fit rates, with mean of 86.26%. The Caneluda and Barbuda breeds had fit rates of 77.26% and 70.83%, respectively.

The grouping analysis through the UPGMA resulted in the formation of two groups (Figure 3) with 100% bootstrap, denoting the difference in the head shape of Barbuda chickens, isolate from the others; and the similarity in head shape between the Peloco and Caneluda breeds, allocated in the same group.

Based on the centroid size, there was a significant difference ($p < 0.001$) in head size among the breeds in the analysis of variance. The mean test showed significant differences ($p < 0.001$) in all comparisons; the Caneluda breed had the largest size (8.49 ± 0.58), followed by Barbuda (7.86 ± 0.47), and Peloco (7.38 ± 0.46).

The regression of the shape as a function of the centroid size showed that only about three percent of the variation in shape is explained by the size variation ($R^2 = 3.17\%$). There was a low and significant correlation ($r = 0.17780$; $p < 0.01$) between size and shape, thus, the head shape can be altered by the increase in size, however these changes are small.

4 DISCUSSION

The deformations found along the canonical axes represent the changes in shape as a function of the variation in the scores of the canonical variables, and can be connected with the dispersion of the populations in the bidimensional space (Monteiro & Reis, 1999; Klingenberg, 2013). The Barbuda and Caneluda breeds, which presented negative scores in the first canonical axis, showed shortened and dorsoventrally flattened beaks, with smaller nostrils, and more elongated eyes compared with the Peloco breed. Most chickens of the Caneluda breed are characterized by round eyes and broad beaks with less curvature than the Barbuda breed.

It would be difficult to notice such differences only with measures of length and width. Identifying the nature of the morphological variation is one of the advantages of geometric morphometrics, which was able to identify subtle variations in shape. Berns & Adams (2010) evaluated the sexual dimorphism in bird species with geometric morphometrics and found that it was able to identify beak shape variations between male and female birds that are not observed by traditional methods, recommending this methodology for studies of beak shape variation within and between species. Other studies with wild birds identified variations in skull (Degrange & Picasso, 2010) and beak (Navarro et al., 2009) shapes, confirming the efficiency of the geometric morphometrics.

In the case of domestic chicken, the head has a number of qualitative attributes that characterize their breed, such as type and color of the crest, and eye and beak color. This group has great morphological variation, which makes breed characterization difficult, since it presents diverse phenotypes. The identification of variations in head shape (beak, nostril and eye regions) among the breeds evaluated was important to their characterization. Thus, this study may support future studies, whether with birds or other species of zootechnical interest, in which breed characterization is necessary for the distinction of crossbreed animals without defined breeds or even with deviation from the breed pattern.

In geometric morphometrics, the Procrustes distance is a measure of the difference between groups considering the shape as a whole (Slice, 2005). Thus, the Peloco and Caneluda breeds are more similar in head shape, and the Peloco and Barbuda breeds are more divergent. However, the cross-validation result indicates that even though the breeds are different, the bird phenotypic patterns are still little defined.

This low phenotypic pattern is a characteristic of native chicken breeds (Almeida et al., 2013, Moreda et al., 2014). However, Peloco chickens had the highest rates of correct classification, i.e., a more characteristic phenotypic pattern. This was due to the fact that the Peloco breed has been managed in the breed conservation center of the State University of the Southwest of Bahia longer than the Caneluda and Barbuda chickens, which were recently introduced to the conservation center.

The cross validation test also showed the variation within each breed. This variation is a result of the lack of phenotypic pattern of these naturalized birds, their genetic diversity, variations depending on the location where the birds were breed, geographic isolation, the artificial selection to which these animals were subjected (Bett et al., 2014), and the high crossbreeding level in the formation of these breeds (Almeida et al., 2013).

Regarding their difference in size, the Caneluda ecotype emerged from birds selected for larger size, long legs and broad breast. Thus, it stood out with larger body size due to its phenotypic pattern, which may explain its largest centroid measures. In contrast, chickens of the Barbuda and Peloco breeds are smaller and have lower weights when adults.

The larger size of the female Caneluda chickens, and their shape similarities with the female Peloco breed indicate that their head shape variation were more depended on other factors than the head size variation. The shape variation was relatively small, compared to the size variation, which differ depending on the sex, between populations, species and individuals in the same group (Slice, 2005). Generally, size is greatly affected by environmental factors, such as type of breeding and feeding, and age.

In traditional morphometry there is no distinction between shape and size, consequently, many measures used to describe the animals' variation in body shape are affected by size variation. Thus, differences may occur among animals under different nutritional managements or ages. Studies using traditional morphometry in domestic animal species have found variations among populations of different herds or locations, considering the body measurements of these animals (Yakubo et al., 2010; Ogah et al., 2011; Texeira-Neto et al., 2012;). However, it is difficult to say whether the variation found is due to differences between individuals or environmental effects. Although some studies using linear measures correct the size effects on the shape (Medrado et al., 2008; Ordonez-Garza et al., 2010; Almeida et al., 2013), geometric morphometrics answer this question by separating shape and size, more accurately identifying shape variations among populations of different environments.

The chicken breeds found in the region evaluated are generally classified as *galinha-terra* (land chickens). However, this description does not include the proper distinction of the breeds, i.e., disregarding the breed variation and characteristics of each group. In this study, despite the chickens share several morphological characters due to the great phenotypic variation of each group, it was possible to separate them through differences in the shape and size of morphological structures that are not observed visually.

The methodology used allowed the identification of head shape variations in the chickens, and can help in their characterization. The beak and eye regions had the greatest variation in shape; females of the Peloco and Caneluda breeds were similar, characterized by their wide, curved beak and round eyes. The Barbuda breed had shortened and dorsoventrally flattened beaks and elongated eyes.

The information on the breed characterization of naturalized chickens is still scarce. Thus, the results obtained in this study will be useful to identify groups of naturalized chickens in the region evaluated, characterize these chicken breeds, and indicate possibilities of implementing use and conservation strategies. Moreover, this methodology may contribute to studies on diversity and breed characterization of other domestic animal species.

ACKNOWLEDGEMENTS

Thanks to the Bahia State Research Foundation (Fundação de Amparo à Pesquisa do Estado da Bahia – FAPESB) and the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq) for granting research scholarships and financial support. Thanks to the State University of Southwestern Bahia (Universidade Estadual do Sudoeste da Bahia – UESB) for providing the experimental animals and facilities.

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ANNEXES

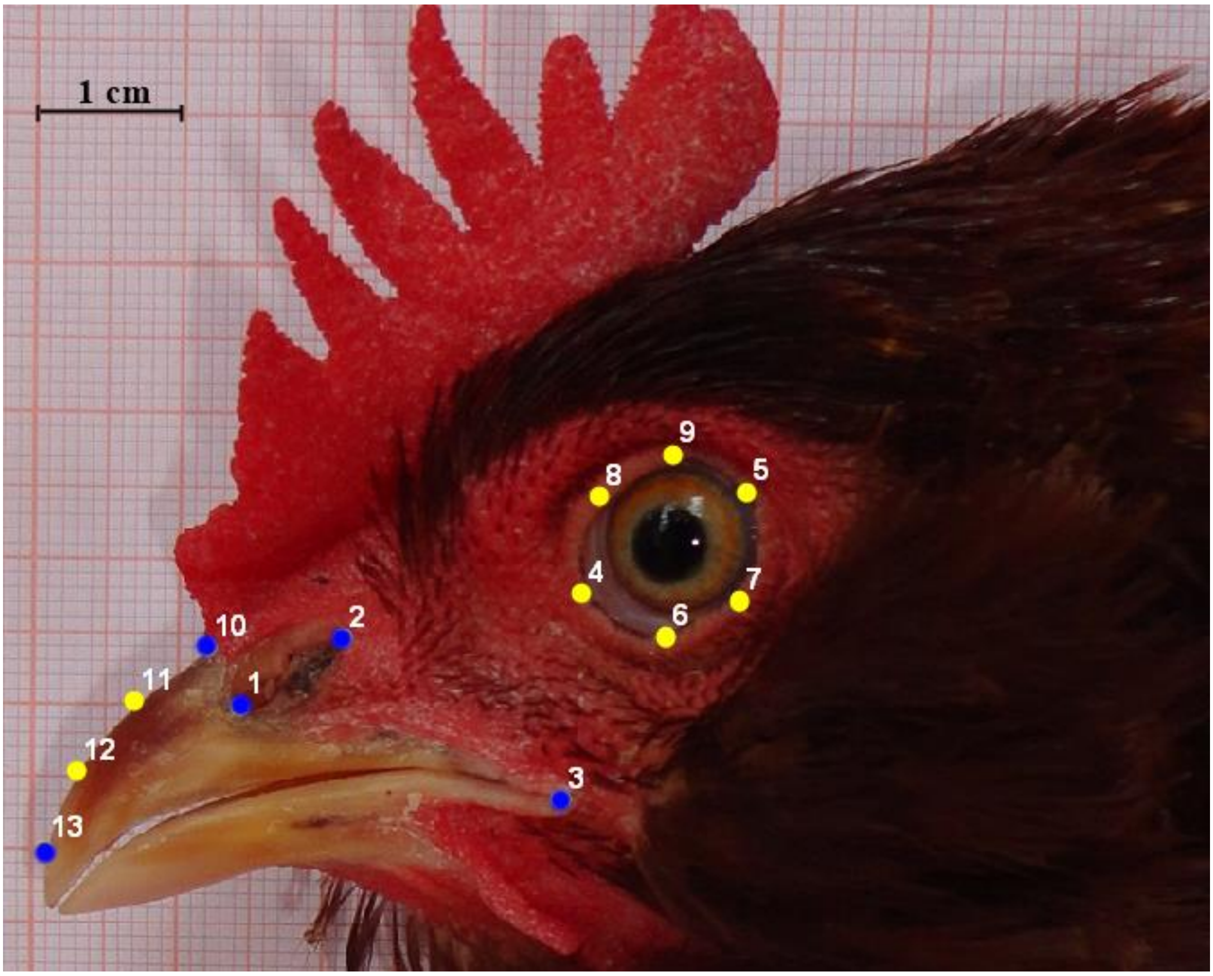
Table 1. Procrustes distances (upper diagonal) and Mahalanobis distance (lower diagonal) between female native chicken breeds.

Breed	Barbuda	Caneluda	Peloco
Barbuda		0.0221*	0.0266*
Caneluda	3.4926*		0.0219*
Peloco	3.7013*	2.6445*	

*($p < 0.01$)

Fonte: Autores (2016)

Figure 1. Left profile of the head of a female Barbuda chicken with five type-I landmark and eight semi-landmark coordinates. Blue dots (1, 2, 3, 10 and 13) are type-I landmarks. Yellow dots (4, 5, 6, 7, 8, 9, 11 and 12) are semi-landmarks.



Fonte: Autores (2016)

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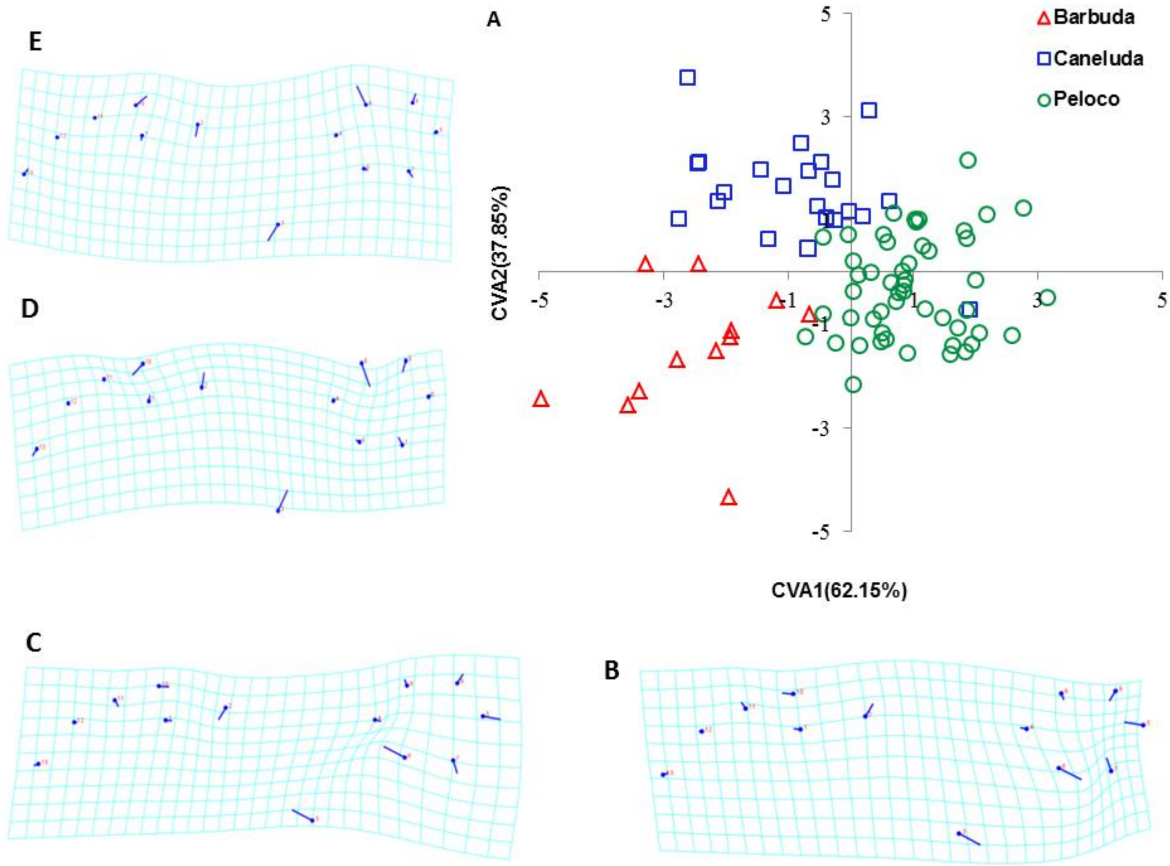
Figure 2. Graphic dispersion and deformation grids of the head shape of female chickens of the Barbuda, Caneluda and Peloco breeds. (A) Graphic dispersion related to the first two canonical axes (CVA1 and CVA2); (B) Representation of the deformation associated with the positive end of the CVA1; (C) Representation of the deformation associated with the negative end of the CVA1; (D) Representation of the deformation associated with the negative end of the CVA2; (E) Representation of the deformation associated with the positive end of the CVA2.



Fonte: Autores (2016)

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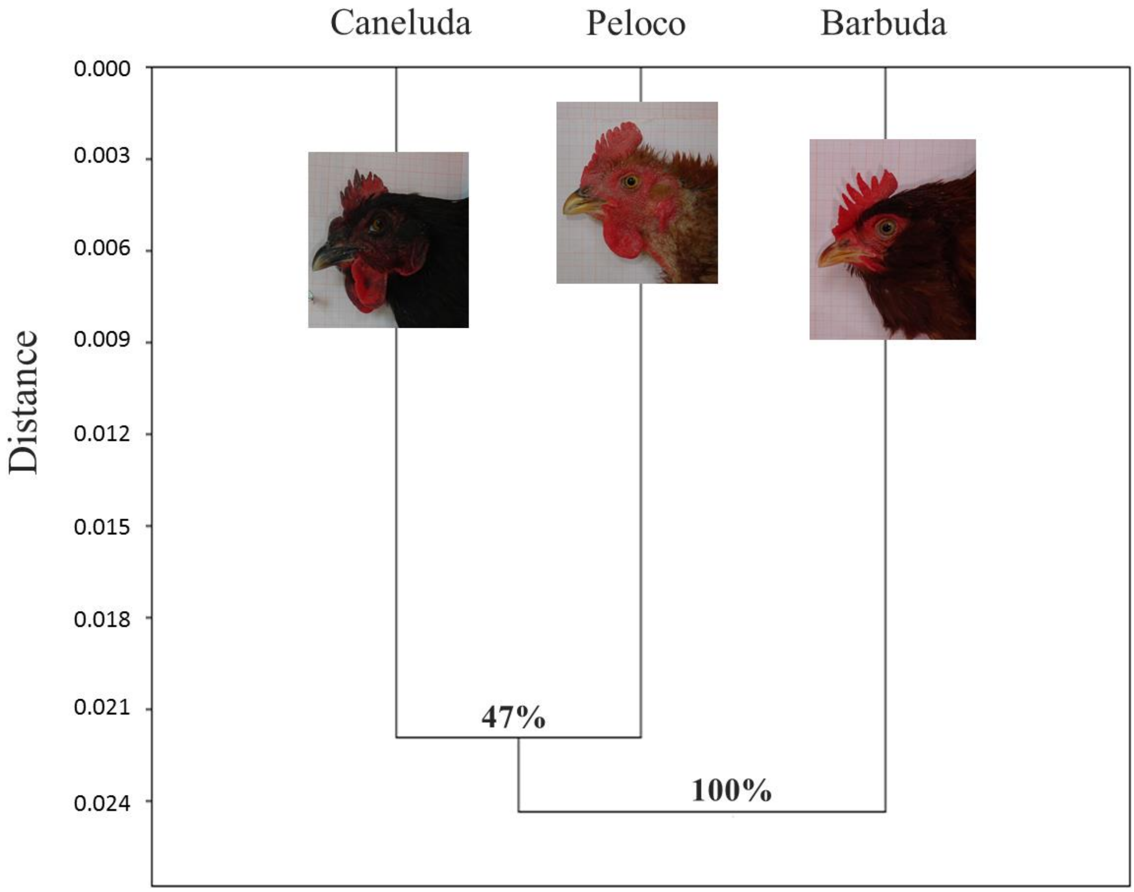
Figure 3. Dendrogram based on the head shape of female chickens from the Barbuda, Caneluda and Peloco breeds, obtained by the UPGMA (Unweighted Pair Group Method with Arithmetic Mean).



Fonte: Autores (2016)